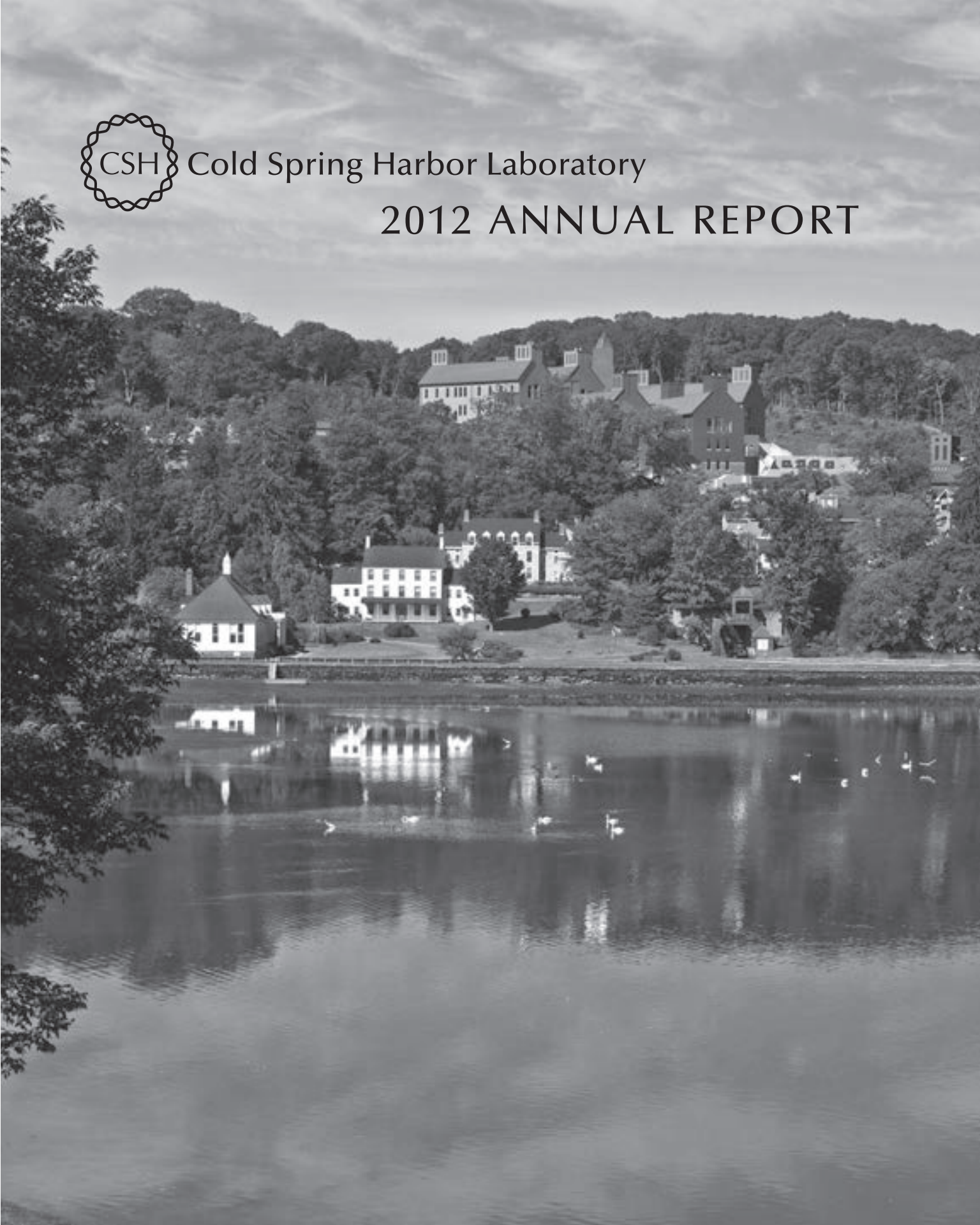
CSH Cold Spring Harbor Laboratory

2012 ANNUAL REPORT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Cold Spring Harbor Laboratory
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www.cshl.edu

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

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John J. Phelan, Jr. (1931–2012)

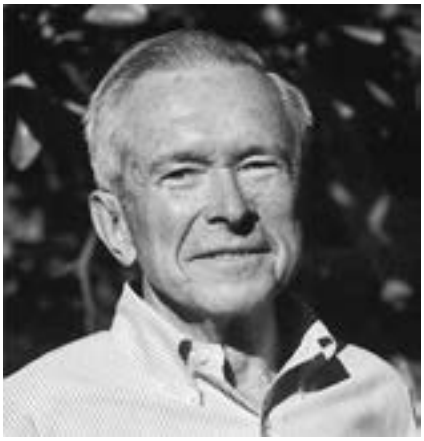
Remembered on Wall Street for his ability to both calm and transform markets, John is remembered at Cold Spring Harbor Laboratory (CSHL) for his long-standing support of genetic research and education. After a long and successful career on Wall Street that culminated in his oversight role as chairman of the New York Stock Exchange throughout the 1980s, John served two terms as CSHL trustee from 1992 to 1999.

His tenure as head of the New York Stock Exchange saw the beginning of major technology changes, most notably the ascendancy of computer technologies that would later so dominate trading. He literally oversaw the transition from the way stocks were traded by specialists to modern computer-driven trading. John was universally praised for his calming response to the stock market crash of October 1987 and the market's recovery.

A graduate of the class of 1970 from Adelphi University, John served his country as a sergeant in the Marines in Korea. He began his career on Wall Street in 1955, becoming managing director of Phelan & Company in his father's specialist firm. As a CSHL trustee, he was active on many board committees, including Executive, Audit, and Commercial Relations, of which he was chairman.

John and his wife, Joyce, provided seed money for the BioMedia program at the CSHL DNA Learning Center, which has grown into a suite of 24 websites providing free education services to millions of students and teachers. The couple also supported the founding of the Watson School of Biological Sciences, our innovative graduate school that was begun during John's tenure and, as of this year, counts a total of 56 Ph.D. recipients, many of whom serve as faculty at universities in the United States and around the world.

We are indebted to John and his family, including his three sons, John, Peter, and David. John's insight was invaluable to the development of innovative science education programs at CSHL. He also provided wise business counsel to the Laboratory at a time of significant growth in its research programs. He is missed but well remembered.



Townsend Jones Knight (1928–2012)

With the passing of our dear friend and Honorary Trustee Townie Knight, Cold Spring Harbor Laboratory also lost a very special connection to the institution's historic roots on Long Island. A descendant of Major Thomas Jones, whose descendants are most famous for owning land from Oyster Bay on the north shore of the island to what today is Jones Beach on the south shore, Townie's DNA was literally part of the Laboratory's DNA.

In 1890, John Devine Jones donated land from an obsolete whaling enterprise to help establish the Biological Laboratory (Bio Lab) at Cold Spring Harbor, one precursor to the modern day Cold Spring Harbor Laboratory (CSHL). With an additional contribution of $5000, the Jones Laboratory was completed in 1893 and still stands as a state-of-the-art neuroscience research lab. Eighty years later, in 1970, Townie joined the Cold Spring Harbor Laboratory Board of Trustees, which he served in many leadership roles until 1995. He was elected Honorary Trustee in 1995 and continued to attend Board meetings for many years. While proudly carrying on the Jones family legacy, Townie left his own distinguished mark on the Laboratory; he oversaw the transfer to CSHL of the assets and land of the Wawapex Society, established in 1892—a Jones Family legacy that supported the Bio Lab and the adjacent Fish Hatchery. As a consequence, CSHL manages the land used by the Friends of the Fish Hatchery.

Educated at Harvard College and having earned a law degree from Columbia University, Townie served in the Air Force before becoming a Senior Partner at the New York law firm Curtis, Mallet-Prevost, where he specialized in international and domestic banking and corporate and financial areas. He was generous in sharing this expertise with us as Assistant Secretary, Audit Committee Chairman, and an early member of the Commercial Relations Committee, on which he helped to oversee some of the first biotechnology spin-off companies established from CSHL research. Townie assisted in the initiation of CSHL's collaborative plant biology research relationship with Pioneer Hi-Bred International, Inc., known today as DuPont Pioneer. He also provided wise legal counsel before CSHL had a lawyer on staff.

Townie and his wife Elise were always great friends and consistent supporters of the CSHL Annual Fund. They also understood the strategic importance of our endowment. In 1993, they donated a home in Lloyd Neck, establishing the Knight Trust to benefit the CSHL endowment. In 2000, we were happy to honor Townie with the dedication of the Townsend J. Knight House, which was once a Jones family home, general store, and home to a young Townie that now serves as student housing for our Watson School of Biological Sciences Ph.D. candidates.

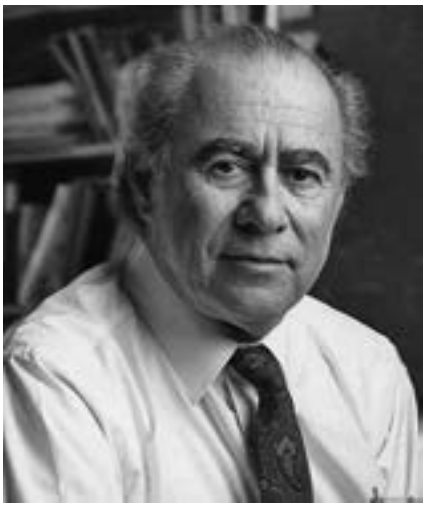
Robert Van Cleef Lindsay (1926–2012)

Rod Lindsay was one of the founding board members of the contemporary Cold Spring Harbor Laboratory (CSHL) that was established in 1962, beginning his relationship with us when we were still the Long Island Biological Association (LIBA) and the adjacent Department of Genetics of the Carnegie Institution of Washington, both predecessors of CSHL. From 1959 to 1965, Rod was treasurer of the LIBA and was later appointed chairman of the Executive Finance Committee of the new CSHL board—the perfect fit for a man who spent his entire career climbing the ranks to become president of J.P. Morgan & Co. and its principal subsidiary, Morgan Guaranty Trust Company of New York. It was Rod whom we thank for engaging the Lindsay family dynasty so integrally in the evolution of CSHL.

Rod is remembered by his family as a Renaissance man, embodying a rare combination of intelligence, loyalty, humility, humor, and practicality—an exceedingly bright gentleman with great common sense and goodwill. He was always concerned with the morale of then Laboratory Director John Cairns, for when John started at CSHL, there was no endowment and the Laboratory did not even have the funds for the director's salary. It was incumbent upon Rod and his fellow trustees to establish and nurture the newly formed hybrid organization that merged the two historic organizations into one. With a degree from Yale, Rod was a lifelong student of history and human nature. Without him, Cold Spring Harbor Laboratory might not have survived.

Rod and his wife, Nancy, supported the construction of the Mary D. Lindsay Child Care Center on our campus, a project that has made an enormous impact on our ability to recruit and retain early-career scientists. Rod's sister-in-law, Mary, also CSHL trustee and honorary trustee, was the instigator of that initiative that has literally transformed how our scientists interact with their children while working at the cutting edge of science.

Following in his father's footsteps, Rod's son Bob continues the Lindsay family support of CSHL and he is currently vice chairman of the Board of Trustees. Like his father, Bob is charting the future path of this great institution that has grown so successfully since the early guidance of Rod and his contemporaries. It is quite certain that Rod and Nancy are also smiling on Bob and wishing us continued success.



Norton Zinder (1928–2012)

In June 1953, Norton Zinder, then 25 years old and a newly minted Ph.D., attended *Viruses,* that year's Cold Spring Harbor Symposium on Quantitative Biology. He was going to be, he was sure, the star of the show; he was going to describe transduction, a new way in which genetic information carried by a phage passed from bacterial cell to bacterial cell. It was not to be—this was the Symposium where Jim Watson described the double helix. As Norton put it with considerable understatement many years later, "I was disappointed by being overshadowed by the Watson-Crick model of DNA."

Zinder's association with Cold Spring Harbor predated the 1953 Symposium. He had taken the Phage Course in 1949 while still a graduate student with Joshua Lederberg and had attended the 1951 Symposium. By the time Zinder participated in the classic 1961 Symposium on *Cellular Regulatory Mechanisms,* things were in turmoil at Cold Spring Harbor. Milislav Demerec had stepped down as director of the two institutions on the Cold Spring Harbor site: the Carnegie Institution of Washington's Department of Genetics and the Biological Laboratory. Zinder was now a well-established researcher at the Rockefeller Institute and sufficiently senior to be invited to become director of the Department of Genetics. Zinder decided that for the department to be effective, a capital investment of $500,000 was needed, but all Caryl Haskins, President of Carnegie, would offer was $200,000. Zinder declined the invitation and wrote an analysis of the situation, recommending that the two institutes be amalgamated. Whether Zinder's suggestion carried any weight, his recommendation came to pass 2 years later and the Cold Spring Harbor Laboratory of Quantitative Biology was formed.

Zinder took on a formal role in the affairs of the Laboratory in 1967 when he became a Trustee representing the Rockefeller Institute. Rockefeller was one of the participating institutions that had pledged $5000 each to support the recently formed Cold Spring Harbor Laboratory. Zinder served a full term until 1973 and returned for a second stint from 1979 to 1984.

In 1966, John Cairns, Director of the Laboratory, announced his resignation, and Zinder played a key role in the transition of the directorship from Cairns to Jim Watson. Watson had joined the Trustees as the Harvard representative and prepared a document describing what he thought needed to be done about the Laboratory. As Zinder reported, "I said to Jim if the job was so easy, why didn't he take it over. To everyone's surprise he said he would think it over. The next day he accepted the post." One of Zinder's other roles, he claimed, was "to defuse Jim's resignations—when upset by the Board, he would resign and it was my job to bring him back."

Norton was interested in the social responsibilities of scientists. He was a key figure in the discussions about recombinant DNA and later chaired a committee to advise the United States government about the disposal of its chemical warfare stockpiles. He made few friends when

the National Cancer Institute committee that he chaired, reviewing the Virus Cancer Program, reported that only 50% of the annual $50 million program was worth supporting. He was a key figure in the establishment of the Human Genome Project (HGP), acting as chairman of the National Institutes of Health Human Genome Advisory Committee (1988–1999). He helped to mediate the short-lived rapprochement between the public project and Celera at the time of the 2003 announcement of the completion of the HGP.

Norton Zinder was born on November 7, 1928, in New York City. He graduated from Columbia University at age 18 and completed his Ph.D. in 1952. He moved to the Rockefeller Institute that year and remained there for the remainder of his career. He was the recipient of numerous awards and honors, including election as a member of the National Academy of Sciences in 1969. He received the Eli Lilly Award in Microbiology and Immunology from the American Society of Microbiology and the NAS Medal in Molecular Biology from the National Academy of Sciences.

Jan Witkowski

PRESIDENT'S REPORT

Cold Spring Harbor Laboratory continues to produce breakthrough science and provide outstanding science education. Of particular note, 2012 saw the recruitment of six new faculty, two of whom, David Tuveson and Gholson Lyon, are not only outstanding scientists, but are clinicians who see patients. David Tuveson will oversee our exciting new Cancer Therapeutics Initiative and focus his research on pancreas cancer, melanoma and carcinoid tumors. He will also work closely with the nearby Lustgarten Foundation that focuses on pancreas cancer, where he will head its science program. Gholson Lyon works in pediatric neurology and studies inherited neurological disorders, principally in families in Utah. Molly Hammell, Jesse Gillis, Dan Levy, and Ivan Iossifov strengthen our basic science research, notably in the field of genomics and in quantitative biology, an area that has emerged as a strong component of our research portfolio. CSHL continues to be recognized by Thomson Reuters as the leading institution in the world in molecular biology and genetics, evidence that our fundamental research is having great impact. One example is Adrian Krainer's years of dedicated inquiry on how genes are differentially expressed—basic research that has now resulted in a promising drug he co-developed for the children's genetic disorder spinal muscular atrophy, which now enters Phase 2 clinical trials.

As 2012 drew to a close, however, the United States approached the edge of a financial precipice popularly called "the fiscal cliff." Amid the fractious partisan politics surrounding that term and the unresolved issue of our federal budget deficit, the implications were clear for the Laboratory. We faced the prospect of yet another year in which our elected national representatives would not find a way to pass a budget, once again leaving in doubt the status of federal support for basic research.

We entered the current year, therefore, acutely aware of the urgency of attracting substantial new philanthropic contributions in order to avoid the possibility that critical cancer, brain and plant biology research be curtailed, even for a moment. That would mark an historic and tragic retreat. Although American philanthropy leads the world in support of basic and applied science, it cannot replace the substantial, stable and predictable support that historically has been provided by the federal government. Unfortunately, stagnant or even declining federal support of science is now the norm. If Cold Spring Harbor Laboratory is to remain at the forefront of research in molecular biology and genetics, we need a larger endowment as a buffer to variations in federal funding that are now occurring and will likely occur for at least the next decade.

Our nation has been without a budget for 4 years. To pay bills since approving its last budget in April 2009, Congress has passed a series of what lawmakers euphemistically call "continuing resolutions." Failing to provide the fundamental guidance and stability that an annual budget provides, our leaders have thus defaulted on a constitutionally specified responsibility to the electorate. This failure has had a deleterious impact on many aspects of national life, including the pursuit of new knowledge in the biological and life sciences. These reductions are also coming at a time when private industry has dramatically cut back on fundamental and basic scientific research, in favor of primarily supporting applied research that leads directly to commercial products. American industry is increasingly looking to academia to carry out the basic research and discovery that will benefit our economy.

For our Laboratory and other research institutions, the absence of macro-level budgetary guidance has had two broad effects. One has been an implementation of fiscal austerity, by default. The other is that the lack of guidance from the federal government has thrown into limbo attempts by the scientific community to plan the course of future, long-term research projects.

In 2012, federal support for research at CSHL, mostly through grants to individual investigators by the National Institutes of Health (NIH) and the National Science Foundation (NSF), amounted to $53.9 million, over 49% of the total funding that we received in support of research

from external sources. To state the obvious: One cannot effectively prepare a plan for future work when a key funding source cannot make choices and plans of its own. Even after the 112th Congress in its waning hours passed a tax package that averted the "cliff," a budget still was not forthcoming. We therefore once again had to guess at the expected level of support for research, essentially figuring out how to deal with uncertainty, including the issuance of some grants on a 6-month basis (normally grants are awarded for 4 years and funds release 1 year at a time). Any scientist can tell you that half a year of funding means nothing when the experiments you are conducting will take several years to plan and execute.

American science will fall behind, without a doubt, if the current situation continues into the future. That much is certain.

My purpose in this letter is to report the present situation and to make suggestions about how federal funding for research might be dispensed on a more stable and predictable basis. I want to discuss not only the federal role in sustaining our common enterprise, but also to encourage members of the research community to help strike a crucial balance by acknowledging our own responsibility to make realistic plans for future work, in view of the ongoing fiscal constraints.

I also want to appeal to philanthropy, to those private individuals who are able to provide much-needed support to CSHL in these austere times to fulfill the vision held by Carnegie, Rockefeller, and other great American philanthropists: that helping to advance science will secure the future strength of the society that our children will inherit. Fortunately for Cold Spring Harbor Laboratory, our Trustees and supporters have recognized this need, but we do need to broaden our base of philanthropic support.

Endless Frontier, Finite Funding

Science is indeed the "endless frontier," as the great American proponent of federally supported basic research, Vannevar Bush, successfully argued the year the Second World War was won. But no one, including Bush, ever said that public funds to support the exploration of the scientific frontier would be limitless.

We in the research community must acknowledge that public funding is finite. Yet in the area of basic and applied biomedical research, as channeled through the NIH, it is important to establish that funding has been essentially frozen since 2003. In that year, legislators from both parties could boast that over the previous 5 years they had authorized a doubling of NIH funding. Spurred by excitement over assembly of the complete human genome sequence and the practical benefits that many expected quickly to materialize from that work, the doubling was an example of lay enthusiasm running ahead of scientific and economic common sense.

What biological scientist would *not* favor a doubling of the main source of research-grant funding? Actually, many of my colleagues and I were not sanguine about the prospect, much preferring instead a federal commitment to a steady and predictable increase in basic research funding each year, while keeping up with or slightly exceeding biomedical cost inflation, which tends to run higher than the national consumer price index.

What some feared in 2003 has come to pass: Federal funding for the NIH has flatlined since the completion of the doubling. Measured from a 1998 baseline, the 2003 NIH appropriation of over $27 billion represented a 100% increase in nominal dollars. But since that time, now nine funding cycles in the past, the NIH appropriation has actually *declined* over 16%, taking biomedical cost inflation into account: the $30.86 billion appropriated for NIH in 2012 was over *$5 billion less* than the 2003 figure, when inflation is factored in.

If we had possessed the foresight and nerve in 1998 to eschew the doubling concept and had instead secured commitments for modest 3% annual increases in federal support for NIH, while pegging base funding to inflation, the NIH budget in 2012 would have exceeded $32 billion—

The Laboratory has always prospered because of philanthropic foresight and civic mindedness. Charles Robertson, seen here with the Watsons in 1974, seeded our endowment.

about $1.5 billion, or 5%, more than the actual 2012 estimate. These numbers assume continued funding of science during the dramatic financial crisis in 2008, but over $10 billion was added to the NIH budget in 2009 from economic stimulus funds, which would not have been necessary if continuous and predictable funding had instead occurred. With such steady support, scientists could have planned long-term projects with confidence. As the situation exists now, many scientists are closing their laboratories because of lack of funding since the sudden spurt of NIH funding has withered and was not sustained. Fortunately, at Cold Spring Harbor our sources of private support have so far prevented shutting down research laboratories. Indeed, philanthropic support has even enabled starting new initiatives that have had a major impact on cancer and autism.

My point is one that prudent financial advisors have been making for as long as capitalism has been around: a simple compounding at modest rates of annual increase is very likely to be more powerful than an occasional fiscal surge, inspired by what are often unrealistic expectations of near-term payoffs. "Slow and steady wins the race"—in science as in building a nest egg.

There are powerful reasons behind this argument as it applies to research funding, and they are not only about numbers. It is instructive to look back for a moment at the history of how our federal government came to vigorously support basic science. Prior to the Second World War, federal contributions were minimal, as weighed against funds provided by the nation's great philanthropists. Most biomedical research was then conducted by scientists based in universities that were supported by endowment income, special research funds, and foundation grants. The year before the start of the Great Depression, The Rockefeller Institute had since its founding in 1902 received some $65 million in endowment funds from the estate of John D. Rockefeller. As noted by the historian Paul Starr, this alone was many times the amount spent by the federal government on medical research during that same interval.[1]

Early in the 20th century, it was the Department of Agriculture that received the lion's share of the government's research attention. To the very limited extent that it invested directly in medical research, the federal government focused on the Hygienic Laboratory, once part of a hospital

[1]Paul Starr, *The Transformation of American Medicine* (Basic Books, 1982), 339.

in Staten Island, and later, after moving to Washington, D.C., the forerunner of the U.S. Public Health Service (PHS). Just after the turn of the 20th century, allocations were less than $50,000 a year. During the Progressive Period, the PHS began to study infectious diseases. In 1930, the Hygienic Laboratory was renamed the National Institutes of Health, and in 1938 it moved to its present location in Bethesda, Maryland. A year prior to that, the government had established the National Cancer Institute (NCI). And in a major departure in 1944, for the first time the NCI authorized federal funds to be allocated to basic researchers not directly in the government's employ. This was the precursor of the modern extramural grant program that provides core research funds for CSHL and many other American research institutions.

On the eve of America's entry into World War Two, the NCI's cancer grants and all other PHS activities received less than $3 million in total federal support. The Department of Agriculture's research budget was almost nine times greater. The war, of course, turned the tables. Vannevar Bush's Office of Scientific Research and Development (OSRD), which played an historic role in developing advanced technologies that made possible the Allied victory, was split in two sections, one dedicated to defense technologies, the other, under the Committee on Medical Research (CMR), focusing on technologies to help meet the medical problems occasioned by war. From a new way to treat malaria, to the isolation of blood derivatives like gamma globulin, to the development of means of mass-producing penicillin, the CMR achieved triumphs that would support arguments following the war for a greatly augmented federal role in biomedical research.[2]

Among the proudest moments in the history of Cold Spring Harbor Laboratory are those chronicling the roles of Demerec, Bryson, and others in laying the foundations for critical advances in penicillin production. Vannevar Bush drew a very important lesson from this and other wartime research efforts. It concerned the society and system that had given rise to academic research institutions like ours where wartime advances were made. The United States was a nation that had granted autonomy to scientists. Open-ended, investigator-initiated basic research had generated the pool of intellectual property from which real-world products were rapidly developed in the urgent wartime context.

Bush's famous report, "Science: The Endless Frontier," written in 1945 in fulfillment of a request from President Franklin Roosevelt, was, however, not an argument for federal funding of *applied* research, but instead the kind of *basic* research that had provided the intellectual capital to fuel those wartime innovations. The war made clear to Bush that only the federal government had sufficient economic power to sustain a world-class basic-science research establishment. Upon this foundation the nation would prosper financially and militarily. But also, importantly, the health and welfare of its citizens would steadily improve. This is the vision behind our modern NIH and NSF.

When Bush later recollected that "[t]he war . . . proved that things could be accomplished in a hurry, given *unlimited* funds, the intensity of war, and most important, a background of basic scientific knowledge ready for application,"[3] he did not mean to suggest this was a reasonable expectation for a nation in peacetime. The question in ordinary times was whether enough funds would be allocated and enough freedom be granted scientists so as to keep new discoveries coming. Today, the NCI budget represents about $16 per person per year for the support of all cancer research and therapy development. About the same amount per person supports all neuroscience research, including all research on Alzheimer's, Parkinson's, all other neurodegenerative diseases, all psychiatric disorders such as autism, schizophrenia, depression, acute disorders such as stroke and trauma, as well as basic brain science. So the annual investment per person is not substantial.

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[2]ibid., 340–342.

[3]G. Pascal Zachary, *Endless Frontier: Vannevar Bush, Engineer of the American Century* (MIT Press, 1999), 288.

Taking Responsibility

At this moment our nation is clearly limited in its capacity to finance basic science. Things that might be done today in areas in which much progress has recently been made, cancer therapeutics being the most notable, are not getting done because of the scarcity of resources, both public and private.

What can we reasonably expect? I would be pleased to see a federal commitment to present levels of NIH and NSF funding, adjusted for inflation plus 3% per year. This would place CSHL and peer institutions on an even keel during the next decade, in which austerity is likely to prevail. It is imperative that we keep up with and at least somewhat exceed the rate of biomedical cost inflation. Otherwise, the nation's science is certain to enter a period of decline. We will not be able to plan, as I have noted. Nor will we be able to support, and therefore will not be able to attract, first-rate minds to enter scientific research. According to a recent report of the NIH-sponsored Biomedical Workforce Committee, of which Cold Spring Harbor's Leemor Joshua-Tor was a member, the number of doctoral students in basic biomedical science continues to soar, with over 15,000 Ph.D.s granted annually. With the success of our science, this number has risen quickly, from a base of about 6500 new Ph.D.s a year in the mid-1980s. The number far exceeds the numbers exiting basic science. In contrast, other fields of science such as chemistry, physics and behavioral and social science do not produce increasing numbers of active Ph.D.s. At the same time, the number of all principal investigators under age 36 in biomedicine who are receiving career-sustaining NIH grants has declined from 18% 2 decades ago to less than 4% today. The number of applications, meantime, for a shrinking number of NIH grant awards of all kinds continues to rise. Nationwide, fewer than 1 application in 6 is successful. Based upon my experience in reviewing grants, I estimate that 1 in 4 or 1 in 3 are of the highest quality and warrant funding. At Cold Spring Harbor, the success rate of proposals by our faculty is more than twice the national average, partly due to the philanthropic support that sustains science through times when federal funding is not obtained and partly due the fact that at Cold Spring Harbor, philanthropic support supplements federal grant support.

Under these circumstances it is imperative for research institutions to take responsibility for their future. The administrative team at Cold Spring Harbor Laboratory provides an example of how ambitious yet realistic planning 5 to 10 years out, combined with a constant effort to husband scarce resources, can yield a program in basic science that—as the statistics continue to show—places our faculty's output at the very top of the field. As mentioned, CSHL's research is the most influential in molecular biology and genetics, as measured by the impact of faculty publications.

The so-called NIH salary cap determines the maximum salary support that scientists can receive. It has been reduced substantially in recent years, and may be reduced some more. We and other institutions must make up the difference if we are serious about retaining faculty, for the NIH cap does not come close to the level of salary that we must pay our senior scientists. We do provide such support to all of our senior faculty, while supporting full salaries for at least the first 5 years for all junior faculty. We also pay for a large portion of our infrastructure costs, in part to meet conditions set but not paid for by the federal government, but mostly to keep the infrastructure capable of supporting cutting-edge science.

How do we manage this? Science at CSHL cannot be done without the proceeds from our fundraising efforts plus annual spending from our endowment. The latter is frankly still too small to provide us with breathing room. So, we have tried to plan our future science programs on the basis of what we can realistically budget, given all the current constraints. It is now time for our political leaders do their part to keep federal support for research predictable. But as I have intimated, the needs of the research community will certainly exceed the government's ability to provide, probably for a decade or more. At Cold Spring Harbor, we are very fortunate to receive generous support from many foundations and individuals. These sources, together with our

endowment draw, covered 50% of our research expenditures in 2012. Such a ratio of federal to private funding of research may have to be the norm for all institutions in the future, not just Cold Spring Harbor and other like-minded research institutions. Medical schools will have to provide more to their scientists, but this change also comes at a time when clinical income is dropping at a rapid clip.

The Power of Philanthropy

Philanthropic support has been a fundamental part of what makes Cold Spring Harbor Laboratory successful. It is clear to all our scientists that federal grant support provides the core funding needed to maintain a research program and its key infrastructure, but it is philanthropic support that allows our scientists to do their most innovative research. Thus we must increase philanthropy, growing our endowment so that key funds can be allocated when needed, not in the year or two that it takes to secure a federal grant, long after a new idea is stale.

Part of the logic for increasing support to Cold Spring Harbor is our track record: we have a long history of major accomplishments and great influence in both research and science education—all achieved through prudent use of very limited funds. The seeds of our success were sowed by the great philanthropists of the last century. The estate of Andrew Carnegie launched our genetics research and sustained it for 60 years. The Carnegie Institution of Washington put the Laboratory on the map as one of the world's leading centers of genetics research. CSHL's future Nobelists Al Hershey and Barbara McClintock were beneficiaries of the Carnegie largesse. When Carnegie monies were withdrawn in the early 1960s, the Laboratory very nearly was forced to close. Not long after that, Jim Watson came to Cold Spring Harbor as Director. The history of the intervening years was possible in large measure because of Jim's great ability to make the case for philanthropic support. He also appreciated the value of defined research focus, most notably cancer research. This research expertise brought in institutionally stabilizing federal funding, much of it via the NCI. Since 1987, we have been designated by NCI as a National Cancer Center for basic research in cancer.

The reality of today, however, is that we have arrived at a point at which, even with an endowment in excess of $300 million, we have little margin for disappointment, whether in the performance of our endowment portfolio or in levels of federal support, or, indeed, in the success of our fundraising efforts. CSHL has prospered because of philanthropic foresight and civic-mindedness: that of Mr. Carnegie and his administrators, but also our local heroes including the Jones family, Louis Tiffany, William K. Vanderbilt, Walter Jennings and George Pratt, Marshall Field III, and J.P. Morgan. Without them, the Delbrück Laboratory would never have been built. Without them, Demerec and Hershey and McClintock, not to mention summer visitors like Salvador Luria and Max Delbrück, also both Nobel laureates, or the then young turks like Norton Zinder and Jim Watson, might never have had the opportunity to change history, right here on the grounds we walk each day.

Charles Sammis Robertson's philanthropic spirit provided the foundation for our current endowment and changed the face of the institution, giving us an endowment and the opportunity to create the Banbury conference center, where small private gatherings of people with the power to change the course of science have been held regularly for over 3 decades. More recently, we have been privileged to have truly major support from the Simons Foundation and the Stanley Medical Research Institute, philanthropic support that has literally changed how we understand the cause of certain psychiatric disorders. Today, we are fortunate to have many other individuals, particularly our current Trustees, who provide significant support to Cold Spring Harbor Laboratory and provide us with the funds that will ensure that we remain a leader in the life sciences. But to maintain our leadership in research and science education we will have to expand greatly the number of such loyal supporters.

"Surplus wealth is a sacred trust which its possessor is bound to administer in his lifetime for the good of the community." That was Andrew Carnegie's "Gospel," and it is one that I fervently hope a civic-minded few will now take to heart so that our great institution might safely navigate some very treacherous fiscal waters. Perhaps our nation's leaders will also smooth out the fiscal waters for research funding, and together, a public-private partnership will continue to ensure that American science remains the most innovative in the world.

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

Research

The year 2012 was another superb one for research at Cold Spring Harbor Laboratory. Here, we provide details of a handful of significant investigations that led during the year to published results in major scientific journals. These are suggestive of the breadth of activity at the Laboratory, as more than 600 scientists and technicians in 52 labs extend our knowledge of cancer cell biology and genetics, as well as cancer treatments; critical circuits and biochemical pathways in the brain that go awry in psychiatric as well as neurodevelopmental and neurodegenerative disorders; and genes and their pathways in plants that will help boost crop yields and extend range. The influence of CSHL faculty publications continues to be extraordinary; once again we were independently rated Number 1 in impact on molecular biology and genetics, worldwide.

Pfizer

DuPont Pioneer

The value of the Laboratory's research is gaining broader recognition outside the academic community. 2012 was marked by the announcement of collaborative agreements with two companies at the top of the Fortune 500, DuPont and Pfizer Inc. CSHL's plant biologists are working with scientists at leading seed producer DuPont Pioneer to better understand the genetic processes that control growth and development. This work is already providing a basis for new ways of boosting plant yields and for extending the range of key food crops. Pharmaceutical giant Pfizer is teaming with our researchers to develop technology for a next-generation library of human short hairpin RNAs (shRNAs). These tiny molecules, which can turn genes on and off by engaging a biological mechanism called RNA interference, are extremely useful in the identification of novel targets for anticancer drugs.

Decision-Making Abilities in Response to Multiple Sensory Cues Are Similar in Rodents and Humans

The ability of humans to make decisions in the most efficient and unbiased way based on perceptual information, for example, from auditory and visual cues, is well documented. Assistant Professor Anne Churchland published work this year showing that other mammals, in this case rodents, perform equally well in similar tests. Rats were trained through receiving rewards to respond to visual cues via the appearance of a white light on an LED screen and auditory cues through a speaker, either separately or in tandem. The results of the tests with rats were compared to similar stimuli presented to humans, with some additional features added that mimicked a real-life situation. Both humans and rats made accurate decisions in response to these combined, multisensory stimuli that were close to optimal based on a statistical prediction. By varying the time between the visual and auditory cues, the team was also able to show that the brain processes each cue in parallel, before fusing them together at a later stage in order to make a decision. These results suggest decision-making processes are evolutionarily conserved and provide a basis for researchers to use rodents as an animal model to study decision making in humans.

A. Churchland

Live-Imaging Microscopy Offers Insights into Modulation of Tumor Response during Therapy

M. Egeblad

Assistant Professor Mikala Egeblad and her team are experts in live-imaging microscopy. This technique was instrumental to a study they published this year showing how cancer cells in mouse tumors and the noncancerous "stromal" cells around them (the tumor microenvironment) respond during chemotherapeutic treatment with the drug doxorubicin. They were able to watch as the tumor and the surrounding tissue changed before, during,

and after drug administration. What they saw in the resulting time-lapse movies was that there is regulation of the permeability, or leakiness, of the blood vessels that wind through and around the tumor, which impacts the local recruitment of inflammatory cells. Resistance to doxorubicin correlated with intermediate-stage tumor development and the relative permeability of the blood vessels supplying the tumor. In mice lacking the gene for a protein involved in regulating blood vessel permeability, matrix-metalloproteinase-9 (MMP9), blood vessels were leakier and mice responded better to doxorubicin. The response was also improved when mice were lacking a gene, *CCR2*, important in the recruitment of inflammatory cells. Mikala's live-imaging techniques point to the possibility of boosting therapy responses using additional targeted factors.

T. Gingeras

Massive Analysis of the Human Transcriptome Suggests Redefining the Gene

Professor Thomas Gingeras and colleagues, including Professor Greg Hannon, published a comprehensive analysis of the messages, or transcripts, produced across the complete genome of human cells. The study was part of the latest coordinated release of data from the Encyclopedia of DNA Elements, or ENCODE, consortium. The new data show that up to three-quarters of the genome is capable of being transcribed and that much of nonprotein-coding RNA is organized in a way that indicates it may be functional, i.e., it may perform biochemical actions, and that smaller functional RNA transcripts can reside within longer transcripts, being released as longer ones are chopped up or "processed." Given the large amount of the genome that is transcribed, and the nested organization of many nonprotein-coding RNAs, the perceived boundaries between genes are shrinking and even overlapping. These observations challenge the previous understanding of what discrete unit makes up a gene and raises the implication that disease-associated mutations may lie in functional nonprotein-coding RNA transcribed from what was once thought to be "spacer" regions of DNA between genes.

G. Hannon

Origin and Migration of Powerful Cortical Inhibitory Cells Determined

Chandelier cells are powerful inhibitors of the excitatory impulses from the pyramidal neurons of the brain's cortex. One chandelier cell makes contact, through chemical junctions known as synapses, with up to 500 pyramidal neurons. Thus, they are critical for managing information flow in the brain. But the origins of chandelier cells had remained enigmatic until a study by Professor Josh Huang and colleagues was published this year. Using new technologies to mark and trace migrating neural cell progenitors, they located the starting place for chandelier cells in a previously unrecognized portion of the developing embryonic mouse brain they dubbed the VGZ (ventral germinal zone). In addition, they identified a gene that is switched on at the birth of a chandelier cell called *Nkx2.1*, the product of which is a transcription factor important to other inhibitory neurons. Josh's team found that after birth, chandelier cells take a stereotypical route of migration into the cortex, where they set up residence at very specific sites in cortical layers 2, 5, and 6. Since it is already known that the number and connective density of chandelier cells is reduced in schizophrenia, work on the developing brain and how its circuits are put together has important implications for disease research.

J. Huang

Proteins Involved in Small RNA Biogenesis and Function

Two proteins important in the biogenesis of gene-regulating small RNAs were the subject of new publications in 2012 stemming from collaborative work between the groups of HHMI Investigators and CSHL Professors Leemor Joshua-Tor and Gregory Hannon. One paper detailed the

L. Joshua-Tor

structure of human Argonaute-2 (Ago2) protein bound to a microRNA (miRNA) guide. The structure revealed a remarkable evolutionary conservation also seen in an archebacteria protein the teams had previously determined, especially in regions important for target recognition and activity of the protein. Structure-based understanding of how the structure is stabilized, now clear, could be important for designing therapeutic small RNAs exploiting this mechanism or for blocking Argonaute activity. A second study published this year involved the biochemical characterization of a protein known as Zucchini, which had been thought to be involved in cleaving phospholipid molecules. However, previous work in the Joshua-Tor and Hannon labs had implicated it in the biogenesis of genome-protecting PIWI-interacting RNAs (piRNAs) found in cells of the germline—sperm and eggs. Their new results show that the mouse version of Zucchini, mZuc, does not have phospholipase activity, but in fact acts as a nuclease—it cuts nucleic acid chains. Structural analysis reveals that the shape of the catalytic binding site is consistent with that of a nuclease. Thus, the evidence implies the Zucchini protein is involved in reducing long precursor RNAs to short piRNAs in germline cells.

A Genetic Check in the Molecular Clock That Controls Timing of Tomato Plant Flower Production

The timing of flowering in plants is important for their successful reproduction. It helps attract pollinators such as bees. Variations in this timing can affect flower, fruit, and seed production, and consequently agricultural yield. Assistant Professor Zach Lippman and his team have previously shown that, in addition to external factors such as light and temperature, there is a molecular clock in the tomato plant responsible for controlling timing of flowering. In new research published this year, they showed that a gene called *Terminating Flower*, or *TMF*, is responsible for acting as a check on this clock, preventing it from running too fast. Mutations in *TMF* result in the reproductive shoots, or inflorescences, of the tomato plant producing just a single flower. This is due to the plant proceeding to flower while still in the vegetative state, the phase in which leaves are still being made. When normal *TMF* is present, the tomato plant produces its characteristic multiflowered inflorescences. Thus, *TMF* is responsible for coordinating and synchronizing the tightly controlled process of flowering by gently slowing down. The important implication is that the genetic control of flowering in plants such as tomato can be manipulated in agricultural crops to improve yield.

Z. Lippman

Toward the First Vertebrate Whole-Brain Image Map

Professor Partha Mitra and colleagues are working toward constructing a whole-brain wiring diagram of the mouse. Earlier this year, they released the first set of gigapixel (1 billion-pixel) images from the project (http://mouse.brainarchitecture.org). The current data set is close to 1 petabyte in size (uncompressed). To visualize the brain, the team labeled neuronal pathways with tracers, cut 20-μm-thick slices from "front" to "back," and then imaged each slice. Using a semiautomated, quality-controlled, light-microscopy-based assembly, they captured ~500 images per brain. These were then arranged to present viewers with a unique journey through a three-dimensional model of the mouse brain, along the neuronal pathways that represent the inputs and outputs of given brain regions. The images were produced at the intermediate, or "mesoscopic," scale of detail, i.e., between the extremely fine detail of electron microscopy and the macroscopic scale of MRI-based imaging. At the mesoscopic scale, Partha's team expects to produce a stereotypical map of neuronal connections, i.e., connections that are similar between individuals and genetically determined in a species-specific manner.

P. Mitra

A Protein That Has a Central Role in Cortical Progenitor Cell Fate

Professor Linda Van Aelst and colleagues have identified a protein key in determining whether cortical progenitor cells will proliferate, make progenitor cells, or differentiate and turn into mature cells. The progenitor cells are known as radial glial cells (RGCs), whereas the mature cells are pyramidal neurons, excitatory nerve cells found in the brain's cortex. The protein Linda's group shows to be central in regulating RGC proliferation and differentiation is called DOCK7. It previously had been shown by the Van Aelst lab to be highly expressed in the hippocampus and cortex of the developing rodent brain, controlling the formation of axons. Now they show that when DOCK7 is silenced in developing mouse embryos, RGCs remain in their progenitor state, but when overexpressed, RGCs differentiate prematurely. These two processes must be finely balanced for proper cortical development, and DOCK7 is the key protein in maintaining this balance. The team found that DOCK7 antagonizes the growth of microtubules through an interaction with a protein called TACC3, thus affecting the movement of the nucleus within the cell. This study illuminates a process central to cortical development and may also help further our understanding of abnormal brain development in conditions such as microencephaly, which is characterized by small brain size.

L. Van Aelst

Genome Analyses of Two Major Agricultural Crops Pave Way for Higher Yields

This year, Cold Spring Harbor Laboratory researchers were at the forefront of two ambitious projects to study the complete genomes of two of the so-called big three agricultural crops: maize, better known as corn, and bread wheat. Associate Professor Doreen Ware and colleagues provided the most comprehensive analysis of maize to date. They analyzed genetic structure and the relationships and sequential ordering of individual genes in more than 100 varieties of wild and domesticated corn. This revealed tremendous variation among varieties, including genome size variation of up to 25%, indicating that the evolution of maize is still ongoing. They were also able to identify hundreds of genes that had a role in the domestication of wild corn and discovered that breeding efforts focused on selecting for hybrid vigor, a phenomenon first described by CSHL plant geneticist George Schull in 1908, were important in this process. The consortium of scientists involved was also able to expand upon a previous study in which they identified more than 1 million genetic markers.

D. Ware

Professor W. Richard McCombie's team was involved in a huge and complex technical analysis of the genome of bread wheat. The challenge was that the bread wheat genome's 17 billion DNA "letters," or nucleotides, are spread across six copies of each of its seven chromosomes and up to 80% consists of repeat sequences. The team overcame this using next-generation sequencing techniques in which the DNA was randomly broken up into smaller pieces and assembled for analysis by identifying the overlapping ends. They were then compared to known sequences from a range of grasses, including rice and barley. The analysis revealed 94,000 to 96,000 genes, a large number of gene fragments, and 132,000 SNPs (single-nucleotide polymorphisms). This suggests that bread wheat underwent rapid and significant changes during the process of domestication. In both maize and bread wheat, newly identified structural variations and expanded gene families are implicated in many traits important for crop growth and survival, providing an important framework within which to significantly improve breeding efforts.

W.R. McCombie

A Striking Link between the Fragile-X Gene Product and Autism Mutations

Continuing his laboratory's important investigations of the role in autism causation played by spontaneously occurring, or *de novo,* gene mutations, Professor Mike Wigler and colleagues

M. Wigler

published results this year that revealed an interesting linkage with genes implicated in Fragile-X syndrome. The most common cause of inherited intellectual disability, Fragile X is also counted among the autism spectrum disorders (ASDs) due to the co-occurrence of autism-like symptoms in some patients. It occurs when a gene called *FMR1* fails to direct nerve cells to manufacture a protein called FMRP. Mike's team demonstrated, with help from Ivan Iossifov and other computational biologists at the Laboratory, that ~20% of the genes found to be disturbed in a sample of 343 autistic children appear to be regulated by the FMRP protein. The team finds that small de novo mutations—as small as a single DNA letter or areas of small insertions or deletions of genetic material up to 15 letters in length—could be traced in the majority of small children with ASD to the father's germ cells (sperm) and that their occurrence correlated directly with the father's age, older dads being more likely to contribute sperm that will result in a child with small autism-related de novo mutations. As Wigler points out, because of research connecting FMRP to neuroplasticity—the sensitization and desensitization of nerve cells to repetitive inputs—the new results indicate a possible convergence of mechanisms in autism.

Using DNA Barcodes and Gene Sequencing to Map Circuits in the Brain

A multiyear project conceived by Professor Tony Zador that in 2011 earned him a prestigious National Institutes of Health (NIH) "Transformative Research" grant this year yielded its first results. The project seeks to develop a novel method to map circuits and pathways throughout the mammalian brain at extremely fine resolution, that of individual neurons and their estimated one trillion synaptic connections. Zador and colleagues propose to use high-throughput DNA sequencing, which is both inexpensive and fast, to probe the connectivity of neural circuits at single-neuron resolution. As set forth in a provocative paper, Tony and colleagues introduce a technique they called BOINC, an acronym for the barcoding of individual neuronal connections. Now in proof-of-concept testing, BOINC is designed to provide immediate insight into the computations that a neural circuit performs. It consists of three steps: (1) neurons are labeled with a specific DNA "barcode;" (2) barcodes are exchanged among neurons connected across synapses, by exploiting a deactivated virus that can move genetic material across these gaps; and (3) barcodes from connected neurons are joined to make single pieces of DNA, which can then be read by DNA sequencers. A full set of such sequences would be analyzed computationally to reveal the synaptic wiring diagram of a whole mouse brain. It is a bold idea, and the Zador lab will soon be able to report on its success in realizing it.

A. Zador

A Cancer Target That May Also Be Important in Alzheimer's Disease

Professor Yi Zhong and colleagues obtained intriguing results this year in their continuing efforts to identify targets for treating Alzheimer's disease and other neurodegenerative illnesses. Using the fly brain as a model, Yi had previously studied memory loss associated with the expression in brain cells of a peptide called amyloid β-42 (Aβ-42), found in human brain plaques. Yi's flies express the human *Aβ-42* gene and suffer memory deficiencies analogous to those seen in patients. In new experiments, Yi's team demonstrated that enhanced activation in brain cells of the epidermal growth factor receptor, or EGFR, exacerbated memory loss in the Aβ-42 fruit fly model. EGFR overexpression is a defining feature of certain cancers, notably a subset of lung cancers, which can be temporarily reversed with targeted EGFR inhibitors. Yi's team showed that EGFR overexpression in fly neurons correlates with severity of memory loss in Aβ-42-expressing flies. The team dosed the flies with EGFR inhibitors over a week's time, and behavioral tests showed that this prevented memory loss. The results were confirmed in mouse models of Alzheimer's also based on

Y. Zhong

the human *Aβ-42* gene. Other members of Yi's team, working in a parallel but separate process, also identified EGFR as an Alzheimer's drug target, around the same time. That team was testing a library of 2000 synthetic compounds for activity against Aβ-induced memory loss in fruit fly models. Of these, 45 showed positive results after two months of dosing, and three of these, tested in vitro, specifically prevented Aβ-42 from activating human EGFR.

Cold Spring Harbor Laboratory Board of Trustees

New York Governor Andrew Cuomo said it best when he visited the campus in October: "[For] what it does for the soul, the people it gives hope to, Cold Spring Harbor has always been synonymous with accomplishing the impossible." We do this at all levels of the institution, starting with the Board of Trustees.

A. Cuomo and B. Stillman

Led by Chairman Jamie Nicholls, we are attracting leaders of the highest caliber to our governance bodies. The board welcomed Charles "Casey" Cogut, senior corporate partner at Simpson Thacher & Bartlett LLP, with expertise in M&A, private equity, and governance.

Re-elected to the board for an additional 4-year term were Dr. David Botstein, Jacob Goldfield, Leo A. Guthart, Thomas D. Lehrman, Dr. Charles L. Sawyers, Dr. Marilyn H. Simons, Dr. James M. Stone, Paul J. Taubman, and Roy J. Zuckerberg. Officer elections continued the terms of Chairman Nicholls, Vice Chairs Robert D. Lindsay and Marilyn H. Simons, Dr. Leo A. Guthart, Bruce Stillman, Ph.D., and W. Dillaway Ayres. Paul J. Taubman replaced Edward Travaglianti as Secretary.

C. Cogut

Many thanks to our friends who are retiring trustees, having served on the board and numerous committees from 2004 to 2012: Stephen Lessing, Andrew Solomon, and Dr. Jerome Swartz.

We mourned the passing of former CSHL trustees Townsend Knight, Robert Van Cleef Lindsay, and John J. Phelan. Rod was one of the founding members of the contemporary Laboratory, serving from 1959 to 1965. Townie served from 1973 to 1995, when he was named Honorary Trustee. John served two terms from 1992 to 1999.

Development

With the help of our board, CSHL capped another year of record-breaking success in fundraising with an Annual Fund total of $6.6 million raised. The 7th Double Helix Medals Dinner Gala raised $3.7 million and honored Parkinson's disease research activist Michael J. Fox, Apple Chairman Art Levinson, and philanthropist Mary Lindsay, who is also a CSHL Honorary Trustee.

Double Helix Medal winners M.J. Fox, M. Lindsay, and A. Levinson

In the community, the Cold Spring Harbor Laboratory Association made many new friends by organizing events that included a music, art, and food festival called Labapalooza; the 11th Women's Partnership Luncheon featuring Professor Michael Wigler (who spoke about single-cell sequencing in cancer research); and the annual President's Council retreat, which focused

Women's Partnership for Science Luncheon

A. Solomon, G. Welch, and B. Stillman

this year on the topic of medical ethics. CSHL Director of Research David L. Spector and Associate Professor Lloyd Trotman headed the agenda, which also included DNA Learning Center Executive Director David Micklos. CSHL Trustee Andrew Solomon, acclaimed writer on politics, culture, and society, discussed his new book, *Far From the Tree*. Other guest speakers included Drs. Gilbert Welch of Dartmouth, Jeffrey Berger of Stony Brook University, and Hans Sauer of the Biotech Industry Organization.

Of course, doing the impossible would not even be imaginable without private philanthropic support. In 2012, CSHL was grateful for new major gifts from (in alphabetical order) an anonymous donor, Jamie Nicholls and Fran Biondi, Mr. and Mrs. David Boies, Charitable Lead Annuity Trust under the Will of Louis Feil, Laurie J. Landeau Foundation, Mr. and Mrs. Robert D. Lindsay and Family, the Lustgarten Foundation, the Simons Foundation, and Dr. and Mrs. James M. Stone.

Research Faculty

There is no clearer evidence of our ability to achieve what our Governor called "the impossible" than the research accomplishments of our faculty. Many are individually recognized each year by competitive awards and academic honors.

Professor and Howard Hughes Medical Investigator Greg Hannon was elected to the U.S. National Academy of Sciences, which ranks among the highest honors conferred upon scientists in America. Dr. Hannon, a molecular biologist, is recognized the world over as among the foremost authorities on small RNA biology and RNA interference. RNAi, as it is known among scientists, is a natural cellular mechanism implicated in genome defense, in which small RNA molecules act to regulate gene expression. It has been exploited by scientists led by Greg and colleagues for many extremely valuable purposes, including hunting for cancer genes, stopping viral infections, and, most recently, treating diseases in clinical trials.

The AAAS council elected Professor and Howard Hughes Investigator Leemor Joshua-Tor a 2012 AAAS Fellow, recognizing her contributions to the field of nucleic acid enzymes, particularly


A. Krainer


N. Tonks


C. Vakoc


F. Albeanu

in the fields of RNA interference and DNA replication. Leemor's laboratory studies the molecular basis of cell regulatory processes, using the tools of structural biology and biochemistry to examine proteins and protein complexes associated with these processes. Her efforts largely center on nucleic acid regulation, including the process of RNA interference and DNA replication initiation in papillomaviruses.

Professor Adrian Krainer received a MERIT AWARD (Method to Extend Research in Time) from the National Institute of General Medical Sciences. This award rewards highly productive scientists by extending funding for an existing research project grant. Adrian is a leader in the field of RNA splicing, which has significant implications for many human genetic diseases because a high proportion of disease-causing mutations affect messenger RNA (mRNA) splicing and stability. mRNAs are the coded instructions copied from the genetic material that direct cells to manufacture specific proteins. Adrian's current research will result in a better understanding of which mutations cause defective gene expression, and precisely how they do so. Already, his research has guided the development of a drug to treat a fatal childhood disease called spinal muscular atrophy (SMA). Clinical trials to test this drug were begun in 2012.

Professor Nicholas Tonks was named Vallee Visiting Professor by the Bert L. and N. Kuggie Vallee Foundation. This honor is conferred upon those who have demonstrated a record of exceptional creativity, originality, and leadership and sustained success at an elite level. A biochemist and molecular biologist, Nick is well known for having laid the foundations for the identification and functional characterization of a superfamily of 107 regulatory enzymes called protein tyrosine phosphatases, or PTPs. Protein phosphatases recognize phosphorylated amino acid residues and function to remove them; their action is thus complementary to the class of protein kinases, which add phosphate groups to amino acid residues. Together, they play crucial roles in signal transduction pathways.

Anne Churchland received the McKnight Foundation Scholar award supporting neuroscience research. This award addresses a basic problem which, when solved, could immediately and significantly impact clinical issues. Anne plans to use the award to study the neural circuits underlying a process known as multisensory decision making—the integration of information from individual senses such as seeing, hearing, and touch to make more accurate decisions. Anne was also selected for the Janett Rosenberg Trubatch Career Development Award, which rewards promise and achievement in the field of neuroscience for early-career professionals.

Assistant Professor Christopher Vakoc received the "A" award for pediatric leukemia research, one of only four to receive this honor from Alex's Lemonade Stand Foundation. He will work on a project entitled "BET bromodomain inhibition as epigenetic therapy in pediatric leukemia." Chris was also named a "V Scholar," one of 17 of the "best and brightest" cancer researchers, by the V Foundation. His project is entitled "Therapeutic targeting of the Polycomb complex PRC2 in acute myeloid leukemia."

Florin Albeanu was named a Pew Scholar in Biomedical Sciences. Florin, who came to CSHL as a Fellow in late 2008 and was appointed a member of the faculty in 2011, studies how the brain

C. Hammell

encodes stimuli from the outside world, within and across sensory modalities, to generate specific perceptions that, in turn, trigger complex behaviors. He is interested in how the brain is shaped by sensory experience and what modifications occur in neuronal circuits that allow us to learn and remember.

Christopher Hammell was named a Rita Allen Foundation Scholar. As part of the cancer research program, Dr. Hammell is interested in understanding the gene regulatory process that gives rise to normal development in animals as well as alterations in these processes that give rise to diseases such as cancer. Chris also received the special honor of being named the Milton E. Cassel Scholar, a tribute to the memory of a longtime president of the foundation.

CSHL once again teamed up with the National Institutes of Health (NIH) to host a regional conference on funding opportunities and research priorities in the neurosciences. Heading up the agenda were Dr. Thomas Insel, Director of the National Institute of Mental Health; Dr. Robert Finkelstein, Director, Division of Extramural Research at the National Institute of Neurological Disorders and Stroke (NINDS), and Dr. Alan L. Willard, Deputy Director of NINDS. Supported by the Alfred P. Sloan Foundation and organized by the CSHL Office of Sponsored Programs, CSHL faculty members Florin Albeanu, Anne Churchland, and Steve Shea facilitated the discussions with faculty from Columbia, SUNY Stony Brook, New York University, and the Massachusetts Institute of Technology (MIT).

The laboratories of CSHL's 52 principal investigators are supported by an active community of postdoctoral fellows, this year numbering nearly 160. The Postdoc Liaison Committee was created to give the postdoctoral community a formal organization through which to pursue its own agenda of enhancing the postdoctoral education experience at CSHL. Headed by an elected group of six, they are the primary organizers of the twice-yearly postdoctoral collaboration and networking retreats.

Some of committee's most successful activities include initiation of a career development series in which faculty members educate postdocs on "real-life" topics such as how to negotiate for a startup package after landing a faculty position or how to hire staff of a new lab. The newly formed Bioscience Enterprise Club, which welcomes postdocs, graduate students, and the CSHL campus community at large, is off to a great start providing opportunities to learn about

Postdoc Symposium

nontraditional science careers, develop entrepreneurial skills, and network with professionals in the biotech industry, clinical research, intellectual property law and tech transfer, consulting, science education, policy, and administration.

New Faculty

CSHL continues its historic commitment to attracting and promoting world-class research faculty. According to Director of Research David L. Spector, who heads the institution's faculty recruitment efforts, "in the last year, we have strategically invested in research faculty who are at the forefront of cancer therapeutics, genomics of human disease, human genetics, computational biology, and bioinformatics. We look forward to the significant impact that we know these exceptional scientists will have in shaping the future of biomedical research."

D. Tuveson

David Tuveson, M.D., Ph.D., was appointed Professor and Deputy Director of the CSHL Cancer Center. Dave obtained a bachelor's degree in chemistry at MIT, followed by M.D. and Ph.D. degrees at Johns Hopkins. After obtaining a faculty position at the University of Pennsylvania, he moved to the University of Cambridge, England, to develop preclinical and clinical therapeutic strategies. CSHL recruited Dave to direct the Cancer Therapeutics Initiative (CTI). He serves simultaneously as Director of Research for the Lustgarten Foundation, the nation's largest private foundation dedicated to funding pancreatic cancer research. Together with the Lustgarten Foundation, CSHL announced the opening of the Lustgarten Foundation Pancreatic Cancer Research Laboratory located on our Hillside Campus. This laboratory will focus exclusively on pancreatic cancer research, with initial studies centered on early detection, drug development, and drug delivery.

Dave's team investigates fundamental aspects of cancer biology and applies this knowledge to the development of new therapeutic and diagnostic strategies. His lab developed the first mouse models of pancreatic ductal adenocarcinoma (PDAC), which have been instrumental in the discovery of biomarkers of early disease. They also identified pathways and druggable targets involved in the initiation, progression, and metastasis of PDAC and developed new therapeutic strategies. Following the observation made in his lab that PDAC tumors contain a deficient and compressed vasculature, which limits therapeutic drug delivery and therefore efficacy, Dave has uncovered several methods to correct or target these vascular deficits and promote drug response. This work has led to the initiation of several clinical trials. At CSHL, he will continue the search for new vulnerabilities in PDAC neoplastic cells and the tumor surroundings, called the microenvironment. His team will evaluate candidate drug targets in an advanced therapeutics testing facility being developed as part of the CTI. He aims to translate his preclinical results into the design of pivotal investigational clinical studies.

G.J. Lyon

Another scientist-clinician whom we added to the faculty this year is Assistant Professor Gholson J. Lyon, M.D., Ph.D. Gholson received a B.A. in biochemistry from Dartmouth College and shortly thereafter completed an M.Phil. in genetics at Christ's College, University of Cambridge, England. He has a Ph.D. from Rockefeller University in chemical biology and an M.D. from Weill Cornell Medical College. Gholson is affiliated with the Utah Foundation for Biomedical Research.

Here, Gholson focuses on analyzing human genetic variation and its role in severe neuropsychiatric disorders. He does so by studying large groups of related individuals living in the same geographic location. The Lyon lab is using sequencing of whole genomes and of the exome—the small portion of the genome that encodes proteins—to find mutations that distinguish disease syndromes, in populations from Utah and elsewhere. Gholson is interested in the discovery of families with rare diseases and/or increased prevalence for syndromes such as Tourette syndrome, ADHD, obsessive-compulsive disorder (OCD), intellectual disability, autism, and schizophrenia.

J. Gillis

Joining CSHL as an assistant professor is Jesse Gillis, Ph.D. He holds a B.S. in biophysics from the University of Toronto, where he also earned both an M.S. and a Ph.D. in Neuroscience. He was recruited to CSHL from the University of British Columbia, where he did a postdoctoral fellowship at the Centre for High-Throughput Biology.

The Gillis laboratory is working to understand how genes interact and how this relates to gene function and the effect on disease. Using computational biology, he is interpreting the functions of genes in the context of the networks they form, with data derived from gene association studies.

Promotions

Congratulations to Alexei Koulakov, who was promoted to professor. Alexei came to CSHL in 2003 from the University of Utah, where he was an assistant professor in the department of Physics. He received his Ph.D. from the University of Minnesota and was a postdoctoral fellow at the Salk Institute. Alex is interested in computational and theoretical neuroscience and is probing neural circuits to understand the mechanisms of neural computation, the means by which vast networks of nerve cells in the brain encode and are able to understand messages.

Glenn Turner and Lloyd Trotman were promoted this year to associate professor. Lloyd came to CSHL as an assistant professor in 2007, after a postdoctoral research fellowship at Memorial Sloan-Kettering Cancer Center. Glenn joined CSHL in 2006 as an assistant professor, having received his Ph.D. and performing postdoctoral research at California Institute of Technology.

Promoted this year to assistant professor were Molly C. Hammell, who, in addition to contributing her expertise in computational biology to many ongoing research collaborations among CSHL labs, has been manager of the CSHL Cancer Center's Bioinformatics Shared Resource since 2010; Ivan Iossifov, who began at the Laboratory in 2008 as a Quantitative Biology Fellow; and Dan Levy, who joined Mike Wigler's lab as a postdoctoral fellow in 2007 and was then promoted to senior computer scientist.

Isabel Aznarez Da Silva, who works in the laboratory of Adrian Krainer, and Camila Dos Santos, who works in Gregory Hannon's lab, were promoted to research investigators.

2012 WSBS Doctoral graduates F. Rollins, K. Honegger, E. Nakasone, P. Finigan, and Z. Wang

Departures

Assistant Professor Rob Lucito is currently Assistant Professor of Science Education at Hofstra North Shore LIJ School of Medicine and remains associated with CSHL as an adjunct professor.

Education Programs

The Watson School of Biological Sciences (WSBS) celebrated its 9th graduating class, awarding Ph.D.s to Patrick Finigan, Kyle Honegger, Elizabeth Nakasone, Frederick Rollins, and Zhenxun Wang.

This year's Honorary Degree recipient was Sir Kenneth Murray, who was knighted by the Queen of England in 1993 for his discovery of hepatitis B antigens. His long list of achievements includes developing a life-saving hepatitis vaccine, starting the first European biotech company, and creating the Darwin Trust to support biological scientists from


K. Murray

less affluent parts of the world. We were saddened to hear that Dr. Murray passed away before this Annual Report went to print.

In August, WSBS opened its doors to the 14th incoming class of nine students: Nitin Singh Chouhan, William Donovan, Talitha Forcier, Yu-Jui (Ray) Ho, Irene Liao, Paul Masset, Annabel Romero Hernandez, and Abram Santana. These new degree candidates come to us from the United States, France, India, Mexico, and Taiwan.

The WSBS now counts 54 Ph.D. graduates who are thriving in the "outside world." They continue to publish in top journals and secure prestigious independent positions, fellowships, and awards. Eleven of our graduates have secured tenure-track faculty positions, and, as such, they are now receiving federal grants and publishing papers as independent researchers.

Current students continue to win prestigious fellowships and prizes. In 2012, Colleen Carlston was selected into the National Science Foundation's East Asia and Pacific Summer Institutes for U.S. Program. In addition, she received a National Science Foundation Graduate Research Fellowship. John Sheppard also was awarded a National Science Foundation Graduate Research Fellowship and received a National Defense Science & Engineering Graduate Fellowship from the Department of Defense. Melanie Eckersley-Maslin was awarded a Keystone Symposium Travel Fellowship. She was also awarded an American Society for Cell Biology Travel Fellowship to attend the annual conference. WSBS students have published more than 210 papers to date, many in the most prestigious journals.

The National Institutes of Health's National Institute for General Medical Studies renewed the School's Training Grant for a period of 5 years. Despite very tight funding, the NIH recognized the outstanding achievements of the program, the students, and the faculty in the funding of this award. The training grant funds six students and also serves as an endorsement of the School's excellence.

The annual Gavin Borden Visiting Fellow Lecture "Electron transfer in times of stress: New roles for redox active antibiotics," was presented on April 23 by Dianne K. Newman, Ph.D., Professor of Geobiology and Howard Hughes Medical Institute Investigator at the California Institute of Technology.

Twenty-six undergraduates (selected from 884 applicants, the largest pool to date) from around the United States as well Canada, Switzerland, Ireland, and the United Kingdom formed the 53rd cohort of the Undergraduate Research Program (URP). This year's URP Faculty Directors were Anne Churchland and Michael Schatz. The historic 10-week program for undergraduate students convenes in the summer, and it provides some of the finest college students a priceless opportunity to conduct sophisticated research at the side of a CSHL investigator.

Under the direction of Professor David Jackson, the Partners for the Future Program for high school seniors attracts the best and brightest aspiring scientists to an average of about a dozen of our labs each year. Established by Dr. James Watson in 1990, the program provides an opportunity for gifted Long Island high school students to have hands-on experience in biomedical research.

In August, Leemor Joshua-Tor stepped down as Dean of the WSBS after 5 years of outstanding leadership. As the third leader of a school known as one of the nation's most innovative Ph.D.-granting programs, Leemor advanced the curriculum in significant ways, including the addition of timely courses in quantitative biology, physical biology, and imaging. She has also served during her term as a member of the Biomedical Workforce Task Force of the U.S. National Institutes of Health, which recently issued recommendations to support a future sustainable biomedical research infrastructure. A structural biologist who began her career at CSHL in 1995, Leemor is an Investigator of the Howard Hughes Medical Institute. She has made seminal contributions to the understanding of how RNA interference works to silence gene expression and has advanced new therapeutic options for combating papillomavirus, which causes cervical cancer. She continues her

53rd Undergraduate Research Program

research at the Laboratory, studying the molecular basis of cell regulatory processes using the tools of structural biology and biochemistry.

Thank you to Adrian Krainer and Linda Van Aelst, who served as interim deans for the remainder of the year. I was pleased to announce the selection of Alexander A.F. Gann as the new dean, effective January 2013.

We recruited Dean Gann from the CSHL Press, where he served as Editorial Director. At the Press since 1999, he has produced publications ranging from textbooks for undergraduate and graduate education to laboratory manuals and books on the history of science. Alex is a co-author of *Molecular Biology of the Gene,* now in its 6th edition, and of the recently released *Annotated Double Helix,* a new edition of James D. Watson's autobiographical classic. Alex received his Ph.D. from the University of Edinburgh, Scotland, in 1989, after which he continued his postdoctoral training at Harvard and University College, London, and lectured at Lancaster University. A longtime member of the WSBS faculty, he brings a unique combination of inside perspective and broad understanding of the impact of the digital and genomic revolutions upon higher education and the biological sciences.

77th CSH Symposium

Meetings and Courses Program

The CSHL meetings program attracted strong attendance with more than 7100 meeting participants and almost 1300 course participants (trainees, teaching and support faculty). The Cold Spring Harbor Asia program, including 18 conferences and one summer school, has to date attracted more than 3000 participants. This brings the anticipated year-end total for both U.S.- and China-based programs to nearly 11,400.

Our flagship meeting, the Cold Spring Harbor Symposium, focused in 2012 on plant biology for the first time in the 77-year history of the series. Given the importance of plant research in such vital fields as epigenetics and evolution, plants naturally have been discussed at many prior symposia. This year, however, they occupied center stage, with a stellar list of presenters that included four CSHL faculty members.

The year saw the introduction of two new meetings, *Regulatory and Noncoding RNAs* and *Epigenetics and Chromatin,* which drew strong attendance and featured a high proportion of unpublished research. We anticipate these meetings will each become regular biennial series. Two existing meetings were merged in 2012, as well: *Personal Genomes* and *Pharmacogenomics* were combined as *Personal Genomics and Medical Genomes*.

Just how robust our programs are, and how loyal are our attending scientists, was demonstrated when Hurricane Sandy powered up the East Coast at the end of October. Four courses were then due to end and a meeting and a course were due to begin the day of the storm's local landfall, October 30. We made great efforts to accommodate our guests, mostly helping them arrange for departures. But a hearty core group of about 65 braved the elements and held a 2-day version of their scheduled meeting on *Nuclear Receptors and Disease*.

Courses

The CSHL Courses program benefited greatly in 2012 from the opening of the completely rebuilt Hershey Building on the main campus. This fully modern and architecturally striking 18,000-square-foot facility was finished in time to host more than 10 of our annual courses, including a successful new course on *Single-Cell Analysis* and a new *Workshop on Cognitive Aging*. Covering a diverse range of topics in molecular biology, neurobiology, structural studies, and bioinformatics, the courses teach advanced students the latest innovations that can be applied immediately to their research. Instructors are drawn to teach at Cold Spring Harbor from universities, medical schools, research institutes, and companies worldwide.

The Hershey Building was rededicated June 8 in a ceremony that highlighted the history and future of scientific research and education at the Laboratory. Named for the late Dr. Alfred Hershey, a Nobel laureate and CSHL scientist, the new facility was made possible by a $15 million grant provided in 2008 by the Howard Hughes Medical Institute (HHMI).

The new facility allows for a 25% increase in course offerings and participants, including a number of new courses in computational approaches to biological questions. Courses such as *Computational and Comparative Genomics* and *Computational Neuroscience: Vision*, both offered this year, put a new emphasis on the computational aspects of biology, including mathematics, statistics, and computer science.

Harry Anand, mayor of the Village of Laurel Hollow, commended CSHL at the Hershey rededication ceremony, noting the Laboratory's continuing commitment to designing and building facilities that enhance the natural beauty of the local landscape. James Childress, partner of Centerbrook Architects and Planners, led the effort to design a completely new building to replace the original Hershey Building, which was erected in 1979. Other speakers at the rededication were Dr. James D. Watson and Dr. Jack Dixon, Vice President and Chief Scientific Officer of HHMI.

The ceremony also honored beloved CSHL course instructors Dr. Gordon Sato, for whom a new Flow Cytometry Laboratory has been named, and Dr. Mark J. Zoller, whose name now graces one of the facility's modern teaching laboratories. In attendance were Dr. Sato; Dr. Zoller's widow, Ms. Karen Zoller; Senator Carl Marcellino; CSHL Board Chairman Jamie Nicholls; and fellow trustees, faculty, employees, and friends of the Laboratory.

Suzhou Conference Center

Now in its third year of operation, the Cold Spring Harbor Asia (CSHA) program, under the direction of Dr. Maoyen Chi, is headquartered at the Suzhou Dushu Lake Conference Center, a purpose-built academic conference center on the outskirts of old Suzhou,

within a high-technology suburb (SIP). The scientific program includes large symposia and meetings, training workshops and Banbury-style discussion meetings. CSHA is a wholly owned subsidiary of CSHL and is not beholden to outside partners in terms of our scientific programming. The 50% growth in meeting attendance between the first and third year of operations bodes well for the future.

Banbury Center

In its 35th year of operations, Banbury Center continued to have an active role in the Laboratory's educational mission. At the beginning of the year, the Conference Room underwent a major renovation. By autumn, it was the turn of Sammis Hall, which had gone largely untouched since its opening in 1981. These efforts ensure that participants in our programs have the fully modern and up-to-date accommodations that they expect.

Despite the renovation work, Banbury's facilities were used intensively throughout the year, hosting 18 meetings as well as six lecture courses and two Watson School courses. CSHL postdocs came on two occasions for a retreat and the Robertson family came for their annual meeting. As usual, CSHL is happy to help our neighbors; this year, the Cold Spring Harbor School District board twice used the Banbury Center facilities.

We welcomed back the Boehringer Ingelheim Foundation, which, for several years, has brought its fellows for training in writing papers and giving talks. The National Institute for Mental Health returned for its Brain Camp, providing the brightest clinical fellows to high-level neuroscientists, encouraging them to think of taking up research. For the second year, Carl Cohen of Science Management Associates taught a "Leadership in Bioscience" workshop.

Banbury Center's first meeting on patenting was held 30 years ago, just 2 years after the 1980 *Chakrabarty* case (which declared that a modified microbe was patentable). In 2012, the question of patents was brought into sharp focus by the recent Myriad Genetics case involving patents covering the *BRCA* genes. The meeting, *Patenting Genes: New Developments, New Questions*, discussed this and other unresolved issues.

DNA Learning Center

DNA Learning Center (DNALC) Executive Director David Micklos received the 2012 Elizabeth W. Jones Award for Excellence in Education from the Genetics Society of America. David was recognized for bringing "the excitement of DNA science into the educational curriculum for thousands of students, high school teachers, and undergraduate faculty."

Urban Barcode Project grand prize winners

On June 6, nine finalist teams of high school students representing eight public high schools, plus home schooling, from all of the five boroughs of New York City, presented their submissions to the first-ever NYC Urban Barcode Project competition. They were selected from more than 200 students on 75 teams, whose research posters were judged by conservation and genetic biologists and education experts. For most students, it was their first independent research project. Twenty-six percent of contestants were African American or Latino, groups that are underrepresented in science.

New York City institutions, including the American Museum of Natural History, Genspace, New York Botanical Garden, Brooklyn Bridge Park, and the

Rockefeller University, partnered with the DNALC to provide facilities and mentoring to the student teams. Developed and executed by the DNALC with funding from the Alfred P. Sloan Foundation, the Urban Barcode Project was the first large-scale attempt to use barcoding projects to encourage students to explore the urban environment beyond their doorsteps.

It was the biggest summer yet of DNA camps: 1000 5th- to 12th-grade students attended 53 weeks of camps conducted by the Dolan DNALC, DNALC *West,* Stony Brook University, and Brookhaven National Laboratory, the Trinity School, and at the Chapin School in Manhattan. Students from Beijing High School 166 also attended 3 weeks of camps on bacterial genetics, human genomics, and DNA barcoding.

During the summer, the DNALC also extended its NYC *Urban Barcode Project* to the study of biodiversity in the unique glacial landscapes of Long Island. Six student workshops, *Barcoding Biodiversity,* explored the intertidal zone adjacent to the main campus. Naturalist photographer David Liittschwager joined the first workshop, instructing in his methods for examining and photographing life in a one-cubic foot sample, which he published in the February 2010 issue of *National Geographic*. Seventy-seven students collected and processed more than 300 samples, resulting in 165 high-quality DNA barcodes.

DNALC staff continued in 2012 to provide essential support for the National Science Foundation (NSF)-funded *iPlant* Collaborative project, which seeks to develop a national cyberinfrastructure needed for data-heavy 21st-century plant research. As the *iPlant* infrastructure has expanded to support animal genomics research too, the DNALC's *DNA Subway* website has been upgraded to support student projects using animal species. Total visits to the site grew 40% in 2012, to more than 33,000.

This was but a fraction of the more than 4.2 million visits to the 22 DNALC websites in 2012, a figure to which is added 865,000 visits to the DNALC YouTube channel; and 568,000 downloads of the *3D Brain*, *Weed to Wonder,* and *Gene Screen* phone apps, to gauge DNALC's total cyber-impact for the year—more than 5.6 million visits!

In 2012, the team also executed a successful renovation of the museum exhibit at the Dolan DNALC. A fourth teaching laboratory and prep area was added, the lunchroom was enlarged, the basement prep laboratory was renovated, and storage and display spaces were reorganized. The exhibit is evolving into one based on human ancestry. One part will have the theme of "Becoming Human," treating our ancient history and evolution, and the other "Being Human," representing where we are today and comparing characteristics of modern populations.

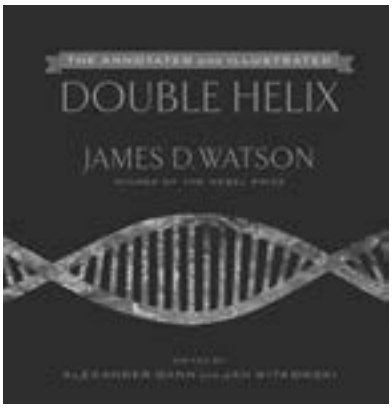
CSHL Press

In 2012, the CSHL Press continued to publish seven journals and added 12 new print and five new electronic titles to its list of 180 print books and 18 online books.

The most recent impact factor analysis continues to rank our journals *Genes & Development* and *Genome Research* at the top of their disciplines; *Genome Research* reached a new high of 13.608, after 4 years of consecutive increases, and now ranks #2 in genetics and #2 in biotechnology. *Genes & Development* currently ranks #2 in developmental biology and #3 in genetics. In 2012, CSHL journal articles were electronically downloaded a remarkable 10 million times, and 38,000 books were shipped to 150 countries.

The first and flagship journal, *Genes & Development*, marked its 25th Anniversary this year. In celebration of this anniversary, the Genetics Society of Great Britain, one of the founders along with CSHL, held its annual autumn meeting in honor of the journal. The 2-day meeting, titled At the Cutting Edge of Molecular Biology: 25 Years of *Genes & Development*, was held November 7–8 at the Royal Society (London). The meeting brought together distinguished scientists from across the biological sciences, focusing on issues such as chromatin, epigenetics,

CSH Perspectives in Biology



The Double Helix

gene regulation, replication, checkpoints and DNA repair, RNA function and control, development, stem cells, and diseases such as cancer, representing the broad scope of science covered by the journal.

In its second year of sales, the review journal *Cold Spring Harbor Perspectives in Biology* continues to increase notably in revenue and usage. The concept of a "new type of review journal" with subject collections that build monthly has been well received, as has its consistent editorial excellence. It received its first impact factor this year. The newest review journal, *Cold Spring Harbor Perspectives in Medicine*, completed its first year of publication with exceptional usage of collections about HIV, Alzheimer's, Parkinson's, and addiction.

New print books and five new e-books included *Genome Science*, a new and long-awaited collection of laboratory exercises by the teaching staff at the DNA Learning Center. In June, a new edition of the laboratory manual *Molecular Cloning* was published. Long established as the gold standard for molecular biology techniques, and by far the most successful book ever published by the Press, with more than 200,000 copies sold, this new edition was a complete revision by authors Joe Sambrook and Michael Green, assisted by a dozen expert contributors. A classic of a different sort also reappeared, co-published by the Press and Simon & Schuster: James Watson's 1968 autobiographical account of the discovery of the structure of DNA, *The Double Helix*, which was named during the year as one of the Library of Congress's 88 "Books that Shaped America." CSHL Professors Alex Gann and Jan Witkowski illustrated and annotated the text with footnotes, photos, correspondence, and other documents that placed this most famous account of scientific discovery in its social, intellectual, and historical context.

Library and Archives

On April 1–3, Library and Archives Department Executive Director Ludmila Pollock and colleagues from Rockefeller University, Memorial Sloan-Kettering Cancer Center, and the Marine Biological Laboratory hosted a meeting at the Banbury Center called "Envisioning the Future of Research Libraries." It brought together the top leaders of scientific research libraries from the United States, United Kingdom, Germany, and France, with a goal of constructing a model for the future (circa 2020) scientific research library.

In 2009, CSHL and the Wellcome Trust initiated a project to create an International Catalog for the History of the Human Genome Project (HGP). On May 3–5, an international meeting about the History of the HGP was held at the Banbury Center, where discussion centered on how to best present the history to different audiences, including scholarly researchers. Phase 1 of this project, which includes identifying all of the potential collections, creating a new, open-access database, and carrying out a pilot project, will be completed in 2013.

Millions of primary-source documents telling the amazing story of the biological revolutions of the 1950s and 1960s are now freely available on the World Wide Web thanks to an effort led by the Wellcome Library of Great Britain. Entitled *Codebreakers: Makers of Modern Genetics*, the resource provides public access to first-hand notes, letters, sketches, lectures, photographs, and essays from those responsible for uncovering the structure of DNA. CSHL's Library and Archives collections, which include the papers of Nobel laureates James Watson and Sydney Brenner among others, are a key source for *Codebreakers*. CSHL was very happy to participate in the project with

partners Churchill Archives Centre Cambridge, the University of Glasgow, King's College London, and University College London.

Infrastructure Projects

Hurricane Recovery

The Laboratory's preparations for Hurricane Sandy were extensive, and apart from an extended power outage that the Laboratory's staffs managed with emergency power, the campus emerged relatively unscathed. There was, however, some damage. The Laboratory has dealt with 40–60 fallen mature trees and the Robertson House slate roof required significant repair, and due to the storm surge, post-storm repairs were required to the mechanical systems of the low-lying Jones Laboratory.

Airslie Renovations

The Laboratory undertook extensive interior renovations to the house, modernizing the kitchen and service areas to accommodate the numerous development activities undertaken at the President's house while maintaining a livable private residence.

Wawepex Alterations

The Office of Sponsored Programs—tasked with an ever-increasing workload—was overcrowded in the circa 1830 Wawepex Building. Interior renovations were completed to provide additional workspace for the department's staff.

Sammis Hall Renovations

Built in 1981, Sammis Hall has housed Meeting and Course participants for more than two decades. New carpet, furnishings, fixtures, and systems were installed to meet contemporary expectations.

Blackford Exterior Renovations

The circa 1905 Blackford Hall has been in continuous operation for more than a century and structural cracks and spalling in the poured concrete structure were evident. It was necessary to repair and stabilize the building to prevent further decay. Of particular concern was preserving the historic nature of the building while conserving the relatively thin four-inch-thick concrete walls. The project was completed with the building remaining in full operation.

Receiving Building

Construction of the new Hershey Building required that the receiving and mail-room facilities be relocated. These functions were temporarily housed in a trailer on-site until the new Receiving Building could be constructed. The 1500-square foot structure was sited close to Route 25A to limit truck traffic on campus and was designed to appear as a complementary outbuilding of the historic Davenport House nearby.

Hershey Building Completion

After 2 years of construction, the Hershey Building was completed in the second quarter of 2012 and hosted courses for the summer. At the same time, both the Flow Cytometry and Microcopy facilities moved their operations into the building.

Community Outreach

On October 5, members of the Long Island Regional Economic Development Council accompanied New York Governor Andrew Cuomo and Lieutenant Governor Robert Duffy to tour the

Receiving building

Hershey building

Hillside laboratory of Professor David Tuveson, one of our latest recruits and a distinguished pancreatic cancer researcher who is also the Deputy Director of CSHL's Cancer Center.

Drs. Tuveson and Stillman briefed the dignitaries on CSHL's new Cancer Therapeutics Initiative, which New York State is helping to fund by seeding the construction of a new Advanced Drug Testing Facility located at the Woodbury Genome Center. The central idea in our Cancer Therapeutic Initiative is to create a translational pipeline that will enable us to help develop a new generation of cancer drugs that will be more effective and less toxic than those currently in use. Another objective is to significantly reduce the time it takes to identify and test candidate drugs, to accelerate their path to the clinic.

Stuart Rabinowitz, President of Hofstra University, and Kevin Law, President and CEO of the Long Island Association, both co-chairs of the Long Island Regional Economic Development Council, highlighted the importance of public support for cutting-edge science in driving economic development of the region and in providing new high-quality jobs. The Governor's next stop that day was the Broad Hollow Bioscience Park, an incubator facility that CSHL helped to found and where CSHL spin-off biotech companies have made their start.

CSHL is pleased to join with Brookhaven National Laboratory, SUNY Stony Brook, Hofstra University, and North Shore LIJ in Accelerate Long Island, an initiative to expand on the success of the Broad Hollow Science Park and strengthen the bioscience economy of Long Island.

Governor Cuomo (*center*) tours Hillside Laboratory

R.J. Duffy, W.D. Ayers, Jr., and B. Stillman

The Governor's visit came just a month after the visit of Lieutenant Governor Duffy, who chairs the state's regional economic development councils. "This is a great intersection of public health and economic development," said Mr. Duffy of CSHL. "This is a jewel in New York State and to see the brain power and expertise we have here makes us all very proud."

As I have already mentioned in brief, thanks to well-laid preparation plans and hard work—not to mention some luck associated with the outgoing tide—the Lab was able to ride out Hurricane Sandy at the end of October. By moving equipment and data storage to higher ground, bringing up elevators, sandbagging, and using diesel-powered backup generators for electricity, all essential scientific operations continued through the storm and in its aftermath. Even attendees of the Nuclear Receptors and Disease meeting continued undaunted, producing a defiant slogan that attendees and the Lab community will long remember: "Science vs. Sandy. . .Science Wins!"

Our preparedness allowed us to help our neighbors. We were in constant contact with the leadership of Laurel Hollow village, firefighters, police, friends, and residents, who benefitted from our food services, WiFi access, and warm spaces. CSHL even hosted TV coverage of the 2012 Presidential Election in Grace Auditorium, for CSHL families as well as community residents who were still without power more than a week after the storm.

A special thank you to those on our campus who helped us all weather the storm, in particular: Art Brings, Peter Stahl, Gerry Holler, Lisa Bianco, Culinary Services staff led by Jim Hope, Payroll staff led by Lari Russo and Damian Desiderio, IT staff led by Sean Kelly, and the Meetings and Courses team with David Stewart, Maureen Morrow, and Andrea Newell. Above all, a warm thank you to COO Dill Ayres, who spent many a sleepless night on watch in his office.

The year was a big one for CSHL in social media. The Laboratory's Twitter following grew to 2500 and friends on Facebook surpassed 1000. The monthly e-mail newsletter we fondly call the Netletter continues to attract new subscribers—more than 7000 by year end—and we consistently outperform other newsletters based on how many of our subscribers actually read the

Meeting attendees defy Hurricane Sandy

R. Martienssen

stories we deliver. The CSHL blog, Labdish, grew too, and we now host guest bloggers who represent the CSHL campus community from grad students to professors. Thanks, Michael Schatz, Anne Churchland, Clare Rebbeck, Antoine Molaro. Hats off to CSHL faculty who are active in social media, promoting their own ideas and providing perspectives on the latest developments in biology and genetics.

Adding to our public lecture series, both on our own campus and in other venues, are appearances by faculty on the World Wide Web. You can visit www.bigthink.com to watch professors Rob Martienssen and Michael Schatz explain how they're applying their research to solve some very big problems. Rob describes how he's using the principles of epigenetics that his group has uncovered to "persuade" a tiny weed to produce biofuel. Mike talks about his efforts to modify Google's "secret sauce" to manage the DNA data deluge brought on by the revolution in genome sequencing.

M. Schatz

We also launched the *Harbor Transcript* interactive iPad app this year, enriching the print version with additional multimedia content. We encourage you to download the *HT* and other apps produced by the DNA Learning Center for free.

Harbor Transcript
iPad App

While active in the global arena, we also pursue many local community outreach opportunities. In addition to tours that are conducted for participants in the CSHL Meetings and Courses program, more than 600 "nonscientific" guests participated in the CSHL Walking Tour program, visiting the campus from near and far, including Singapore, Italy, Japan, and China. The success of the program is largely due to the enthusiasm of guides, who this year totaled 13 graduate students and postdocs.

The Laboratory collaborated again with the Cold Spring Harbor Village Main Street Association to celebrate DNA Day on April 21–22 with a scavenger hunt that led participants to the Cold Spring Harbor Library, the Cold Spring Harbor Whaling Museum, the Firehouse Museum, and our own DNA Learning Center. DNA Day is celebrated across the country, with educational events sponsored by the National Human Genome Research Institute (NHGRI), a part of the National Institutes of Health. The day commemorates the completion of the Human Genome Project in April 2003, and the discovery of DNA's double helix, events closely tied to the Laboratory.

2012 Long Island 2-Day Walk

On April 17–18, first graders from local public and private schools Goosehill Primary and Friends Academy came to main campus for a special science fair. At each of six "experiment" stations, the children learned about scientific principles (from non-Newtonian fluid dynamics to cell structure to brain anatomy!) through hands-on activities and instruction conceived, planned, and led by 10 WSBS graduate students and four DNALC instructors. About 140 students, accompanied by 15 teachers and 120 parents, participated during the 2 days.

On August 8, CSHL and 13 local breast cancer organizations received checks from funds raised by the 2012 Long Island 2-Day Walk To Fight Breast Cancer. CSHL actively participates in the event, which attracted more than 400 participants. Each year, LI2DAY earmarks $15,000 for six scholarships that are awarded to local high school seniors with a parent or guardian who has been affected by breast cancer. Thanks to the CSHL volunteers who head

R. Sordella

up the LI2DAY Scholarship Committee. To date, $120,000 has been granted in LI2DAY Scholarships.

Associate Professor Raffaella Sordella participated in the Nassau Suffolk County Swim Across America event, swimming one mile to raise money for cancer research. We salute Raffaella's commitment.

Our employees lent a helping hand to the community in many ways. On campus, we conducted a blood drive in August, collecting 28 pints. CSHL also participated in Daffodil Days for the American Cancer Society. Members of our scientific community volunteer in many local elementary, middle, and high school science fairs.

CSHL Public Lectures

March 14–Alea Mills, Ph.D., CSHL Professor: *Where Will the Future of Genetic Technology Take Us?* Hosted by The Secret Science Club, Brooklyn, New York.

April 16–Brian Skotko, M.D., M.P.P., Board-certified medical geneticist at Children's Hospital Boston, Massachusetts General Hospital, Brigham & Women's Hospital, and Dana Farber Cancer Institute; Instructor, Children's Hospital Boston, Harvard Medical School, Boston, Massachusetts: *Keeping Children and Adolescents with Down Syndrome Healthy: Medical Updates for Physicians, Parents, and Educators*. Co-sponsored by CSHL and Down Syndrome Connection of Long Island.

A. Mills

April 19–David Spector, Ph.D., CSHL Professor and Director of Research: *The Laboratory @ Your Library: Discussion of "The Immortal Life of Henrietta Lacks*." Hosted by the Cold Spring Harbor Library, Cold Spring Harbor, New York.

June 26–W. Richard McCombie, Ph.D., Professor, CSHL and Director, CSHL Stanley Institute for Cognitive Genomics; **Diane Esposito, Ph.D.**, Research Investigator/Research Compliance Specialist, Cold Spring Harbor Laboratory; **Kenneth Offit, M.D., M.P.H.**, Chief, Clinical Genetics Service, Memorial Sloan-Kettering Cancer Center; **Kasmintan Schrader, M.B.B.S.**, Research Fellow, Cancer Biology and Genetics Program, Memorial Sloan-Kettering Cancer Center; **Peter K. Gregersen, M.D.**, Center Head, Robert S. Boas Center for Genomics and Human Genetics, The Feinstein Institute for Medical Research: *Follow Your Genes—Decision Making and Your Personal Genome.* Co-sponsored by CSHL, Bank of America-Merrill Lynch, North Shore-LIJ, and St. Johnland Nursing Center.

July 11–David Liittschwager, Photographer, *National Geographic* and Guest Instructor, DNALC Summer Camp: *A World in One Cubic Foot: Portraits of Diversity*.

November 18–David Spector, Ph.D., CSHL Professor and Director of Research: *Henrietta Lacks and HeLa Cells: Impact on Biological Research and Informed Consent*.

CSHL Public Concerts

March 23:	Charlie Albright, Piano
April 13:	Mischa Bouvier, Baritone (w/piano)
April 27:	Louis Schwizgebel, Piano
May 18:	Michael Brown, Piano
August 17:	Southampton Festival Chamber Orchestra
September 7:	Benjamin Beilman, Violin (w/piano)
September 21:	Narek Arutyunian, Clarinet (w/piano)
October 12:	Sarah Wolfson, Soprano (w/piano)

Looking Forward

Thanks to the entire CSHL community for a remarkable year. The institution continues to set world standards in biological and genetic research and education and that is the result of hard work and dedication on the part of our board, faculty, students, and staff. On behalf of CSHL, I thank our generous donors for allowing the best and brightest scientific minds to push the boundaries of research in cancer, neuroscience, plant genetics, genomics, and quantitative biology.

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

CHIEF OPERATING OFFICER'S REPORT

W. Dillaway Ayres, Jr.

While unusually severe weather events are occurring with increasing frequency, it is unlikely that we will soon forget "Superstorm Sandy." The good news for Cold Spring Harbor Laboratory is that we survived the storm relatively unscathed, experiencing no loss of scientific materials, animals or assets. This was thanks to exceptionally good preparation on the part of our facilities personnel and good luck with the timing of the peak storm surge. The adage, "the harder we work, the luckier we get" comes to mind. Perhaps most memorable will be our campus rallying cry, "Science versus Sandy—Science wins!"

In his President's Message, Bruce Stillman describes a more serious and immediate threat to our winning streak than the weather—the alarming decline in federal research funding and its implications for institutions like ours. This is a challenge that we have seen coming and one for which we have been planning and acting proactively from an operational and financial perspective. The primary defense is to continue to do exceptional science so that we can attract more than our share of the shrinking federal pie. We can continue our successful efforts to generate private funding from foundations and generous individuals. We can continue to manage our expenses as aggressively as possible and maintain a lean, efficient administration. We can grow our endowment through effective investing and fundraising.

The Laboratory's ratio of public to private funding has shifted with changing circumstances. During the period 2005–2012, federal funds as a percentage of our total research budget have declined from 59% to 43% while private funding has increased from 18% to 29%. While the dramatic increase in private funding has driven healthy growth in the research enterprise and is reflective of excellent science and effective fundraising, it is not always a predictable revenue stream. In addition, many foundation grants do not come with the requisite amount of support for indirect or research operating costs.

Endowment is our lifeline for the future. While spending from the funds represented only 11% of the 2012 research budget, endowment is a critical buffer against the uncertainties of federal and private funding. What may be less obvious is the historic role of the endowment in sustaining CSHL's unique approach to research that has emphasized the nurturing of early-career scientists.

Since the original gift of $7 million from the Charles Robertson family in 1973, the total assets of the funds (net of spending, plus contributions) have grown to $313 million at the end of 2012, an all-time high. Investment performance is one of two keys to growth, and, for our success, we have a hardworking committee of our Board of Trustees to thank. The goal is to achieve healthy appreciation of our investment assets while maintaining an appropriate degree of liquidity, and avoiding undue risk and volatility.

Since assuming the chair of the Investment Committee in 2007, trustee John Phelan has overseen a restructuring of the portfolio's asset allocation strategy from a plain vanilla mix of stocks and bonds to one designed to increase diversification and decrease volatility. Toward that end, allocation to long equity and fixed income asset classes has been substantially reduced while we have added considerably to hedged equity and absolute return investments. Investment performance has validated the strategy. The fund returned 12.5% for the calendar year 2012 with an allocation that incorporated a good deal of downside protection. Fundraising is at least as critical to endowment growth as investment performance. Consequently, development efforts over the next several

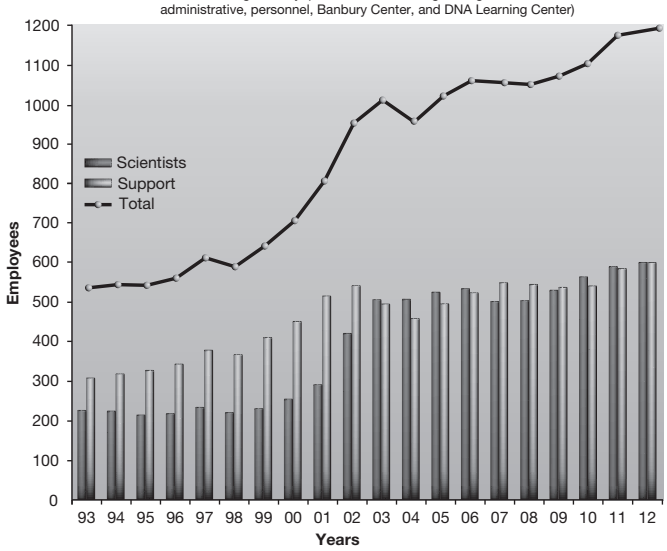
years will place particular emphasis on endowment. These are difficult funds to raise, so success in this area will require much hard work.

The political and fiscal machinations in Washington and the prospect of the "sequester" demand extraordinary measures to control operating and administrative expenses. Management has developed a detailed austerity plan that will kick in if necessary. Unfortunately, this takes a toll on our most important asset—our talented and dedicated staff.

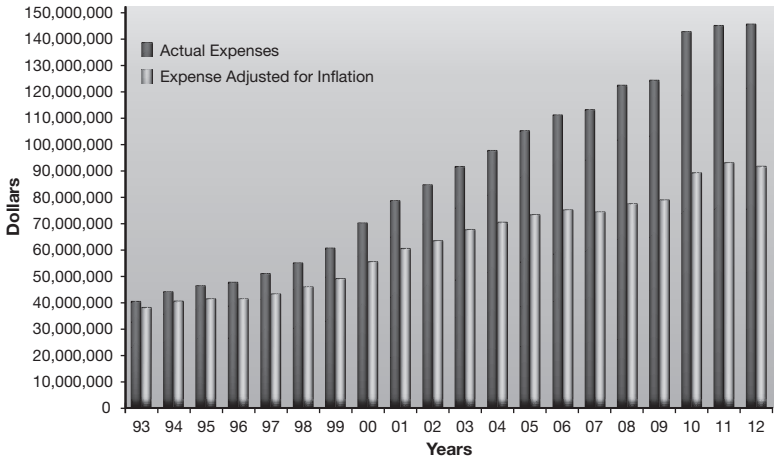
W. Dillaway Ayres, Jr.
Chief Operating Officer

Staff

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

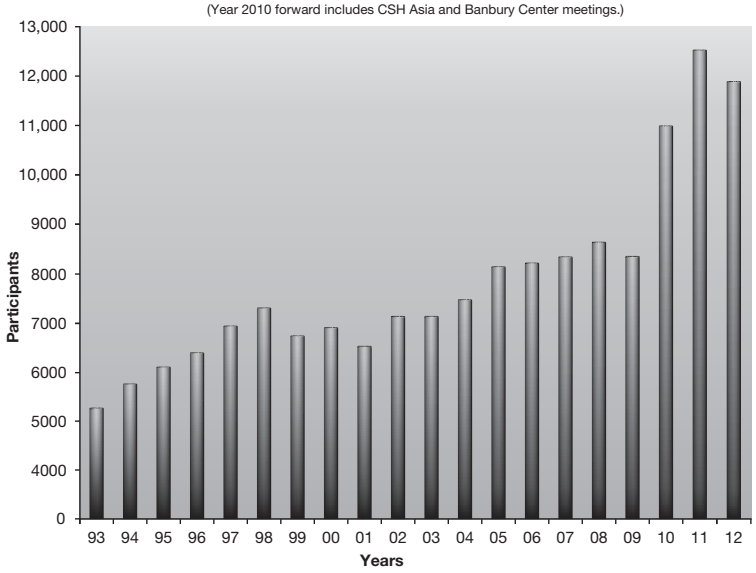


Operating Expense



Meetings and Courses Participants

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



Long-Term Service

Front row (left to right) Mary Smith, Art Brings, Patty Bird, Sadie Arana, Pat Urena, Terri Grodzicker, Bobbie Peters, Inez Sialiano, Nancy Hodson, Jan Witkowski, Julie Ehrlich. *Second row (left to right)* Yi Zhong, Randy Jones, Cliff Sutkevich. *Third row (left to right)* David Spector, Dill Ayres, James Watson, Bruce Stillman, John Inglis, Dick McCombie.

The following employees celebrated milestone anniversaries in 2012:

40 years	Terri Grodzicker
35 years	Margaret Wallace
30 years	Patty Bird, Art Brings, Dave Micklos, Cliff Sutkevich
25 years	Sadie Arana, Lisa Bianco, Jim Duffy, Julie Ehrlich, John Inglis, Bobbie Peters, Inez Sialiano, Pat Urena, Jan Witkowski
20 years	Robert Collins, Greg Hannon, Nancy Hodson, Randy Jones, Dick McCombie, John Pisciotta, Claudia Schmid, Mary Smith, Leslie Wenzel, Curtis Williams, Yi Zhong

Long-Term Service: 15 Years

Front row (left to right) Denise Weiss, Maria Mosquera, Liz Janow, Kim Bronson, Priscilla Wu. *Top row (left to right)* Joseph Houser, Robert Dickerson, Sal Serafino, Joseph Simorowski, James Watson, Bruce Stillman, Aigoul Nourjanova, David Spector, Bill Dickerson, Dill Ayres.

15 Years	Benjamin Boettner, Kimberly Bronson, Peggy Calicchia, Bill Dickerson, Robert Dickerson, Robert Eifert, Joseph Houser, David Jackson, Liz Janow, Tatyana Michurina, Maria Mosquera, Andrea Newell, Aigoul Nourjanova, Jorge Ramirez, Wilson Ramones, Sal Serafino, Joseph Simorowski, Denise Weiss, Priscilla Wu


RESEARCH

See previous page for photos of the following scientific staff.

Row 1: S. Qiao (Lippman Lab); D. Bahel (Hammell Lab); C. Zepeda-Mendoza (Spector Lab); M. Penzo (Li Lab)

Row 2: M. He, J. Tucciarone (Huang Lab); D. Pal (Sordella Lab); Y.K. Lee, D. Ware (Ware Lab); I. Falciatore (Hannon Lab)

Row 3: A. Kepecs, E. Demir (Kepecs Lab); N. Shirole (Sordella Lab); S. Cho Lee (Martienssen Lab); L. Zheng (Zheng Lab)

Row 4: I. Lindsted (Sordella Lab); C.-I. Hwang, D. Tuveson (Tuveson Lab); C. Fernandez-Marco, (Timmermans Lab); V. Pinskiy (Mitra Lab)

Row 5: B. Stilllman, M. Hossain (Stillman Lab); L. Trotman, T. Herzha (Trotman Lab); H. Oviedo (Zador Lab); H.-Y. Jeon (Krainer Lab)

CANCER: GENE REGULATION AND CELL PROLIFERATION

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

In the **Leemor Joshua-Tor** lab, members study the molecular basis of nucleic acid regulatory processes by using the tools of structural biology and biochemistry to examine proteins and protein complexes associated with these processes. They use X-ray crystallography to obtain three-dimensional structures of individual proteins and complexes. Biochemistry and molecular biology enable them to study properties that can be correlated with protein structure and function. It was Joshua-Tor and her team who first obtained the structure of a full-length Argonaute protein, work that instantly solved a long-standing puzzle in the RNA interference (RNAi) field. By observing the structure, they realized that Argonaute was the long-sought Slicer. This year, they added a critical insight into the mechanism of the key slicing event in RNAi, cleavage of the mRNA, by solving the structure of human Argonaute 2 bound to a microRNA (miRNA) guide. In looking at a similar process, the generation of genome protecting PIWI-interacting RNAs (piRNAs), in a collaboration between the Joshua-Tor and Hannon labs, this year they have determined the structure and function in fruit flies of Zucchini, showing it to be a key nuclease in the initial generation of piRNAs. Joshua-Tor and colleagues determined the three-dimensional structure of a protein complex called RNA-induced initiation of transcriptional gene silencing (RITS), discovering new details of how its various domains contribute to heterochromatin assembly and gene silencing. In addition, they solved the crystal structure of the complex of Gal3p–Gal80p with α-D-galactose and ATP, a classic transcription complex with its two ligands, in the yeast *Saccharomyces cerevisiae*. This work revealed that the Gal3p transducer of the *GAL* regulon interacts with the Gal80p repressor in its ligand-induced closed conformation. Joshua-Tor is also well known for her work on the E1 helicase enzyme, which acts to unwind DNA strands during the DNA replication process.

Adrian Krainer's lab studies the mechanisms of RNA splicing, ways in which they go awry in disease, and the means by which faulty splicing can be corrected. One area in which they study this is spinal muscular atrophy (SMA), a neuromuscular disease that is the leading genetic cause of death in infants. Their ability to correct an mRNA splicing defect in SMA that makes a gene called *SMN2* only partially functional forms the basis of a potentially powerful therapeutic approach. It is possible to stimulate SMN protein production through altering RNA splicing by the

introduction of chemically modified pieces of RNA called antisense oligonucleotides (ASOs) into the spinal cords of mice. This year, using ASOs in mice carrying a transgene of human SMN2, they developed a model for SMA using a technique they called TSUNAMI (shorthand for targeting-splicing using negative ASOs to model illness). This method can be used as a template to develop models for the study of other diseases caused by splicing defects, including familiar dysautonomia. The Krainer lab has also worked to shed light on the abnormal glucose metabolism of cancer cells, sometimes referred to as the Warburg effect. They recently published results showing that targeting cancer cell metabolism by modulating the splicing of the gene PK-M kills cancer cells grown in culture. The lab's expertise in splicing also recently uncovered a new exception to the usual rules regarding its mechanism. They found that at some splice sites, one or more bases in the U1 splicing guide RNA or its target RNA could bulge out to better facilitate a match. Some of the sites at which this can occur are within genes in which mutation can lead to diseases such as cancer.

David L. Spector's lab studies the spatial organization and regulation of gene expression. Their studies are focused on live single-cell analysis of the dynamics of chromatin and the recruitment of members of the gene expression machinery to a stably integrated genetic locus in human cells, as well as the analysis of endogenous genes in mouse embryonic stem cells (mESCs). In addition, the Spector lab is characterizing long nuclear retained noncoding RNAs (lncRNAs) that exhibit altered levels of expression as mESCs transition from the pluripotent state to neural progenitor cells. During the past year, the team focused on characterizing a *Malat1* knockout mouse that was developed in the laboratory. *Malat1* is among the most abundant lncRNAs, and previous studies identified a novel mechanism of 3′-end processing of this RNA. Cell biological and biochemical analyses indicated that *Malat1* is not essential for nuclear speckle assembly/maintenance or the level and phosphorylation status of SR splicing factors. Although RNA-Seq analysis of the *Malat1* knockout mouse did not reveal changes in global gene expression or alternative pre-mRNA splicing, 12 genes exhibited statistically significant changes in expression in brain cortex. Interestingly, among these 12 genes, five, including the lncRNA *Neat1*, reside adjacent to *Malat1* and are up-regulated ~1.5- to 2.3-fold. The up-regulation of *Neat1* suggests a potential compensation between *Malat1* and *Neat1*, which will be pursued in future studies.

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

Bruce Stillman's lab studies the process by which DNA is copied within cells before they divide in two. Working with yeast and human cells, Stillman and colleagues have identified many of the cellular proteins that function at the DNA replication fork during the S phase, the portion of the cell-division cycle when DNA synthesis occurs. Among these proteins are those that facilitate the assembly of chromatin, the protein–DNA complexes that form the chromosomes. The prime focus of current research, however, is the mechanism that initiates the entire process of DNA replication in eukaryotic cells. At the heart of this mechanism is a protein that binds to "start" sites on the chromosomes, called the origin recognition complex, ORC. Stillman's research also focuses on the process by which duplicated chromosomes are segregated during mitosis. The team has found ORC at centrosomes and centromeres, structures that orchestrate chromosome separation during

mitosis. This year, Stillman's team discovered that mutations that alter the largest protein found in this complex, Orc1, alter the ability of ORC to regulate both DNA replication and centrosome duplication. These mutations have been linked to Meier-Gorlin syndrome, a condition that results in extreme dwarfism, small brain size, and related characteristics of abnormal growth.

Acute myeloid leukemia (AML) is a particularly devastating and aggressive blood cancer that is currently incurable in 70% of patients. Research in **Chris Vakoc**'s lab seeks to understand this disease as well as others, such as lymphoid leukemias and epithelial tumors, by studying them at the level of genomic regulation. He is particularly interested in the proteins that regulate chromatin–DNA and associated proteins in the nucleus of the cell. To identify proteins involved in this process, which may also be targets for drug therapy, he deploys large-scale genetic screens using RNA interference (RNAi) as well as genetically engineered mouse models that display the hallmarks of human cancer. In collaboration with Jay Bradner at the Dana-Farber Cancer Institute, Vakoc has shown that the small-molecule drug candidate JQ1 has potent anti-AML activity. It works by suppressing the protein BRD4, which is a critical regulator of the potent oncogene c-*Myc*. Targeting BRD4 with JQ1 as a therapeutic strategy is currently being evaluated in clinical trials. More recently, Vakoc's team identified another chromatin regulator, PRC2, that if blocked with small-molecule inhibitors may reactivate a powerful tumor suppressor mechanism that can protect against AML.

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

C.M. Hammell C. Aquirre-Chen D. King
D. Bahel M. Konta
C. Carlston R. Perales

The temporal regulation of gene expression is an indispensable characteristic of metazoan development. At its core, this regulation is the basis for the progressive control of developmental programs and is essential for the coordination of animal growth. *Caenorhabditis elegans*, as a model organism, has provided an unparalleled system in which the principles of temporal cell-fate specification can be examined. Its strength derives from the fact that *C. elegans* development has an essentially invariant cell lineage and that each of the cell divisions occurs at a prescribed time in wild-type animals. The experimental tractability of *C. elegans* has led to the identification of a variety of conserved gene products that control temporal gene expression and represent components of the heterochronic (hetero: change; chrono: timing) developmental pathway. Heterochronic genes can be classified into two broad subgroups according to how they affect temporal aspects of development. Heterochronic mutations can alter the *patterns* in which major changes in temporal gene expression occur (Fig. 1), by lengthening or contracting the *relative timing* between individual cell divisions. Phenotypes associated with each class of heterochronic mutation are strictly elaborated within the context of the continued larval molting programs. Genes that control the *patterning* of cell-fate specification function within *modular regulatory programs* that are limited to a single stage of development. Importantly, the activity and sequential expression of these individual modules are integrated into a much larger, linear gene regulatory network (Fig. 1A). Precocious heterochronic mutants execute later stage-specific cell division programs at earlier times by skipping intermediate stages of development and display adult-specific gene expression programs during larval development. Conversely, retarded heterochronic mutants reiterate individual cell-fate programs and inappropriately retain larval features in adult-stage animals. The modular nature and regulatory logic of temporal patterning genes are underscored by a simple regulatory strategy that is used multiple times throughout development. The transition from one pattern of larval development to the next is coordinated by the sharp transcriptional induction of specific microRNAs (miRNAs) and the subsequent and rapid reduction of expression of their target transcripts. Transitions from the L1–L2-stage developmental programs are controlled by the induction of *lin-4* and the subsequent down-regulation of *lin-14* expression. Likewise, transitions from L2–L3- and L4–Adult-staged events are controlled by the expression of the middle-timer miRNAs (*mir-48, mir-241,* and *mir-84*) and late-timer miRNA, *let-7* and the subsequent down-regulation of their targets, *hbl-1* and *lin-41*, respectively. This serial and progressive regulation of temporal patterning genes limits the activity of individual cell-fate specification programs to a single larval stage.

The mechanisms and components that ensure the temporal precision of miRNA-mediated expression (and therefore couple development processes with chronological aging) are unknown. Furthermore, virtually nothing is known about how the relative timing of specific developmental events (molting cycle, cell cycle, cell migration, etc.) is controlled throughout larval development and how these specific developmental events are coupled to the temporal patterning of development.

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

C. Carlston, D. King

During the past 2 years, we have leveraged the invariant cell lineage, fixed intervals of postembryonic cell divisions, and the genetic tractability of *C. elegans* to identify factors that coordinate temporal gene expression and the specification of distinct chronological cell fates. To identify components that control the

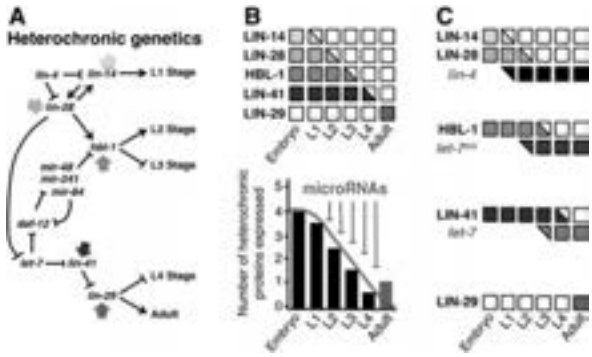


Figure 1. (*A*) The genes regulating the heterochronic pathway that controls specific cell-fate transitions are ordered into a large, linear gene-regulatory logic. (*B*) The ordered down-regulation of most heterochronic genes is orchestrated by the sequential activity of miRNAs. (*C*) The transcriptional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

biogenesis of miRNAs, we intentionally focused on finding suppressors of specific miRNA mutants that produce mature 21-nucleotide regulatory RNA products yet fail to efficiently down-regulate their target transcripts. Animals harboring *lin-4*(*ma161*) or *let-7*(*n2853*) mutations (which alter a single nucleotide in the mature "seed sequence" of the miRNA) display specific retarded cell-lineage phenotypes at early and late stages of development and underaccumulate functional *lin-4* and *let-7* miRNAs, respectively. Phenotypes associated with these alleles are indistinguishable from those displayed in animals harboring null mutations of these genes. In addition to mutations in specific heterochronic miRNAs, we have used a novel, antimorphic allele of the *C. elegans* miRNA-specific argonaute (*alg-1*[*ma192*]) that is globally compromised in miRNA activity and exhibits specific retarded heterochronic phenotypes due to the inappropriate perdurance of a single miRNA target. Although each mutation at the cellular and molecular levels disrupts a specific regulatory interaction required for normal development, each of these mutant animals fails to express adult-specific gene expression programs and is developmentally retarded. These macroscopic defects can be easily monitored through the use of an adult-specific fluorescent reporter (*col-19pro::GFP*) that is transcriptionally activated at the end of the normal fourth larval stage. Because *lin-4*(*ma161*), *alg-1*(*ma192*), and *let-7*(*n2853*) animals reiterate specific larval developmental programs, they fail to express c*ol-19::GFP.* Candidate suppressors of each of these distinct mutants were identified as genes that, when mutated or depleted via RNA interference (RNAi), recapitulate the normal *col-19::GFP* expression patterns in each mutant background. This screening step by itself is analogous to previous methods used to isolate heterochronic genes. A key element that distinguishes our strategy from prior screens is a refinement procedure that isolates, from the suppressors identified in primary screens, gene products that normally function to promote the precision of temporal miRNA expression. This secondary screening process is both powerful and direct, as the retarded developmental phenotypes displayed by each of these genetic contexts result from a common inability to express an unambiguous reporter of adult-specific expression (*col-19::GFP*). Suppressors identified in an initial genetic background (i.e., in primary screens above) can be queried for their ability to correct phenotypes associated with the mutations affecting other cell-fate specification events occurring at different larval stages (secondary screens). To date, we have, through ethylmethanesulfonate (EMS) mutagenesis, screened ~20,000 haploid genomes of the *lin-4*(*ma161*) genotype for heterochronic suppressors and a similar number of genomes in the *alg-1*(*ma192*) background. We have identified ~28 mutations that pass the primary and secondary refinement filters. Analysis of these mutants indicates that at a minimum, we have identified six complementation groups encoding genes that function to regulate the expression of multiple heterochronic miRNAs. These efforts have been complemented with an RNAi-based screen for suppressors of the *lin-4*(*ma161*) heterochronic phenotypes. This genome-scale screen, querying the function of ~86% of the *C. elegans* protein-coding genes, has identified ~10 candidate suppressors.

The primary outcome of these efforts is the identification of a conserved developmental clock that directly couples gene expression governing iterated developmental processes (including growth, behavior, and molting) to those controlling sequential cell-fate specification. In contrast to heterochronic genes that are turned on or off at specific developmental transitions, the developmental clock components we have identified are ubiquitously expressed throughout larval development. Importantly, they are also expressed in an oscillatory fashion and directly control the expression of multiple heterochronic miRNAs. Components of this developmental

clock include (1) *lin-42*, the *C. elegans* homolog of the circadian master regulator, Period, that functions to transcriptionally repress the expression of multiple miRNAs, (2) *lin-67*, encoding a conserved RNA-binding protein implicated in both human cancer and alternative mRNA splicing, and (3) *pqn-59*, a ubiquitously expressed and essential *C. elegans* "prion-like" gene.

lin-42, the *C. elegans* Period Homolog, Is a Transcriptional Repressor and Negatively Regulates miRNA Expression

C. Aquirre-Chen, D. King, R. Perales, M. Konta

Five alleles from the EMS-based screens contain genetic lesions that lie within a single complementation group on chromosome II. Analysis of the molecular lesions within this region identified alterations in a single gene, *lin-42*. In addition to these genomic alterations in the *lin-42* gene, we identified *lin-42* (with two independent RNAi clones) as a potent suppressor of *lin-4*(*ma161*) heterochronic phenotypes in our genome-scale RNAi-based strategy. *lin-42* is a large, complex locus that encodes the *C. elegans* homolog of the human and *Drosophila* Period genes implicated in circadian gene regulation. Through the use of transcriptional reporters, we have demonstrated that the *lin-42* locus produces multiple protein products from at least two promoters and that both promoters are temporally regulated. *lin-42* is ubiquitously expressed in embryonic development. During the larval stages of development, the transcriptional activation of the two *lin-42* promoters occurs once per larval stage, each peaking in expression just prior to each larval molt (Fig. 2). *lin-42a* and *lin-42b/c* promoter activity is curtailed after animals transition into adulthood. Translational reporters indicate that in addition to the oscillatory transcription patterns of the *lin-42a* and *lin-42b/c* promoters, the subcellular localization of each LIN-42 isoform is temporally regulated. LIN-42A protein accumulates in the cytoplasm during the middle portions of larval development and rapidly accumulates in the nuclear compartment as LIN-42B and LIN-42C expression begins. The molecular mechanisms controlling this temporal redistribution of LIN-42A from the cytoplasm to the nucleus and physical interactions between the LIN-42A, LIN-42B, and LIN-42C isoforms are an active focus of investigation in the lab.

Analysis of *lin-42* phenotypes suggests that it functions throughout larval development to inhibit the precocious expression of adult-specific genes. Specifically, phenotypes associated with *lin-42* require the expression and activity of multiple heterochronic miRNAs, and analysis of the small RNA expression patterns in *lin-42* mutants indicates that LIN-42 functions to negatively regulate their expression. In an effort to understand the temporal regulation of *lin-42* and to determine the mechanism by which it controls miRNA expression, we constructed a series of fluorescent reporters that would allow us to monitor the expression of multiple miRNAs in living animals throughout development. These constructs include the promoter regions of protein-coding genes or miRNAs driving nuclear-localized green fluorescent protein–pest or mCherry–pest sequence fusions. The addition of the PEST sequence reduces the half-life of the transcriptional reporter to ~1 h and allows the transcriptional activity of each gene to be monitored in real time. This dual-reporter system allows us to directly compare an expression pattern of an unknown gene to a highly characterized transcript of defined spatial and temporal pattern. Through this analysis, we found that most miRNAs are temporally regulated and that their expression, like the expression of *lin-42,* oscillates (Fig. 2). Because each LIN-42 protein can be localized to the nucleus, we hypothesized that it may function as a transcriptional regulator of miRNA expression. To directly test this hypothesis, we immunoprecipitated LIN-42/chromatin complexes and characterized the binding patterns of LIN-42 on a global scale. Consistent with

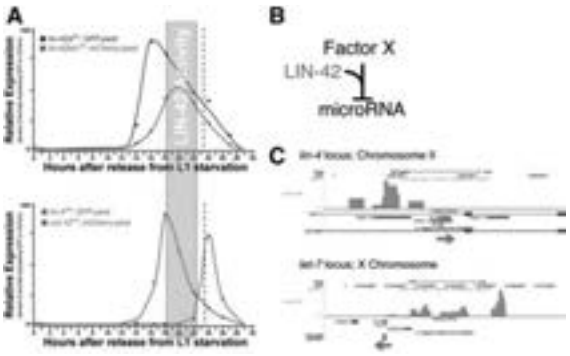


Figure 2. (*A*) *lin-42* and *lin-4* miRNA expression peaks at the end of each larval molt. (*B*) *lin-42* functions genetically to negatively regulate the expression of several miRNAs and does so by binding to chromatin. (*C*) LIN-42B binds the elements in the *lin-4* and *let-7* promoters.

LIN-42 directly regulating the oscillatory expression of multiple miRNAs, we found that LIN-42/chromatin interactions were enriched in the upstream regulatory sequences of miRNA promoters (Fig. 2). In addition to binding to the promoter regions of key genes that control developmental timing, LIN-42 binds to the putative regulatory regions of mRNAs and long intergenic noncoding RNAs (lincRNAs). The impact and mechanism by which LIN-42 regulates *C. elegans* transcription are a major focus of our laboratory.

lin-67, the *C. elegans* Homolog of the Human Proto-Oncogene Sam68, Controls Developmental Timing

R. Perales

Analysis of postembryonic mRNA expression patterns suggests that a significant number of transcripts are temporally regulated and that much of this regulation is coupled to the molting cycles of larval development. Although oscillatory expression patterns of many of these genes are maintained through both transcriptional (by differential expression of transcription factors) and posttranscriptional mechanisms (e.g., miRNAs), analysis of sequencing data and the isolation of additional regulators of heterochronic gene expression from our screens indicate that RNA processing may also have a major role in controlling temporal gene expression. The primary example of this class of regulators is *lin-67,* which was isolated in our RNAi-based screens for suppressors of the retarded heterochronic phenotypes of *lin-4*(*ma161*). *lin-67* encodes a highly conserved RNA-binding protein containing a single KH domain. Depletion of *lin-67* expression via RNAi also suppresses phenotypes associated with multiple miRNA mutants, including *alg-1*(*ma192*) and *let-7*(*n2853*). *lin-67* RNAi in wild-type animals results in the precocious expression of adult-specific fates, directly implicating *lin-67* function in normal temporal gene expression. Deletion of the *lin-67* gene also results in fully penetrant sterile phenotype and suggests that *lin-67* functions to control temporal gene expression throughout somatic development and contributes to germline maintenance. Translational reporters indicate that GFP::LIN-67 is a nuclear protein (Fig. 3) that is expressed throughout development. In a manner reminiscent of the temporal expression patterns

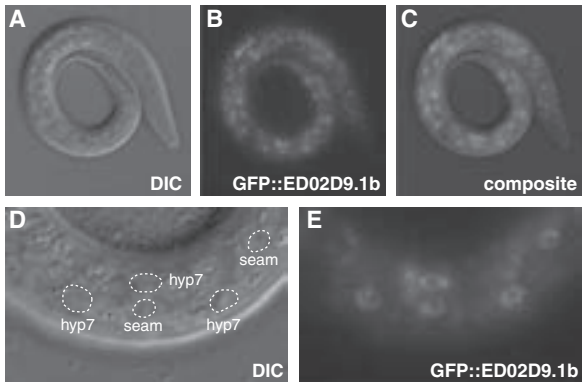


Figure 3. The *C. elegans* STAR family protein GFP::LIN-67 is a ubiquitously expressed, nuclear-localized RNA-binding factor.

of LIN-42, GFP::LIN-67 is expressed in an oscillatory fashion and accumulates once during each larval stage. LIN-67's closest homolog is the proto-oncogene Sam68, which is overexpressed in a variety of cancer types and is a member of the STAR (signal transduction activator of RNA) family of proteins. Sam68 has been implicated in controlling the alternative splicing of a variety of mRNA targets, many of which have been implicated in essential tumor maintenance, epithelial to mesenchymal transitions, neuronal development, and animal fertility. In addition, Sam68 functions to control the stability of many targets and directly contributes to aspects of cell cycle control and cell-fate specification. The roles of Sam68 in both normal development (neurogenesis and fertility), as well as its role in tumor biology, are also controlled at the posttranscriptional level, where phosphorylation contributes to both a regulated RNA-binding capacity and its differential nuclear and/or cytoplasmic localization. Through the study of the *C. elegans* Sam68 homolog, we intend to broaden our understanding of the roles of the STAR family proteins to encompass the temporal regulation of gene expression by characterizing changes in mRNA splicing in *lin-67* mutants and identifying the direct RNA targets of LIN-67.

Functions of PQN-59, a *C. elegans* "Prion-Like" Protein, in the Heterochronic Pathway

D. Bahel

Analysis of temporal gene regulation during *C. elegans* development has revealed that many of the repetitive

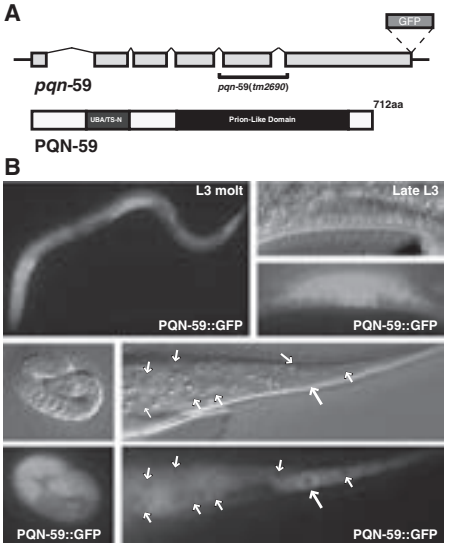


Figure 4. (*A*) *pqn-59* encodes a *C. elegans* "prion-like" protein with an amino-terminal ubiquitin-associated domain. The *tm2690* deletion allele of *pqn-59* disrupts *pqn-59* activity. (*B*) PQN-59::GFP is a ubiquitously expressed protein that functions in the cytoplasm to regulate temporal gene expression.

developmental events (including behavioral changes and molt cycles) and those involved in cell-fate specification are controlled by genes that directly control transcription or RNA metabolism. One of the surprising finds in our RNAi-based screens of *lin-4(ma161)* suppressors was that a *C. elegans* "prion-like" gene is essential for this process. Depletion of *pqn-59* (via RNAi) completely suppresses the gene expression and cell-fate defects associated with multiple miRNA mutants and alters the temporal patterning of normal animals, again suggesting that genes identified in our screens function throughout development. PQN-59::GFP, like other developmental clock components, is ubiquitously expressed and reaches peak levels just prior to the end of each molting period. Our current hypothesis is that, like other "prion-like" proteins, PQN-59 can form higher-order structures and that, during each molting period of postembryonic development, this structure serves as a scaffold that facilitates the regulated expression of downstream developmental activities.

Deanna Bahel

STRUCTURAL BIOLOGY OF NUCLEIC ACID REGULATORY PROCESSES

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

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

Mechanisms of RNAi

T. Schalch, C. Faehnle, E. Elkayam, C.-D. Kuhn, J. Ipsaro, C. Kuscu, J. Walleshauser, C. Ashar, E. Greene, C. Logan [in collaboration with G.J. Hannon, R.A. Martienssen, Cold Spring Harbor Laboratory; J. Partridge, St. Jude Children's Research Hospital]

RNA interference (RNAi) has made an enormous impact on biology in a very short period of time. Not only are we still discovering new cellular pathways for the regulation of gene expression that use these pathways, but RNAi has become an extraordinarily useful and simple tool for gene silencing. Almost from its beginnings, people have used genetics, biochemistry, molecular biology, and bioinformatics to study the mechanism of RNAi and related pathways. We argued, however, that in order to 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 (dsRNA) is processed to yield short (~19–31 nucleotides) dsRNAs that trigger the RNAi response. These short RNAs get incorporated into effector complexes called the RNA-induced silencing complex (RISC), wherein, in the mature complexes, a single-stranded RNA (ssRNA), the anti-sense strand of the original dsRNA, is retained in the complex. This short RNA (small interfering RNA [siRNA] or microRNA [miRNA]) then acts to guide the RISC complex to its target through base complementarity. In all cases, these complexes contain a small ssRNA and an Argonaute protein, which serve to define the RISC complex. In the past few years, we have been studying Argonaute family proteins, their complexes, and their roles in various RNAi silencing pathways.

Human Argonaute-2 in Complex with the Tumor Suppressor miRNA, miR-20a

Several years ago, we determined the first structure of a full-length Argonaute protein. This archael protein was composed of four domains: N, PAZ, Mid, and PIWI. The similarity of the PIWI domain to RNase-H-like nucleases revealed Argonaute as the key effector protein in the RISC complex and it was identified as Slicer—the enzyme that produces the endonucleolytic cut in an mRNA when the guide and target mRNA are fully complementary. Since then, a structure of a bona fide eukaryotic Argonaute protein has evaded structure determination until recently, when the first structure of human Argonaute-2 (hAgo2) bound to a heterogeneous mixture of RNAs was solved by N.T. Schirle and I.J. MacRae at Scripps. Ago2 is the only essential Argonaute protein in mammals. Shortly thereafter, we determined the structure of human Argonaute-2 in complex with the tumor suppressor miRNA, miR-20a (Fig. 1). The structure of human Ago2 shows a remarkable similarity to its bacterial counterparts, demonstrating the same domain organization. The RNA interacts with all domains of the protein as well as the interdomain linkers. The 5′ end of miR-20a is bound to the Mid domain, with the 5′-nucleotide placed in a well-defined binding site and swung away from the rest of the RNA. Bases 2–8,

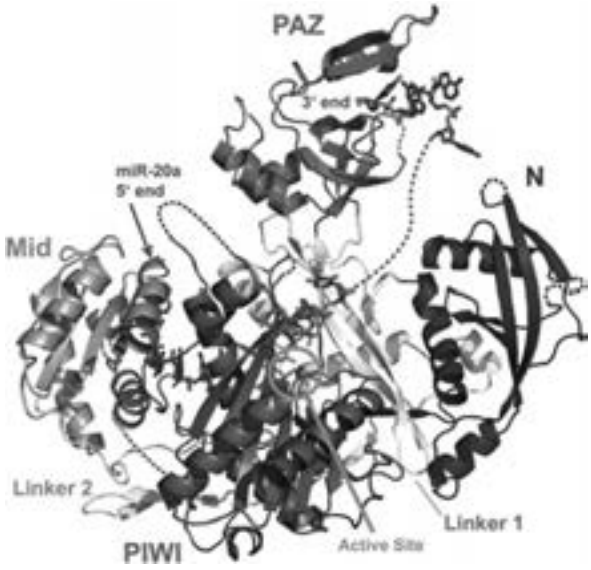


Figure 1. Structure of human Argonaute-2 in complex with the tumor suppressor miRNA, miR-20a.

known as the "seed region" necessary for target binding, are threaded along the PIWI domain, whereas the 3′ end is tethered to the PAZ domain. Apart from the first nucleotide, two major kinks are visible along the path of the guide RNA. One is between bases 6 and 7, with an Ile residue from the linker that connects the PAZ and Mid domains, intercalating between them. Another kink between bases 9 and 10 has two arginine residues from the PIWI domain stacked on these bases with an Ile residue from the linker in between the bases. The RNA confers great stability on human Ago2, which is susceptible to proteolytic digestion in the absence of the RNA. We continue to investigate features of Argonaute that arose from our structural work to further our understanding of the Argonaute proteins in their essential roles in human development and diseases.

Zucchini: A Nuclease Implicated in piRNA Biogenesis

Maintenance of germline integrity is critical to the reproductive success of a species. To protect against instability caused by mobile genetic elements, PIWI proteins and their associated small RNAs (Piwi-interacting RNAs [piRNAs]) form functional RISCs to repress the deleterious activity of such transposable elements. Silencing by piRNAs can be divided into three phases: biogenesis of piRNAs, a subsequent effector step, and an adaptive "ping-pong" cycle. In primary piRNA biogenesis, long, ssRNA transcripts are first parsed into smaller 5′- monophosphorylated fragments. This piRNA precursor then appears to be loaded into its PIWI-family partner. The 3′ ends of the piRNAs are formed by a Mg^{2+}-dependent trimming activity. To mount a response commensurate to the quantitative load of transposons, piRNAs can be amplified in a feed-forward "ping-pong" mechanism generating so-called secondary piRNAs. Once mature piRNAs are loaded into PIWIs, the resulting effector complexes are able to direct silencing at either the transcriptional level or posttranscriptional level. Although a general scheme for piRNA biogenesis and action has been established, the identities and activities of many of the molecular players remain unknown. Zucchini was suggested by genetic studies as a factor involved in piRNA biogenesis. However, previous reports indicate that Zucchini may be acting indirectly as a lipase. We produced a soluble amino-terminal truncation of the mouse homolog of the protein (mZuc) and showed that it has single-strand-specific nuclease activity. In addition, we determined the crystal structure of mZuc to 1.75 Å resolution (Fig. 2). The structure of mZuc displays a striking architectural similarity to HKD nucleases and is consequently dissimilar to HKD lipases. Additionally, the structure revealed a long, positively charged groove that can accommodate single-stranded nucleic acid substrates and a zinc finger motif that is frequently found in nucleic-acid-binding proteins. Taken as a whole, these biochemical and structural findings implicate Zucchini as a primary

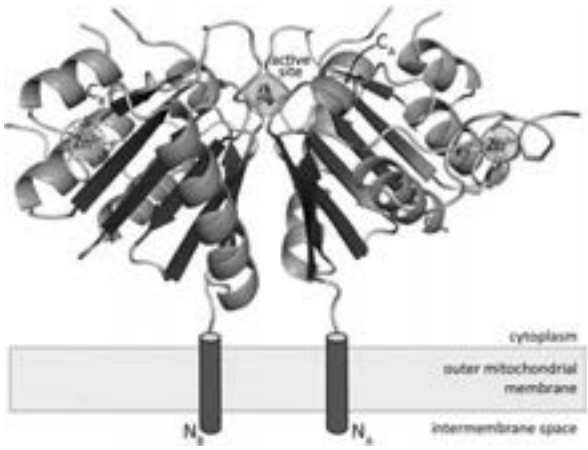


Figure 2. Structure of the mouse zucchini dimer.

piRNA biogenic nuclease perhaps being responsible for generating the 5′ ends of primary piRNAs.

The Different Faces of E1: A Replicative Hexameric Helicase

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

During DNA replication, two complementary DNA strands are separated and each becomes a template for the synthesis of a new complementary strand. Strand separation is mediated by a helicase enzyme, a molecular machine that uses the energy derived from ATP hydrolysis to separate DNA strands while moving along the DNA. Our early studies focused on recognition of the DNA replication origin by the helicase. Our crystal structure of the replicative helicase E1 from papillomavirus bound to single-stranded DNA and nucleotide molecules at the ATP binding sites provided a unique look into the mechanism of translocation of this molecular machine along DNA. We continue to study this helicase and the role other domains of the protein might have in helicase assembly and activity. We are using biophysical studies in solution—in bulk and at the single molecular level—to understand this helicase in mechanistic detail and correlate our findings with the structural framework.

A Transcriptional Switch: Revisited

T. Lavy, **P.R. Kumar, H. He**, D. Wah

A wealth of genetic information and some biochemical analysis have revealed an elegant model for the *GAL* regulon of the yeast *Saccharomyces cerevisiae*, a beautiful model system to understand transcriptional activation in eukaryotes. While attempting to place these studies into a structural and biochemical framework, we discovered the involvement of a dinucleotide in the induction of this classical transcriptional switch. Specifically, nicotinamide adenine dinucleotide phosphate (NADP) appears to jumpstart the switch, which is then sustained by the previously known transducer–repressor (Gal3p-Gal80p) interaction. This represents a real shift in how we think about the *GAL* transcriptional switch and our textbook view of how these switches work. In light of this discovery, we would like to find the link between galactose induction and the metabolic state of the cell that is sensed by the repressor, Gal80p. Our goal is to further delineate the molecular interactions and possible enzymatic activities involved in induction, activation, and repression in order to provide a mechanistic view of this switch. We are using a combination of X-ray crystallography, biochemistry, genetics, and metabolomics to more fundamentally understand this biological paradigm. The most recent addition is our structure of the complex among the Gal80p repressor, the Gal3p transducer, and the two small molecule ligands—galactose and ATP. We have found that the interaction between the proteins occurs only when Gal3p is in a "closed state" induced by ligand binding. Comparison to the free Gal3p structure that we have also determined and a previously determined structure of a galactose and ATP-bound form of the very similar active galactokinase, Gal1p, clearly shows that ligand binding induces the closed state of Gal3p. Furthermore, examination of the sites of Gal3p constitutive mutations shows that these would favor a closed state for Gal3p without necessitating ligand binding. On the other hand, Gal80p superrepressor mutants appear to result from their inability to form a stable complex with Gal3p, rather than tighter association with the activator, Gal4p. We are now examining possible enzymatic activities as well as other complexes involved in this classic transcriptional switch.

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RNA SPLICING

A. Krainer
M. Akerman, O. Anczuków, I. Aznárez, F. Bezirci, S. Brøner, D. Chatterjee, A. Chaudhury, S. Das, L. Hu, I. Hua, Y. Hua, M. Jensen, H.-Y. Jeon, K.-T. Lin, Y.-H. Liu, L. Manche, T. Nomakuchi, J. Novatt, A. Raimer, K. Sahashi, R.-Y. Tzeng, Z. Wang, J. Wu, A. Xu, L. Zepeda

Mechanisms of Constitutive and Alternative Pre-mRNA Splicing

RNA splicing is required for expression of most eukaryotic protein-coding genes. The spliceosome selects authentic splice sites with very high fidelity, relying on limited sequence information present throughout introns and exons. In humans, >90% of genes are expressed via alternative splicing, giving rise to multiple protein isoforms. The choice of alternative splice sites is commonly regulated to alter gene expression, either tissue-specifically or in response to a developmental program or to signaling pathways. The fact that multiple protein isoforms can be expressed from individual genes demonstrates that the classical "one gene–one enzyme" paradigm is no longer valid and provides an explanation for the unexpectedly small number of protein-coding genes uncovered by genome-sequencing projects. Both constitutive and alternative splicing mechanisms involve numerous protein components, as well as five noncoding RNA components that are part of small nuclear ribonucleoprotein (snRNP) particles. The work in our lab focuses on the identification and molecular characterization of protein factors and sequence elements that are necessary for the catalysis and fidelity of splicing and/or for the regulation of alternative splice site selection. We are interested in how the spliceosome correctly identifies the exons on pre-mRNA, and how certain point mutations in either exon or intron sequences cause aberrant splicing, leading to various human genetic diseases. Related areas of interest include the remodeling of mRNP architecture as a consequence of splicing, which influences downstream events, such as nonsense-mediated mRNA decay (NMD); the various roles of alternative splicing misregulation in cancer; and the development of effective methods to correct defective splicing or modulate alternative splicing, especially in a disease context. A summary of some of our recently published studies is provided below.

Alternative Splicing and Cancer

Alternative splicing has an important role in cancer, partly by modulating the expression of many oncogenes and tumor suppressors, and also because inactivating mutations that affect alternative splicing of various tumor suppressor genes account for some of the inherited and sporadic susceptibility to cancer. In addition, alternative splicing controls a metabolic switch characteristic of all cancer cells, known as the Warburg effect. Cancer cells preferentially metabolize glucose by aerobic glycolysis, characterized by increased lactate production. This distinctive metabolism involves re-expression of the embryonic M2 isozyme of pyruvate kinase, in contrast to the M1 isozyme normally expressed in differentiated cells, and it confers a proliferative advantage to tumor cells. The M1 and M2 isozymes are expressed from a single *PK-M* gene through alternative splicing of a pair of mutually exclusive exons (exons 9 and 10, respectively). We previously demonstrated that splicing enhancer elements that activate exon 10 are mainly found in exon 10 itself, and deleting or mutating these elements increases the inclusion of exon 9 in cancer cells. To systematically search for new enhancer elements in exon 10 and develop an effective pharmacological method to force a switch from PK-M2 to PK-M1, we carried out an antisense oligonucleotide (ASO) screen, in collaboration with Isis Pharmaceuticals. We found potent ASOs that target a novel enhancer in exon 10 and strongly switch the splicing of endogenous PK-M transcripts to include exon 9.

The ASO-mediated switch in alternative splicing leads to apoptosis in glioblastoma cell lines, and this is caused by the down-regulation of PK-M2, and not by the up-regulation of PK-M1, as expression of PK-M2 cDNA in the ASO-treated cells prevents apoptosis. These results suggest the potential of ASO-mediated splicing modulation for cancer therapy.

SRSF1 (formerly SF2/ASF) is a prototypical serine- and arginine-rich (SR) protein, with important roles in splicing and other aspects of mRNA metabolism. We previously determined that SRSF1 is a potent oncoprotein with increased expression in many tumors, including breast cancer. In collaboration with Senthil Muthuswamy (Ontario Cancer Institute) and Rotem Karni (Hebrew University, Jerusalem), we have investigated SRSF1's ability to transform human and mouse mammary epithelial cells in vivo and in vitro. We showed that SRSF1-overexpressing mouse COMMA-1D cells form tumors after orthotopic transplantation to reconstitute the mammary gland. In organotypic three-dimensional culture, SRSF1-overexpressing human MCF-10A cells form larger acini than control cells, reflecting increased proliferation and delayed apoptosis during acinar morphogenesis.

We found that these effects require the first RNA-recognition motif and nuclear functions of SRSF1. SRSF1 overexpression promotes alternative splicing of *BIM* and *BIN1* isoforms that lack pro-apoptotic functions and contribute to the acinar phenotype. We also demonstrated that *SRSF1* cooperates specifically with *MYC* to transform mammary epithelial cells, in part by potentiating activation of the translation initiation factor oncoprotein eIF4E. Furthermore, *MYC* and *SRSF1* show correlated expression in human breast tumors. SRSF1 is also up-regulated in lung carcinomas, where it likewise shows significant correlation with MYC expression. We have now demonstrated that *SRSF1* is a direct transcriptional target of MYC, providing a mechanistic basis for their correlated expression in cancer. MYC knockdown down-regulates *SRSF1* expression in multiple lung cancer cell lines. MYC directly activates transcription of *SRSF1* through two noncanonical E-boxes in its promoter. The resulting increase in SRSF1 protein is sufficient to modulate alternative splicing of a subset of its target transcripts. In particular, MYC induction leads to SRSF1-mediated alternative splicing of the signaling kinase *MKNK2*. We further showed that SRSF1 knockdown reduces MYC's oncogenic activity, decreasing proliferation and anchorage-independent growth. These results suggest a mechanism for SRSF1 up-regulation in a subset of tumors with elevated MYC levels and identify *SRSF1* as a critical MYC target that contributes to its oncogenic potential by enabling MYC to regulate the expression of specific protein isoforms through alternative splicing.

As part of a systematic analysis of the SRSF1 protein-interaction network, we used immunoprecipitation and quantitative mass spectrometry (i-DIRT) and found a novel interaction between SRSF1, the ribosomal protein RPL5, and the ubiquitin E3 ligase MDM2. We demonstrated that SRSF1 stabilizes the tumor suppressor protein p53 by abrogating its MDM2-dependent proteasomal degradation. Moreover, SRSF1 is a necessary component of the complex, which functions in a p53-dependent ribosomal-stress checkpoint pathway. Consistent with the stabilization of p53, increased SRSF1 expression in primary human fibroblasts decreases cellular proliferation and ultimately triggers oncogene-induced senescence (OIS). These findings underscore the deleterious outcome of SRSF1 overexpression and identify a cellular defense mechanism against its aberrant function. Furthermore, they implicate the RPL5-MDM2 complex in OIS and demonstrate a link between spliceosomal and ribosomal components, functioning independently of their canonical roles, to monitor cellular physiology and cell cycle progression.

Targeted Antisense Modulation of Alternative Splicing for Therapy and Disease Modeling

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

Phase I clinical trials with our ASO compound, ISIS-SMN_{Rx}, have now been completed; the drug, which was administered to SMA patients by lumbar puncture, was well tolerated at all doses tested. Phase II trials to further establish appropriate dosing and begin to assess effectiveness are ongoing.

We also described the use of different ASOs that exacerbate *SMN2* missplicing to phenocopy SMA in a dose-dependent manner when administered to *SMN2*-transgenic $Smn^{-/-}$ mice. Intracerebroventricular ASO injection in neonatal mice recapitulated severe SMA-like progressive motor dysfunction, growth impairment, and shortened life span, with α-motor neuron loss and abnormal neuromuscular junctions. These SMA-like phenotypes were prevented by intracerebroventricular (ICV) injection of the above therapeutic ASO. We uncovered starvation-induced splicing changes—particularly in *SMN2*—which likely accelerate disease progression. These results constitute proof of principle that ASOs designed to cause sustained splicing defects can be used to induce pathogenesis and rapidly and accurately model splicing-associated diseases. This approach allows the dissection of pathogenesis mechanisms, including spatial and temporal features of disease onset and progression, as well as testing of candidate therapeutics. By targeting splicing of endogenous genes, the method can potentially be used to phenocopy diseases in wild-type animals.

Noncanonical 5′-Splice-Site Recognition

An established paradigm in pre-mRNA splicing is the recognition of the 5′ splice site by canonical base pairing to the 5′ end of U1 small nuclear RNA (snRNA). We previously reported that a small subset of 5′ splice sites base pair to U1 in an alternate register that is shifted by one nucleotide. Using genetic-suppression experiments in human cells, we have now demonstrated that many other 5′ splice sites are recognized via noncanonical base-pairing registers involving bulged nucleotides on either the 5′ splice site or U1 RNA strand. By combining experimental evidence with transcriptome-wide free-energy calculations of 5′ splice site/U1 base pairing, we estimate that 10,248 5′ splice sites (~5% of human 5′ splice sites) in 6577 genes use "bulge registers." Several of these 5′ splice sites occur in genes with mutations causing genetic diseases and are often associated with alternative splicing. These results call for a redefinition of an essential element for gene expression that incorporates these registers, with important implications for the molecular classification of splicing mutations and for alternative splicing.

Analyzing Splicing by Next-Generation Sequencing

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

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CELL BIOLOGY OF THE NUCLEUS

D.L. Spector G. Arun, J. Bergmann, M.S. Bodnar, M. Eckersley-Maslin, S. Hearn, M. Huebner, R.I. Kumaran, Z. Lazar, J. Li, G. Ruiz Buendia, C. Zepeda-Mendoza, B. Zhang

Most cellular processes can trace their beginnings to the nucleus, where a gene is activated resulting in the production of an RNA molecule, some of which encode for proteins, whereas others are functional in the form of RNA. Although much biochemical information is available regarding many of the factors involved in gene expression, the spatial and temporal parameters that influence gene expression and the role of noncoding RNAs in regulating this multifaceted process are just beginning to be identified. During the past year, our research has focused on elucidating various mechanisms of regulating gene expression and DNA repair and the role of long nuclear retained noncoding RNAs in development and cancer progression.

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

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

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

Previously, we presented evidence that a 3.6-kb genomic sequence in the 5′ regulatory region of the *Oct4* gene is sufficient to mediate pairing of a transgene with the endogenous *Oct4* alleles. During the past year, we assessed a series of DNA elements within the 3.6-kb region to determine if any were necessary for *Oct4* allele pairing to occur. On the basis of their roles in homologous chromosome pairing in other biological paradigms, Ctcf, E2A, Oct4, and Yy1 were identified as candidate proteins capable of binding the 3.6-kb DNA region in a combinatorial fashion to mediate pairing. Putative protein-binding sites were identified using the MatInspector program, and site-directed mutagenesis yielded four new DNA fragments, with the putative binding sites for either Oct4/Sox2, Ctcf, Yy1, or E2A replaced with scrambled DNA sequences. Each of the four mutated DNA fragments were individually targeted to the *Col1A* locus in KH2 ES cells via FRT-mediated recombination. ES cell lines with successfully targeted mutated transgenes were then differentiated using retinoic acid (RA), and DNA FISH (fluorescence in situ hybridization) was performed using probes specific for the transgene, *Col1A* locus, and *Oct4* locus. The ability of the mutated transgenes to associate with endogenous *Oct4* was assessed after 1 day of RA differentiation. Ctcf, E2a, and Yy1 mutants were still capable of pairing with endogenous *Oct4* loci. In contrast, the transgene with mutated Oct4/Sox2 consensus binding sites exhibited a reduced level of transgene pairing to a frequency equivalent to the wild-type *Col1A* locus. These results demonstrated that the Oct4/Sox2 consensus binding sites are necessary for *Oct4* allelic pairing to occur. Ongoing in vitro studies are confirming this interaction.

Monoallelic gene expression describes the transcription from only one of two homologous alleles of a particular gene. We previously performed an unbiased RNA sequencing screen to identify random monoallelically expressed genes, taking advantage of a hybrid ESC (embryonic stem cell) line that is an F_1 cross between C57BL/6 and CAST/Ei strains, so that the expressed single-nucleotide polymorphisms (SNPs) would reflect from which allele the transcript is derived. Libraries for RNA sequencing were generated from seven single-cell ESC clones and seven single-cell NPC (neural progenitor cell)

clones from three separate differentiation experiments. The libraries were amplified and sequenced using the Illumina platform, generating ~80 million unique reads per clone, of which ~80% mapped to a custom-built C57BL/6 × CAST/Ei genome using a Burrows-Wheeler Aligner (in collaboration with Paul Flicek's group, EBI, Hinxton, U.K.). Although only 30 transcripts were identified as monoallelic in ESCs, representing less than 0.29% of assessable genes, this increased to 478 transcripts during differentiation to NPCs. This corresponds to ~4% of expressed transcripts. Of particular interest, genes showed variations in expression patterns between clones from true monoallelic expression to various levels of allelic imbalance, implying that this regulation is rather plastic yet controllable. Two methods are being used to validate the 478 NPC transcripts. First, polymerase chain reaction (PCR) amplification of exonic SNPs followed by Sanger sequencing has been performed for more than 87 gene-clone combinations, giving a 91% concordance with the RNA-Seq screen analysis. Second, RNA FISH was performed to validate monoallelic candidates at the single-cell level. Comparison of the 30 ESC candidates and 478 NPC candidates revealed only five transcripts that overlapped between the two data sets.

Because, once established, the choice of active allele is maintained across cell generations, a memory must be in effect to maintain this monoallelic state. Three possible mechanisms have been investigated (DNA methylation, nuclear organization, and histone modifications). Thus far, DNA methylation and nuclear positioning do not appear to have a role in maintaining the monoallelic state. Ongoing studies are assessing the role of histone modifications in maintaining the active and silent states of the respective alleles.

When the levels of expression of these transcripts were compared between ESCs and NPCs, the majority did not show any change in expression levels, suggesting that the switch to monoallelic state does not change total expression levels during differentiation. To further address whether monoallelic expression changes overall mRNA expression levels, quantitative (Q)-PCR analysis of individual NPC clones was performed for four candidate genes. The relative expression in NPC clones did not correlate with the extent of allelic imbalance as determined by D-score. Furthermore, global analysis of expression levels (FPKM) versus D-score showed no difference between monoallelic transcripts versus all assessable transcripts. This lack of correlation between the number of active alleles and total level of gene expression suggests that monoallelic expression is not a mechanism for regulating mRNA levels in the cell, but instead there is transcriptional compensation of the single active allele, likely through a feedback mechanism, such that total level of expression in the cell is maintained.

Long noncoding RNAs (lncRNAs; >200 nucleotides in length) represent a relatively recently studied class of RNAs for which functional insight is currently available for only a few candidates out of potentially thousands. The majority of lncRNAs are expressed at very low levels, some as low as one copy per cell, and these RNAs generally exhibit poor primary sequence conservation over evolution. lncRNAs have been implicated in numerous molecular functions, including modulating transcriptional patterns, regulating protein activities, serving structural or organizational roles, altering RNA processing events, and serving as precursors to small RNAs. We performed a next-generation sequencing screen of poly(A)+ RNA to identify putative lncRNAs that are differentially expressed in mouse ESCs and NPCs. Our goal is to uncover new mechanisms by which lncRNAs regulate gene expression, differentiation, and/or nuclear organization. We identified 638 lncRNAs expressed in ESCs and NPCs, of which 135 were greater than threefold down-regulated and 65 were greater than threefold up-regulated during differentiation toward NPCs. Of these transcripts, ~70% are enriched in the nucleus, indicating potential roles in the regulation of gene expression or nuclear organization. Using stringent criteria, we generated a short list of 46 presently uncharacterized and moderately abundant lncRNAs.

We have begun to characterize these lncRNAs with respect to their expression in embryonic and adult tissues, as well as to examine their subcellular localization using RNA fluorescence in situ hybridization protocols with single-molecule sensitivity. Using antisense oligonucleotide (ASO) technology, we are specifically depleting the level of individual lncRNAs and are in the process of determining cellular phenotypes including maintenance of pluripotency and differentiation potential under knockdown conditions, as well as by assessing global changes in gene expression patterns. Furthermore, we are working on establishing ESC-derived cell lines that will allow us to track the dynamics of candidate lncRNP complexes in living

cells, as well as to determine the composition of specific lncRNP complexes on the protein level. Our overall aim is to provide a thorough and comprehensive characterization of individual lncRNAs to dissect functional interactions of these RNAs with nuclear proteins and their mechanisms of action at the molecular level.

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

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Malat1 (metastasis-associated lung adenocarcinoma transcript 1), also known as *Neat2* (noncoding nuclear-enriched abundant transcript 2), is among the most abundant lncRNAs and is located on mouse chromosome 19qA (human chromosome 11q13.1). It is evolutionarily conserved among mammals and is highly expressed in many tissues. *MALAT1* is up-regulated in several human cancers, suggesting that it may have an important function during development and cancer progression, yet the physiological function of *Malat1* lncRNA has not been determined.

To assess the in vivo function of mouse *Malat1*, we previously established a loss-of-function mouse genetic model using homologous recombination in ES cells. *Malat1*$^{-/-}$ mice are grossly normal and fertile. Offspring of heterozygous breedings follow Mendelian segregation, suggesting that deletion of *Malat1* does not affect mouse pre- and postnatal viability. Breedings between *Malat1* homozygotes and wild type produce normal-sized litters, indicating that *Malat1*$^{-/-}$ mice are fertile. Further cell biological and biochemical analyses indicated that mouse *Malat1* is not essential for nuclear speckle assembly, regulating the level and phosphorylation of SR splicing factors, or cell proliferation. Genome-wide expression and splicing profiling demonstrated that *Malat1* loss results in minimal alterations in global gene expression and pre-mRNA splicing. However, deletion of *Malat1* resulted in a nearly twofold up-regulation of a small group of genes in mouse brain cortex. A lack of major phenotype upon the loss of *Malat1* transcripts could be attributed to functional redundancy with other RNA transcripts (e.g., *Neat1* lncRNA) or to compensatory mechanisms during development, as occurs with respect to many protein-coding genes. Alternatively, some lncRNAs, including *Malat1*, could have a subtle role and regulate cellular processes via a fine-tuning mechanism while exhibiting more elaborate manifestations upon specific stresses such as cancer.

To precisely identify the mechanism by which *Malat1* functions in cancer, studies were initiated with the mouse mammary tumor virus long terminal repeat (LTR)-driven polyoma middle T antigen (MMTV-PyMT) mouse model, which phenocopies human luminal breast cancer. Similar to human breast disease, PyMT mice exhibit all stages of the disease from premalignant to malignant stages and, more importantly, distant metastasis. Tumors have very high penetrance, and the majority (>80%) of mice exhibit lung metastasis. MMTV-PyMT mice develop tumors in both FVB/n and C57BL/6 backgrounds, with the latter being delayed. The spontaneous tumors developed in MMTV-PyMT mice were independently subjected to two different *Malat1* antisense oligonucleotides (ASOs) or scrambled ASO treatment by subcutaneous injection of 25 mg/kg per dose for 5 days with a rest period of 2 days for 6 weeks. Tumor growth was monitored for up to 6 weeks and at the end of the sixth week, the animals were sacrificed and mammary glands and lungs were harvested. *Malat1* RNA levels were reduced by more than 60% in primary mammary tumors from mice treated with *Malat1* ASOs compared to scrambled ASO-treated mice, confirming that the ASOs are efficacious for in vivo studies. We observed slower growth of primary tumors in ASO1- or ASO2-treated mice, resulting in reduced tumor volume as compared to controls. Interestingly, upon ASO treatment, only two out of 18 mice developed >10 metastatic lung nodules compared to scrambled ASO-treated control mice in which six out of 12 mice developed >10 macroscopic metastatic lung nodules. Ongoing studies using this system will delineate the role of *Malat1* at different stages of the disease, in particular during primary tumor progression and lung metastasis. In addition, we have recently begun crosses between our *Malat1*$^{-/-}$ mice and the MMTV-PyMT mouse model. Our current results indicating a potential role of *Malat1* in metastasis are extremely exciting, and with the appropriate tools in place, we are poised to elucidate the role of this lncRNA in cancer initiation and progression.

Evaluation of DNA Repair Pathway Choice upon Zinc Finger Nuclease-Induced Double-Strand Breaks

R. Kumaran, J. Li, Z. Lazar [in collaboration with M.H. Porteus, Stanford University School of Medicine]

DNA double-strand breaks (DSBs) are the most dangerous class of DNA damage. If DSBs are left unrepaired, they can result in genomic instability or cell death. Therefore, cells have evolved complex DNA-damage response pathways to repair DSBs. In mammalian cells, two major and mechanistically distinct DSB repair pathways are nonhomologous end-joining (NHEJ) and homologous recombination (HR). NHEJ and HR can be regulated by multiple factors, including cell cycle phase. Although cell cycle regulation of the DSB repair pathway choice on a single-copy locus is thought to be restricted to G_1 phase for NHEJ and to late S/G_2 phase for HR, it remains unclear for a multicopy locus. We have investigated DSB pathway choice by developing and utilizing multicopy and single-copy DSB reporter cell systems. Using these systems, we initiated a DSB with a specific zinc finger nuclease and then visualized DSB repair as it relates to cell cycle phase. We showed that both the NHEJ and HR pathways can simultaneously be recruited to repair DSBs at a specific site in the genome. The frequency of recruitment of the HR repair protein Rad51 is high at a multicopy versus a single-copy DSB substrate. Interestingly, HR can occur in G_1 phase of the cell cycle, if donor template is available for repair. Our findings suggest a competition between the HR and NHEJ repair machineries throughout interphase of the cell cycle, which could be a critical factor for the stability of mammalian genomes that are highly enriched with repeat sequences. Understanding the mechanisms of DNA repair is vital for developing improved treatment options for cancer, immune, and neurodegenerative disorders.

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Michael Huebner and David Spector

MOLECULAR BIOLOGY OF PAPILLOMAVIRUSES

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The papillomaviruses are a group of viruses that infect and transform the basal epithelium, inducing proliferation of the cells at the site of infection. The resulting tumors (warts) are in most cases benign and will usually regress after some time, but certain types of human papillomaviruses (HPVs) give rise to tumors that are prone to progress toward malignancy, especially frequently cervical carcinoma. Indeed, HPV infection appears to be a necessary cause of invasive cervical carcinoma and thus represents one of the few firmly established links between viral infections and the development of cancer. An impediment to the study of papillomaviruses has been the inability to define simple in vitro cell culture systems for analysis of the viral life cycle. These viruses normally require specialized differentiating cells that only with difficulty can be generated in cell culture. However, for a bovine papillomavirus (BPV-1), a convenient cell culture system exists where viral gene expression, oncogenic transformation, and viral DNA replication can be studied. Thus, BPV has become a useful model for these aspects of the viral life cycle. The DNA replication properties of the papillomaviruses show some unique and interesting characteristics. As part of their normal life cycle, these viruses can exist in a state of latency, which is characterized by maintenance of the viral DNA as a multicopy plasmid in infected cells. The copy number of the viral DNA is tightly controlled, and the viral DNA is stably inherited under these conditions. Papillomaviruses therefore provide a unique opportunity to study plasmid replication in mammalian cells. In addition, the viral DNA replication machinery represents one of the most promising targets for antiviral therapy. In previous years, we have reported the characterization of the papillomavirus replicon and the identification of the viral components that are required for viral DNA replication. In recent years, we have directed our attention toward the biochemical events that are associated with initiation of DNA replication. We are studying the biochemical properties of the viral E1 and E2 proteins and how these two proteins interact with the viral origin of DNA replication and with the cellular replication machinery to generate initiation complexes. Our studies demonstrate that the E1 protein has all of the characteristics of an initiator protein, including ori recognition, DNA-dependent ATPase activity, and DNA helicase activity. The transcription factor E2, whose precise function has remained more elusive, appears to serve largely as a loading factor for E1. Through direct physical interactions with both E1 and the ori, E2 provides sequence specificity for the formation of the initiation complex.

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

Function of the Amino-Terminal Domain of E1 in Viral DNA Replication

The papillomavirus E1 protein is a multifunctional protein that carries out essential functions in viral DNA replication. Although E1 is well-studied, functions have not been assigned to all parts of the protein. The function of the amino-terminal ~150 residues has especially remained mysterious. Although a part of this sequence is involved in nuclear import and export, these sequences account for only a fraction of the amino-terminal domain. Given the parsimony that usually characterizes viral proteins, it is likely that additional functions are present in the amino-terminal domain.

To determine the function of the amino-terminal domain, we generated small successive deletions from

the amino terminus and expressed and purified the resulting proteins from *Escherichia coli*. We tested the proteins by electrophoretic mobility-shift assay (EMSA) for the formation of a double-trimer (DT) complex on the origin of DNA replication. This complex is essential for viral DNA replication. Although deletion of the first 35 residues had no effect on DT formation, deletion of the first 55 residues ($E1_{55–605}$) resulted in a failure to form the DT, demonstrating that sequences amino-terminal to residue 55 are important for DT formation. However, a further deletion up to residue 110 restored the ability to form DT. This result led us to conclude that the sequences present between residues 55 and 110 interfere specifically with DT formation but that these sequences are not exposed in the intact amino-terminal domain. To identify activities present in the amino-terminal domain, we generated fragments from this domain and expressed these as GST fusion proteins in *E. coli*. In this manner, we could identify a nonspecific DNA-binding activity that we initially mapped to between residues 70 and 120. Interestingly, this activity was not detectable in the context of the intact amino-terminal domain ($E1_{1–150}$). However, successive deletions from the amino terminus gradually increased the DNA-binding activity of this fragment. These results demonstrate that the DNA-binding activity present in $E1_{70–120}$ is hidden or sequestered in the presence of the 55 amino-terminal residues. To determine whether the exposure of this DNA-binding activity was responsible for the failure to form the DT complex with $E1_{55–605}$, we knocked out the DNA-binding activity with a specific point mutation. Surprisingly, this mutation did not restore the ability of $E1_{55–605}$ to form a DT.

This result indicated that an activity other than DNA binding is present in the amino-terminal domain and is responsible for the defect for DT formation in $E1_{55–605}$. We performed pull-down assays to look for interactions between fragments from the amino-terminal domain and the rest of E1. We were able to identify a strong interaction between the amino-terminal domain and the oligomerization and helicase domain in E1. Interestingly, the region of the amino-terminal domain involved in this interaction overlaps with the DNA-binding activity present in the amino-terminal domain and is contained between residues 70 and 120. Furthermore, this interaction shows the same behavior as the DNA-binding activity, i.e., the interaction surface is sequestered in the presence of the amino-terminal 55 residues. We could knock out the interaction between the amino-terminal domain and the oligomerization and helicase domain by generating a double point mutation in residues 112 and 113. This double mutation, when introduced into the $E1_{55–605}$, restored the ability to form a DT complex. This result indicates that the defect in $E1_{55–605}$ is the result of an illegitimate interaction that results in faulty oligomerization. These results indicate that the amino-terminal domain most likely has a regulatory role in viral DNA replication, possibly by functioning as a negative regulator of viral DNA replication. This conclusion is supported by the in vivo phenotype of the 112/113 double mutation that results in greatly elevated replication of the viral genome.

DNA REPLICATION AND CHROMATIN INHERITANCE

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Each human cell contains ~2 m of DNA in its genome, and ~2200 cells can fit on top of a pinhead. When extracted, the DNA from these cells stretches to 4.5 km in length. These numbers indicate the nature of the complex problem of copying genetic information each time a cell divides, making sure that DNA replication is both accurate and occurs only once per cell division. For 32 years at Cold Spring Harbor, this laboratory has been investigating the replication of DNA and associated chromatin structures and has identified many of the key proteins that participate in the duplication of DNA. They include proteins at the DNA replication fork that cooperate to synthesize DNA and proteins that initiate the entire process of genome duplication. This year, progress has been made on understanding how the origin recognition complex (ORC), an initiator protein we identified 20 years ago, cooperates with Cdc6, Cdt1, and MCM2-7 proteins to form a prereplicative complex on DNA and how it controls the initiation of DNA replication. These studies also extend the function of ORC protein subunits in control of centrosome duplication that link to a rare recessive human syndrome called Meier-Gorlin syndrome. Other studies have identified a new regulator of cell proliferation in some cancer cells that may be an effective therapeutic target for human cancer.

Origin Recognition Complex

ORC is a DNA-regulated ATPase containing six subunits that in yeast associate with each other during the cell division cycle. ORC binds to origin DNA in the genome and forms a prereplicative complex (pre-RC) at these origins during the G_1 phase of the cell division cycle, in cooperation with the Cdc6 protein and Cdt1. Cdt1 is bound to a six-subunit, barrel-shaped hexamer containing the MCM2-7 proteins, and this heptameric complex is topologically loaded onto double-stranded DNA by ORC-Cdc6 ATPase proteins. In fact, two MCM2-7 hexamers are loaded per origin in yeast and they form a head-to-head structure with double-stranded DNA passing through the middle of the barrels. Upon activation of DNA replication in S phase, at each origin, the MCM2-7 proteins bind other proteins, including Cdc45 and the GINS complex, to become an active DNA helicase that unwinds the DNA in preparation for its replication. We have been investigating how this process occurs and have collaborated with structural biologists Jingchuan Sun and Huilin Li at nearby Brookhaven National Laboratory, along with former postdoctoral fellow Christian Speck and his group at the MRC in London, to study the structure of various intermediates in the assembly of pre-RCs.

Previous cryo-electron microscope (EM) studies have determined the structure of ORC from yeast and ORC bound to DNA. The structure of an ORC-Cdc6 complex bound to DNA was recently completed. The structure revealed that Cdc6 induces major structural changes in ORC, including the rotation of the amino terminus of Orc1 containing a bromo-associated homology (BAH) domain to the back of the structure, allowing Cdc6 to enter. Cdc6 localizes next to the Orc1 subunit. Also adjacent to Orc1 is the Orc4 subunit, but it was too far from Orc1 to be capable of activating the Orc1 ATPase activity via a known arginine (R) finger. The ORC-Cdc6 complex also bends the DNA, and we have shown that yeast ORC can induce supercoiling in DNA, analogous to the supercoiling of DNA observed with *Drosophila* ORC by other investigators. Thus, the ORC-Cdc6 complex bound to DNA is primed to load the MCM2-7 hexamer, but additional structural changes are required.

In more recent studies, again in collaboration with Christian Speck and his colleagues in London and Jingchuan Sun and Huilin Li, a cryo-EM structure of ORC-Cdc6 bound to Cdt1 and MCM2-7 has shown that ORC and Cdc6 undergo further dramatic restructuring to form a helical, right-handed

structure with double-stranded DNA passing through the middle of the complex. The Cdt1-MCM2-7 complex is bound to ORC-Cdc6 via the MCM2-7 carboxy-terminal ATPase domains, with their amino-terminal collar ready to bind another MCM2-7 to form a double hexamer. What is remarkable about the structure of ORC and Cdc6 in this complex is that the right-handed spiral of the ORC-Cdc6 ATPase subunits formed a 34-Å helix with the same pitch as double-stranded DNA. This type of structure resembles the right-handed spiral of replication factor C (RFC) ATPase that has primer-template DNA passing through its center. RFC is a five-subunit ATPase that loads the ring-shaped PCNA (proliferating cell nuclear antigen) DNA polymerase clamp onto the primer-template DNA in preparation for it to activate DNA polymerase at a DNA replication fork. RFC and PCNA were discovered in this laboratory ~26 years ago, and the X-ray crystallographic structure of the RFC-PCNA complex, determined by John Kuriyan, Mike O'Donnell, and colleagues, shows how an ATPase can open a ring-shaped protein to allow it to encircle double-stranded DNA. On the basis of the structure of the ORC-Cdc6 ATPase bound to Cdt1-MCM2-7, it is likely that ORC-Cdc6 uses a similar ring-opening mechanism to load the MCM2-7 ring on DNA.

Biochemical studies using recombinant ORC, Cdc6, Cdt1, and MCM2-7 expressed in either baculovirus or *Escherichia coli* have shown that an assembled pre-RC can be activated in vitro to load Cdc45 and replication protein A (RPA), a single-stranded DNA-binding protein, in the presence of extracts from cells arrested in S phase. These biochemical studies suggest that it will be possible to investigate the mechanism by which DNA synthesis starts at an origin of DNA replication.

Previous studies by this laboratory demonstrated that Orc1, the largest subunit of the human ORC, controls centriole and centrosome duplication in a cyclin E/CDK2-dependent manner during the G_1 phase of the cell division cycle. In the absence of Orc1, centrioles and centrosomes reduplicate in the absence of ongoing DNA synthesis. Purified Orc1 inhibited the kinase activities of both cyclin A/CDK2 and cyclin E/CDK2 enzymes, both of which were required for DNA replication and centriole and centrosome duplication. The ability of Orc1 to block cyclin A/CDK2 activity was abrogated when a mutation in the Orc1 Cy-motif (KRL) was made; however, cyclin E/CDK2 inhibitory activity remained intact.

In the last year, bioinformatic analysis found that Orc1 has a centrosome localization signal sequence that is also found in pericentrin and the AKAP-450 or CGNAP proteins that are known to localize exclusively to centrosomes. The small bipartite sequence, called a PACT domain, localized to the carboxyl terminus of Orc1, was both necessary and sufficient to localize green fluorescent protein (GFP) to the centrosomes, indicating that it is an authentic centrosome localization signal.

Also mapped within the Orc1 protein was the cyclin E/CDK2 and cyclin A/CDK2 inhibitory domain, and Orc1 was found to be localized to the amino terminus of the protein that contained a previously characterized BAH domain that interacts with dimethylated lysine 20 (K20) histone H4 in a nucleosome. By combining the CDK2 inhibitory domain (CID) with the PACT domain and removing most of Orc1 that is required for the initiation of DNA replication, the recombinant protein was able to localize to centrosomes and block hydroxyurea-induced centriole and centrosome reduplication within a single cell division cycle. Both the PACT and the CID domains were required for blocking centriole and centrosome reduplication induced by inhibiting DNA replication with hydroxyurea.

A striking coincidence occurred because other investigators had mapped mutations in a human syndrome called Meier-Gorlin syndrome to genes encoding components of the pre-RC, including ORC subunits, Cdc6, and Cdt1. This syndrome causes microcephalic primordial dwarfism, a condition in which the patients are of very short stature and have proportionally small head size and other abnormalities such as ear and patella developmental defects. For the most part, Meier-Gorlin syndrome patients have near-normal intelligence. Many patients had mutations in one of three different amino acids in the amino terminus of Orc1, and patients with these mutations had the shortest stature and smallest head size of all Meier-Gorlin syndrome patients.

Orc1 Meier-Gorlin syndrome mutations were tested for their ability to block cyclin A/CDK2 and cyclin E/CDK2 kinase activities. Most interesting was the R105Q mutant and, to some extent, the other two mutations, because it failed to inhibit cyclin E/CDK2 kinase activity but retained the ability to block cyclin

A/CDK2 kinase activity. The mutations in Orc1 also failed to block hydroxyurea-induced centriole and centrosome duplication in cells, correlating with the ability to inhibit cyclin E/CDK2 kinase activity.

Because Meier-Gorlin syndrome mutations in Orc1 also localized to the BAH domain, they were tested for the ability to bind to dimethylated K20 histone H4 peptides. All were able to do so within twofold of the wild-type sequence. Thus, although other investigators have suggested that Meier-Gorlin syndrome mutations cause short stature and small head size due to defects in binding histone H4 K20 dimethyl residues, evidence for this conclusion is lacking. In contrast, the new data suggest that the severe short stature of Meier-Gorlin syndrome patients with an Orc1 mutation is a result of defects in both DNA replication and centrosome and centriole duplication. Furthermore, the altered regulation of centriole and centrosome duplication could cause defects during mitosis in proliferating cells or excessive duplication of centrioles that form the basal bodies in cells that form cilia.

The study of Meier-Gorlin syndrome mutations has implications for the regulation of tissue size and shape in humans. These patients are short and have proportionally small head and, hence, brain size, as well as other tissues and organs that are in proportion. These observations suggest that the mechanisms that form tissue size and shape must be coordinated with the ability of the cells to proliferate.

DDX5, a Regulator of Cell Proliferation in Cancer Cells

As part of a project to identify new participants and regulators of the initiation of DNA replication in human cells, an RNA interference (RNAi) screen was used to discover new proteins that either participate in or regulate DNA replication in human cells. These studies resulted in the observation that inhibition of either RPA or ribonucleotide reductase (RNR) in cancer cells provided the most potent inhibition of cell proliferation of all genes tested. Because RNAi has revolutionized loss-of-function genetics by enabling sequence-specific suppression of virtually any gene, and because tetracycline response elements (TREs) can drive expression of short hairpin RNAs (shRNAs) for inducible and reversible target gene suppression, we collaborated with Scott Lowe's group to develop mice that had regulated and reversible inhibition of essential genes using RPA3, the smallest subunit of RPA, as the test gene. The feasibility of transgenic inducible RNAi for suppression of essential genes was demonstrated directly by targeting expression of RPA3 shRNAs to all tissues in mice. Transgenic mice were generated with TRE-driven RPA3 shRNAs, whose expression enforced a reversible cell cycle arrest. In adult mice, the block in cell proliferation caused rapid atrophy of the intestinal epithelium that led to weight loss and lethality within 8–11 days of shRNA induction. Upon shRNA withdrawal, villus atrophy and weight loss were fully reversible. Thus, shRPA3 transgenic mice provided an interesting tool to study tissue maintenance and regeneration. These collaborative studies developed a robust system that serves the purpose of temperature-sensitive alleles in other model organisms, enabling inducible and reversible suppression of essential genes in a mammalian system in vivo.

During the shRNA screen for genes that encode proteins controlling DNA replication, a new role for the DEAD-box RNA helicase enzyme DDX5 in G_1-to-S phase progression was uncovered. DDX5 was found to regulate directly DNA replication factor expression by promoting the recruitment of RNA polymerase II to E2F-regulated gene promoters. In collaboration with Scott Powers, Alex Krasnitz, and other investigators, it was shown that the DDX5 locus was amplified in ~25% of breast cancers and that breast-cancer-derived cells with amplification of DDX5 were much more sensitive to DDX5 depletion than breast cancer cells and a breast epithelial cell line that lacked DDX5 amplification. Indeed, inhibition of DDX5 in cells that lacked DDX5 amplification did not affect cell proliferation. The results demonstrated a new role for DDX5 in cancer cell proliferation and suggest DDX5 as a therapeutic target in breast cancer treatment. To test whether depletion of DDX5 in adult animals might cause a problem if an antagonist of DDX5 activity were to be used for cancer treatment, mice expressing regulated DDX5 shRNAs in all tissues were made. Upon addition of the inducer of the shRNAs that inhibit DDX5 expression, the level of the protein in bone marrow, small intestine, liver, and kidney decreased by up to 90%, and in tissues such as the heart and

lung, DDX5 expression decreased by 50%. Nevertheless, extensive pathology analysis of these mice by collaborator Dr. John Erby Wilkinson at the University of Michigan demonstrated that they lacked any side effects of inhibition of DDX5 in normal tissues.

As part of a survey to determine whether DDX5 inhibition might affect other cancers, it was found that inhibition of DDX5 kills human and mouse leukemia cells—specifically, a very lethal form of acute myeloid leukemia (AML). Unlike breast cancer cells, blocking DDX5 in leukemia cells caused apoptosis. DDX5 inhibition did not affect normal blood cell biology. Interestingly, DDX5 did not control E2F1-regulated genes in these leukemia cells. Using a mouse model for AML developed in Scott Lowe's laboratory and regulated shRNAs that control DDX5 gene expression, it was found that depletion of DDX5 promotes survival of the mice with this lethal form of leukemia. Coupled with the observation that DDX5 inhibition did not cause problems in normal tissues, these studies suggest that drugs that block DDX5 activity may be a safe and effective cancer therapy.

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MECHANISTIC STUDIES OF THE CHROMATIN AND TRANSCRIPTIONAL REGULATORY MACHINERIES IN HEMATOLOGICAL MALIGNANCIES

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

Chromatin, composed of DNA in complex with protein, is the fundamental packaging system for eukaryotic genomes. The basic organizational unit of chromatin is the nucleosome, composed of an octamer of core histones (H2A, H2B, H3, and H4) wrapped by 147 bp of DNA. In addition to protecting the genetic code from genotoxic insult, the structure of chromatin has a profound impact on all DNA-templated processes, including transcription, DNA replication, and recombination. Hence, an elaborate regulatory system has evolved to animate chromatin structure in the wake of such fundamental cellular activities. Two principal categories of chromatin regulators are the enzymes (e.g., acetyltransferases, deacetylases, methyltransferases, and demethylases) and the reader domain-containing proteins (e.g., bromodomains, chromodomains, and PHD domains).

The central long-term goal of research performed in our lab is to explore the mechanistic basis of chromatin regulation and how it relates to the control of gene transcription. We have had a long-standing interest in the relationship between DNA-binding "master-regulatory" transcription factors and the chromatin regulatory machinery, and how this interplay governs cell-fate programs. The biological context in which we study chromatin is in the hematopoietic system, where a hierarchically organized cell lineage program is specified in large part by transcriptional regulators. In addition, studying the hematopoietic system affords a number of powerful biochemical as well as genetic tools for evaluating chromatin and transcription in vivo and ex vivo. An approach taken by our laboratory for studying transcriptional/chromatin regulators has been to compare their action under normal physiologic settings with their role under pathologic conditions, which can deviate demonstrably from the cellular context under which these components evolved. As an example, normal tissues in vivo generally possess robust gene regulatory networks, such that redundancy in gene function can exist under laboratory conditions, which can often obscure studies of molecular mechanisms. In contrast, in aberrant disease states that deviate from normal homeostasis, the nonredundant function of specific regulators can often emerge, thus enabling unique insight into gene function. In addition, studying the action of chromatin regulators in the pathophysiology of disease affords opportunities to translate laboratory observations into biomedical applications, as will be illustrated below. To this end, we have spent the last several years working to investigate the function of chromatin regulators in the setting of hematopoietic cancer—specifically, the disease acute myeloid leukemia (AML). Interestingly, the hematopoietic cell-lineage transcriptional program is often corrupted during leukemogenesis, frequently by direct somatic mutation of transcription factors and chromatin components. The result of this deregulation is an acquired inability of myeloid progenitor cells (a type of white blood cell) to complete terminal differentiation into macrophages and neutrophils, thereby gaining the capability of infinite self-renewal normally only seen in stem cells. We seek to understand how chromatin regulators directly participate in this unique pathophysiology of AML. Research in our laboratory investigates how chromatin regulators are integrated within the oncogenic signal transduction cascades that drive cancer cell growth.

Our principal focus is on acute myeloid and lymphoid leukemias, with an expanding interest in epithelial tumors. To this end, we use genetically engineered mouse models of cancer that recapitulate the cardinal features of the human disease, particularly with respect to therapeutic response. We have had an interest in the mixed-lineage leukemia (MLL) proto-oncogene and its leukemogenic MLL-fusion protein derivatives. MLL is both a "writer" and a "reader" of histone H3 lysine 4 (H3K4) methylation: Its SET domain catalyzes H3K4

methylation and its third PHD domain binds to H3K4 methylation. In leukemia, MLL is mutated via chromosomal translocation to form fusion proteins with corrupted chromatin regulatory functions. We previously identified how MLL uses its unique chromatin-binding activity to perpetuate active chromatin states through mitosis, a function called mitotic bookmarking. We continue to investigate molecular mechanisms used by MLL and MLL-fusion proteins to regulate transcription in normal and transformed cell contexts.

Through a genetic screen, we recently identified the BET bromodomain protein BRD4 as a critical vulnerability in AML. BRD4 is a chromatin reader protein that utilizes its tandem bromodomains to recognize acetylated forms of histone H3 and H4. We found that BRD4 functions as a critical upstream regulator of c-MYC expression, thereby sustaining aberrant self-renewal in leukemia. Remarkably, our work coincided with the development of potent small-molecule inhibitors of BET bromodomains. Using these agents, we pharmacologically validated BRD4 inhibition as a therapeutic strategy in a host of preclinical animal models of leukemia, findings that are now being translated into clinical development. Our lab continues to investigate the BRD4 pathway as a major chromatin-based signaling cascade that sustains c-MYC in hematopoietic malignancies. In addition, our genetic screening approach has revealed a plethora of chromatin-regulator vulnerabilities in many cancer types, fueling our continued efforts to understand and exploit these factors as candidate drug targets in human disease.

Role of Brd4 in Leukemia Maintenance

C. Shen, J. Shi, K. Palacios-Flores

Although prior studies in our lab have validated Brd4 as a novel drug target in leukemia, the mechanistic basis for this observation remains poorly understood. In particular, a major unanswered question in the field is why Myc transcription is hypersensitive to BRD4 inhibition. Our efforts in this area have focused in three major areas. First, we have taken an epigenomic approach toward understanding BRD4 in leukemia cells, which has involved performing genome-wide location analysis of BRD4 occupancy in leukemia chromatin. One remarkable observation from this effort has been the realization that Myc harbors leukemia-specific enhancers that harbor profound levels of BRD4 occupancy. Chromosome conformation capture studies have verified that this enhancer region is looped into close proximity with the *Myc* gene, thereby placing BRD4 at a site of enhancer-promoter contact. Our studies suggest that this enhancer is the genomic platform through which BRD4 communicates with Myc, a function that can be interrupted with drug-like BRD4 inhibitors. A second major area of research has been to characterize BRD4-containing protein complexes to identify critical interacting partners. We have carried out immunoprecipitation mass spectroscopy of BRD4 and identified several enriched partners. One of these is the SET/PWWP domain-containing protein NSD3. NSD3 interacts with the ET domain of BRD4 and is likewise essential for self-renewal of leukemia cells. Ongoing work seeks to understand the mechanistic basis of functional collaboration between BRD4 and NSD3. A third area of investigation in our laboratory is to define a broader network of genetic interactions of BRD4 through small hairpin RNA (shRNA) screening. This has involved identifying synthetic lethal interactions combining shRNA-based gene knockdown in concert with exposure to small-molecule BRD4 inhibitors. The unifying goal of these three efforts is to fully understand the mechanistic basis of BRD4 addiction in leukemia, such that therapeutic efficacy of BRD4 inhibitors can be maximized in cancer patients.

The Chromatin Remodeling BAF Complex as a Mediator of Aberrant Self-Renewal in Leukemia

J. Shi, A. Hohmann

Efforts to understand the role of Brd4 in leukemia has led to the realization that leukemia-specific enhancer elements are potential hot spots of intervention for interfering with aberrant self-renewal programs in leukemia. Hence, we seek to identify enhancer-binding proteins that enable aberrant self-renewal in leukemia. One such factor is the ATPase/bromodomain-containing protein BRG1, which we identified through shRNA screens in search of leukemia-specific epigenetic vulnerabilities. We have found that BRG1 is a key factor that acts to sustain Myc expression in leukemia. Suppression of BRG1 leads to severe antileukemia effects in vitro and in vivo with minimal proliferation

effects in heterologous cell types. AML cells deficient in BRG1 undergo terminal myeloid differentiation as a consequence of failing to maintain Myc transcription. Notably, forced expression of Myc can rescue the majority of phenotypes incurred upon BRG1 inhibition. Similar phenotypes are also observed upon suppression of several subunits of the BRG1-containing BAF complex. Furthermore, we detect a physical interaction between the BAF complex and the ET domain of BRD4, suggesting that these factors might be functional partners on chromatin. Consistent with this notion, we find that BRG1 and BRD4 colocalize on chromatin on a genome-wide scale, as revealed by chromatin immunoprecipitation-sequencing (ChIP-Seq). This includes novel distal enhancer elements downstream from Myc, genomic regions that might impart a leukemia-specific reliance on both factors for Myc expression. Importantly, BRG1 and BRD4 stabilize occupancy of one another at Myc enhancer regions and enable long-range enhancer-promoter looping interactions. Together, these findings implicate collaboration between BRD4 and the BRG1-associated BAF complex as a critical regulatory interaction that sustains Myc transcription in the context of leukemogenesis, regulatory functions that can be targeted by small-molecule bromodomain inhibitors.

Dissecting the Interplay between Lysine Acetyltransferases and Bromodomain-Containing Proteins

J. Roe

Bromodomains are an emerging class of candidate drug targets in oncology. Hence, a major effort in our lab is to pinpoint the bromodomain vulnerabilities in all cancers. However, since bromodomains engage in acetyl-lysine recognition, a major unanswered question is regarding the nature of enzymatic lysine acetylation in cancer cells and how this might program bromodomain functions. As ~20 different lysine acetyltransferases (KATs) are known to exist in humans, we seek to explore whether specific KATs are functionally tied to specific bromodomain proteins. Moreover, because KATs utilize acetyl-CoA as a metabolic cofactor, we ultimately seek to determine whether histone acetylation is connected in some fashion to the cancer-promoting metabolic program. Through genetic screens, we have pinpointed four KATs involved in leukemia pathogenesis. Ongoing genetic and biochemical studies aim to explore the biological functions of KATs in leukemia and the pathways they regulate.

Investigation of Transcription Factor-Coactivator Interactions

A. Bhagwat, F. Mercan

Leukemia-specific enhancer elements are likely to be organized by sequence-specific transcription factors (TFs) that establish long-range enhancer to promoter communication. We have recently initiated projects in the lab to identify novel TFs that support leukemogenesis. This is occurring through both unbiased genetic screens of known DNA-binding proteins and bioinformatic analysis of enhancer motifs that are actively engaged in leukemia cells. On the basis of the findings described above, we hypothesize that targeting of chromatin regulators such as BRD4 is essentially a means of disrupting enhancer function. To complement our efforts on chromatin regulators, we are now investigating additional coactivator complexes (Mediator, TAFs) and whether they also participate in transcriptional regulatory circuits that support leukemia proliferation. The major goal of these studies is to pinpoint specific TF–coactivator interactions that are unique to leukemia maintenance.

Identifying Epigenetic Vulnerabilities in Therapy-Resistant Acute Lymphoblastic Leukemia

S. Kawaoka

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

studies evaluating chromatin occupancy of TRIM in B-ALL and are expanding these studies into other cell lineages. From this work, we hope to gain a general model for how TRIM governs leukemia-specific transcriptional regulation.

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

E. Wang

MLL-fusions are potent oncogenes that initiate aggressive forms of acute leukemia. As aberrant transcriptional regulators, MLL-fusion proteins alter gene expression in hematopoietic cells through interactions with the histone H3 lysine 79 (H3K79) methyltransferase DOT1L. Notably, interference with MLL-fusion cofactors such as DOT1L is an emerging therapeutic strategy in this disease. We have identified the histone H2B E3 ubiquitin ligase RNF20 as an additional chromatin regulator that is necessary for MLL-fusion-mediated leukemogenesis. Suppressing the expression of Rnf20 in diverse models of *MLL*-rearranged leukemia leads to inhibition of cell proliferation, under tissue culture conditions as well as in vivo. Rnf20 knockdown leads to reduced expression of MLL-fusion target genes, including *Hoxa9* and *Meis1*—effects resembling Dot1l inhibition. Using ChIP-Seq, we found that H2B ubiquitination (H2Bub) is enriched in the body of MLL-fusion target genes, correlating with sites of H3K79 methylation and transcription elongation. Furthermore, Rnf20 is required to maintain local levels of H3K79 methylation by Dot1l at *Hoxa9* and *Meis1*. These findings support a model whereby cotranscriptional recruitment of Rnf20 at MLL-fusion target genes leads to amplification of Dot1l-mediated H3K79 methylation, thereby rendering leukemia cells dependent on Rnf20 to maintain their oncogenic transcriptional program.

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CANCER: GENETICS

Gregory Hannon is a pioneer in the study of RNA interference (RNAi), a process in which double-stranded RNA molecules induce gene silencing. Hannon and colleagues have elucidated key elements of the RNAi machinery. This past year, both the structure of human Argonaute 2 bound to a microRNA (miRNA) guide and the structure and function in fruit flies of the key PIWI-interacting RNA (piRNA) nuclease Zucchini were solved in collaboration with the Joshua-Tor lab. In addition, the Hannon lab has led the way in using RNAi to study cancer biology and genetics, generating libraries of short-hairpin RNAs that have been widely applied in gene silencing studies. During the past several years, the Hannon lab has focused on roles of small RNAs in germ cells, which tend to have the most elaborate set of small RNA pathways of any cell type. They have discovered an essential role for pseudogenes in producing small RNAs that are critical for proper oocyte development and an elegant small RNA-based immune system that guards the genome against transposable elements. The Hannon lab also strives to understand the biology of cancer cells, with a focus on breast and pancreatic cancer. They are exploring the roles of small RNAs as oncogenes and tumor suppressors and in exploiting the RNAi libraries they have developed to identify new therapeutic targets for specific disease subtypes. Additionally, they are taking genetic approaches to understand the biology of resistance to currently used targeted therapies. Another research thrust of Hannon's team exploits the power of next-generation sequencing to understand the biology of the mammalian genome. Their efforts range from the identification of new classes of small RNAs to understanding human evolution and diversity. In the last year, they have placed a major emphasis on the evolution of the epigenome and its role in driving cell-fate specification.

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

Alea Mills is studying genetic pathways important in cancer, aging, and autism, identifying the genetic players and determining how aberrations in their functions culminate in human disease. Through innovative use of a technique called "chromosome engineering," the Mills group discovered that one of the most common genetic alterations in autism—deletion of a 27-gene cluster on chromosome 16—causes autism-like features in mice. This year, they showed that autism-like movement impairments can be identified just days after birth, suggesting that these features could be used to diagnose autism. Previously, Mills used chromosome engineering to identify a tumor suppressor gene that had eluded investigators for three decades. The gene, called *Chd5,* was shown by Mills to regulate an extensive cancer-preventing network. The epigenetic role of *Chd5* in development, cancer, and stem-cell maintenance is currently being investigated. The Mills lab is also studying p63 proteins, which regulate development, tumorigenesis, cellular senescence, and aging in vivo. They succeeded in halting the growth of malignant tumors by turning on production of one of the proteins encoded by the *p63* gene, called TAp63. They recently discovered that a different version of *p63*, called ΔNp63, reprograms stem cells of the skin to cause carcinoma development—the most prevalent form of human cancer. Modulation of these proteins may offer new ways to treat human malignancies in the future.

Scott Powers' work focuses on gene mutations that cause cancer and factors that influence responses to specific anticancer drugs. His lab uses technologies that probe the entire genome to identify candidate cancer genes and evaluate their functional role in cell transformation and tumor biology. They also use whole-genome technologies to guide development of novel cancer diagnostics and therapeutics. Using DNA copy-number analysis, the Powers group pinpoints novel amplified oncogenes and then applies functional studies to address the validity of candidate genes and the mechanisms by which they are implicated in oncogenesis. They have successfully applied this approach in liver, colon, and lung cancer. This past year, Powers and colleagues identified a strategy for targeted molecular therapy in liver cancer based on tumor dependency on amplification of the *FGF19* oncogene. Powers has also had an important role in the development of a distinctive CSHL approach to functional study of cancer genes. Called integrative oncogenomics, it is a rapid, large-scale screen for genes that are deleted or amplified in human cancers and suspected of being tumor suppressors, in the case of deletions, or oncogenes, in the case of amplifications.

Michael Wigler, with James Hicks and Alex Krasnitz among others, studies human cancer and genetic disorders from a population genomics perspective. The cancer effort focuses on breast and prostate cancer (the latter jointly with Lloyd Trotman) and involves collaborative clinical studies (with local, national, and international collaborators) to discover mutational patterns predicting treatment response and outcome. The lab also develops methodology for single-cell genomic and RNA analysis, to detect cancer cells in bodily fluids such as blood and urine. This last has major potential applications to the early detection of cancer and monitoring its recurrence and response to therapy during and after treatment. Single-cell analysis has also led to insights about the clonal evolution and heterogeneity of cancers and may lead to a better understanding of initiation, progression, and metastasis. In collaboration with Scott Lowe, now at Memorial Sloan-Kettering Cancer Center, they recently showed that tumor suppressor genes are often clustered in large regions that are deleted in common cancers. The single-cell methods are also being applied to problems in neurobiology (with Josh Huang and Pavel Osten) and differentiation (with Chris Vakoc). The Wigler lab's genetic efforts are a collaboration with Ivan Iossifov and Dan Levy of Quantitative Biology at CSHL, and they are largely focused on determining the role of de novo mutation in pediatric disorders. They participate in a large study of autism organized by the Simons Foundation and also study congenital heart disease and pediatric cancers with collaborators at Columbia University and Memorial Sloan-Kettering Cancer Center, respectively. In collaboration with Alea Mills, they helped create a mouse model for one of the most common genetic abnormalities contributing to autism. Recent work has confirmed and extended the team's previous observations on the role of de novo copy-number variation in autism, with similar results in the other disorders. In a large-scale exome sequencing project with W. Richard McCombie and the Genome Sequencing Center at Washington University in St. Louis, the team has proven the contribution of small-scale de novo mutations to autism. Overall, results confirm their previous genetic models for autism incidence. This study also uncovered a striking link between the gene that causes fragile-X syndrome, *FMR1*, and those regulated by it that are implicated in autism.

RNA INTERFERENCE MECHANISMS AND APPLICATIONS

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Our lab continues to focus on three distinct areas. First, we study RNA biology, with a focus on noncoding RNAs. In particular, we are interested in a conserved pathway that protects animal germ cell genomes against parasitic genetic elements. Second, we study the roles of noncoding RNAs in cancer, mainly breast cancer, and use small RNAs as tools to uncover tumor-specific vulnerabilities as potential therapeutic targets. Third, we develop technologies, mainly in the areas of mammalian genetics and genomics. Historically, we have had a particular focus on using next-generation sequencing to study epigenetic regulation, to identify disease-associated genes, and to help in the establishment of new model organisms.

We said goodbye to a number of labmates this year. Ted Karginov accepted a position as an Assistant Professor at the University of California, Riverside. Several master's and undergraduate students spent time in the group and then moved on to other positions. These include Rebecca Behrens, who joined Wolf Reik's lab as a Ph.D. student; our URPs, Sarah Andersen, Abigail Samuels, and Francesca Aloisio, who returned to complete their undergraduate degrees; and Sai Chen, who joined the University of Michigan Ph.D. program. Ben Czech received his Ph.D. from Tubingen and chose to return to the lab as a postdoctoral fellow. Nicholas Erand, who spent time with us during his master's, will also return as a Ph.D. student. Giorgia Battistoni, who also spent part of her thesis here, will return as a WSBS student in the fall. Several technicians also left the lab, including Michael Baer for another position and Youngjoo Yang and Christine Peterson to join Ph.D. programs. Joaquina Delas Vives joined the lab as a WSBS student. Poppy Gould came to work for a time during her undergraduate thesis and will leave us this fall to also join Wolf Reik's lab in Cambridge. Ashley Maceli, a former URP, returned as a technician. A selected set of laboratory projects are described in detail below.

The Structural Biochemistry of Zucchini Implicates It as a Nuclease in piRNA Biogenesis

A.D. Haase, S.R. Knott [in collaboration with J. Ipsaro and L. Joshua-Tor, Cold Spring Harbor Laboratory]

PIWI-family proteins and their associated small RNAs (piRNAs) act in an evolutionarily conserved innate immune mechanism that provides an essential protection for germ cell genomes against the activity of mobile genetic elements. piRNA populations constitute a molecular definition of transposons that permits them to be distinguished from host genes and selectively silenced. piRNAs can be generated in two distinct ways. Primary piRNAs emanate from discrete genomic loci, termed piRNA clusters, and appear to be derived from long, single-stranded precursors. The biogenesis of primary piRNAs involves at least two nucleolytic steps. An unknown enzyme cleaves piRNA cluster transcripts to generate monophosphorylated piRNA 5′ ends. piRNA 3′ ends are likely formed by exonucleolytic trimming, after a piRNA precursor is loaded into its PIWI partner. Secondary piRNAs arise during the adaptive ping-pong cycle, with their 5′ termini being formed by the activity of PIWIs themselves. A number of proteins have been implicated genetically in primary piRNA biogenesis. One of these, Zucchini, is a member of the phospholipase D family of phosphodiesterases, which includes both phospholipases and nucleases. We have produced a dimeric, soluble fragment of the mouse Zucchini homolog (mZuc/PLD6) and have shown that it possesses single-strand-specific nuclease activity. A crystal structure of mZuc

at 1.75-Å resolution indicates greater architectural similarity to PLD family nucleases than to phospholipases. Considered together, our data suggest that the Zucchini proteins act in primary piRNA biogenesis as nucleases, perhaps generating the 5′ ends of primary piRNAs.

A Genome-Wide RNAi Screen Draws a Genetic Framework for Transposon Control and Primary piRNA Biogenesis in *Drosophila*

P.M. Guzzardo, F. Muerdter

piRNAs, together with Piwi-clade Argonaute proteins, constitute an evolutionary conserved, germline-specific small RNA silencing system. The piRNA pathway is implicated in gene silencing, particularly of repetitive elements, as well as germline differentiation, and maintenance of germline stem cells. Accordingly, mutations in Piwi almost universally lead to sterility and germ cell loss. In *Drosophila melanogaster*, primary piRNA biogenesis involves the processing of a primary transcript into mature small RNAs by factors that remain elusive. To search for proteins involved in biogenesis or downstream effector pathways, we performed a comprehensive genome-wide RNAi screen of more than 40,000 double-stranded RNAs (dsRNAs) in a cell line derived from *Drosophila* ovaries. We identified and validated 87 genes required for transposon silencing. We revealed novel piRNA biogenesis factors and placed previously unknown proteins at the effector step of the pathway. In addition, by validating a large subset of the candidate hits in vivo, we demonstrated the strength and relevance of the primary data set. This study provides not only an important resource for the scientific community, but also a solid foundation on which future research can be built.

Structural Variations of the Genome in Breast Cancer

A. Canela

Structural variations including copy-number polymorphisms (CNV), as well as insertions, inversions, and translocations, have an important role in tumor development. The mutagenic effects of translocations have long been seen as critical to the development of hematological malignancies, where the fusion genes, generated when the translocation joins two otherwise separated genes, are both drivers and indicators of tumor phenotype and clinical outcome. Recently, technological advances have allowed genome-scale analysis of rearrangements also in several epithelial cancers, and an increasing number of fusion genes have been reported. We study structural variation in breast cancer cells at a single-base resolution level by next-generation sequencing. As a first approach, we focused in regions previously characterized by comparative genomic hybridization (CGH) containing copy-number variation as landmarks for identifying complex chromosomal rearrangements. We purified these regions in breast cancer cell lines by array-based genomic capture and deciphered them by sequencing the precise structure of genomic rearrangements, which led to detection of copy-number loss and gain in the CGH (in collaboration with the Wigler and Krasnitz laboratories at CSHL). Because not all the areas of CNV may contain complex chromosomal rearrangements, we used an unbiased approach featuring large-fragment genomic libraries as "jumping libraries" to scan by sequencing the whole genome for structural variation events. Combining large-fragment genomic libraries with sequencing techniques at the transcriptome level (RNA-Seq), used to discover novel transcribed gene fusions, we have identified 72 fusion gene candidates in a panel of Luminal A breast cancer cell lines. To assess the relevance of these fusion genes as driver mutations in breast cancer, we are evaluating their presence and frequency in clinical samples in collaboration with Winthrop-University Hospital. In parallel, we are investigating whether these rearranged genes contribute to tumorigenesis through a functional approach using RNA interference (RNAi). RNAi screens will identify genes acting either as oncogenes or tumor suppressors in the chromosomal rearrangements using the panel of Luminal A breast cancer cell lines and normal mammary cells. These approaches will reveal the importance of structural variation in breast cancer and will guide the design of diagnostic and therapeutic tools.

Investigating the Evolution and Inheritance of Epigenetic Reprogramming in Mammals

A. Molaro, E. Hodges, I. Falciatori, K. Marran [in collaboration with A. Smith, University of Southern California]

Germ cells (sperm and oocytes in mammals) lie at the core of heredity. Each generation they transmit

genetic and epigenetic (e.g., DNA methylation) information necessary to ignite a new cycle of organismal growth and development. Hence, the germ cell genome and epigenome are under constant monitoring during their maturation.

We have established the single CpG dinucleotide reference methylomes of developing germ cells. In the context of this study, we have characterized the dynamics of de novo methylation establishment, especially over retrotransposable elements, which are dependent on this modification for their silencing. We have found that following a complete erasure of all epigenetic marks, a first wave of default de novo methylation precedes an active wave mediated by piRNAs (A. Molaro et al., in prep.). However, thousands of repeat copies escape both of these events and are transmitted in a hypomethylated state to the next generation. Interestingly, when conserved, a subset of these copies constitutes species-specific epialleles (e.g., SVA elements between humans and chimpanzees (Molaro et al., *Cell 146: 1029* [2011]). These repeat copies have divergent regulatory sequences and display lower expression levels during germ cell maturation when compared to their piRNA-targeted counterpart. To gain a better understanding of the evolutionary events that have led to these features, we extended our previous analysis of human and chimpanzee sperm to gorilla and bonobo. Two to three times average independent CpG coverage methylome libraries were generated and assembled over the recently published reference genomes of these two primates. We are currently comparing these four ape sperm methylomes to ascertain whether changes in regulatory sequences are preceding or following differential epigenetic reprogramming. We will also provide an unprecedented insight into the ongoing mutual canalization between the genome and epigenome during speciation. In addition, using mouse models, we have extended our genome-wide epigenetic road maps to other chromatin features. In particular, using newly described surface markers, we isolated hundreds of thousands of primordial germ cells and subjected them to nucleosome footprinting. This will help elucidate understanding the sequence of changes in chromatin structure leading to retrotransposon reactivation and silencing during germ cell maturation. It will also emphasize the connection between nucleosome positioning and de novo DNA methylation over regions that are naturally protected from reprogramming (e.g., gene promoters).

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

B. Czech, J. Preall

The *Drosophila* genome is scarred with the ravages of an ancestral battle being waged in its germline against selfish genetic elements. Transposable elements mobilize in the early stages of gametogenesis, and if left unchecked would invade the genome and cause widespread disruption of essential genes, leading to collapse of the germline and sterility. The piRNA pathway provides a defense against selfish genetic elements by mounting a sequence-specific, RNAi–like nucleolytic surveillance mechanism. Several key factors of this pathway have been identified, but accumulating evidence has suggested that the piRNA pathway is more complex than was first appreciated, with possible ties to chromatin structure, transcriptional regulation, and RNA localization. We have continued our in vivo reverse genetic screen of unprecedented scale in order to fully describe the set of genes that participate in piRNA-mediated genomic defense. Approximately 8200 genes, spanning the entire ovarian transcriptome, were targeted by RNAi constructs driven by a germline-specific promoter. Our screen has uncovered 74 genes that heavily impact transposon expression. Of those, we find 16 known pathway components and 58 novel genes, including cohorts of genes belonging to shared processes such as epigenetic regulation and RNA metabolism and previously not linked to transposon silencing. We have characterized in detail two genes, *GASZ* and *Del*, and identified both as new piRNA biogenesis factors. Ongoing analysis will serve to validate further candidates and improve our understanding of their impact on the piRNA pathway.

The Epigenetics of Breast Cancer Risk

C.O. dos Santos

During the past 3 years, we have improved the available tools for the study of mouse mammary gland stem cells (MaSCs) and revealed important alterations to DNA methylation levels after pregnancy. Through our studies, we identified a new MaSC cell surface marker, CD1d, which expands our ability to isolate these cells nearly 10-fold over the previous strategy.

The CD1d surface marker preferentially enriched for cells with mammary reconstitution potential enabled the investigation of gene expression fluctuation in between mammary stem cells and progenitor cells. The elucidation of this new strategy to isolate MaSCs allowed the generation of a mammary gland reference methylome, which includes DNA methylation profiles at single-nucleotide resolution, of six distinct mouse mammary gland cell types. The comparison of cells harvested from virgin glands to those exposed to at least two cycles of pregnancy suggested the post-pregnancy inheritance of hypomethylated regions nearby areas predictably occupied by a few transcription factors. We are currently investigating the mechanisms by which these modifications to DNA methylation level contribute to the well-established protection from breast cancer that is afforded by early pregnancy. Our studies may reveal additional information that could aid the development of screening methods for breast cancer risk.

How Breast Tumors Tame the Immune System

C. Rebbeck

We have been investigating the influence and response of macrophages within a mouse breast tumor (tumor-associated macrophages [TAMs]). This past year has seen progress, identifying strongly differentially expressed microRNAs (miRNAs) between tumor and normal macrophages. With these data, I won an award to investigate the expression of these miRNAs using in situ hybridization, looking at their particular location within a tumor and how this changes as the tumor grows in size. I have been working on this technique and dual labeling of both miRNA probes and standard immunofluorescence (IF) for surface expression markers. In addition, RNA transcriptome data have provided candidate genes, which I am currently knocking down in situ to monitor changes within the macrophage, and their response to 4T1 tumor cells. In collaboration with a fellow lab member, we won an award to sequence immune cells within a mouse mammary gland and to look for changes that have occurred due to pregnancy, which may provide protection against tumor development.

LaserTAG: Adding Spatial and Temporal Resolution to the Study of Biological Processes

D. Bressan

During the past year, my research mostly focused on the development of a new technique, named "LaserTAG," for the specific tagging and purification of proteins and their binding partners (especially nucleic acids) from complex tissue at cellular or subcellular resolution. The technique is based on the development (in collaboration with Dr. Graham Ellis-Davies at Mount Sinai School of Medicine) of a photoactivable tag (LaserTAG) that can specifically cross-link, upon laser activation, with epitope-modified proteins in vivo. Using a confocal or two-photon microscope (instruments able to aim lasers with incredible precision), it is possible to "select" a specific area of a cell, or tissue, and catalyze the cross-linking of the tag to the target protein only in that area without the need of fixing, treating, and sectioning the sample. The target protein from the selected area can be subsequently specifically purified. I am planning to use this technique to purify mRNAs bound to the ribosome in brain slices. I am particularly interested in studying RNA

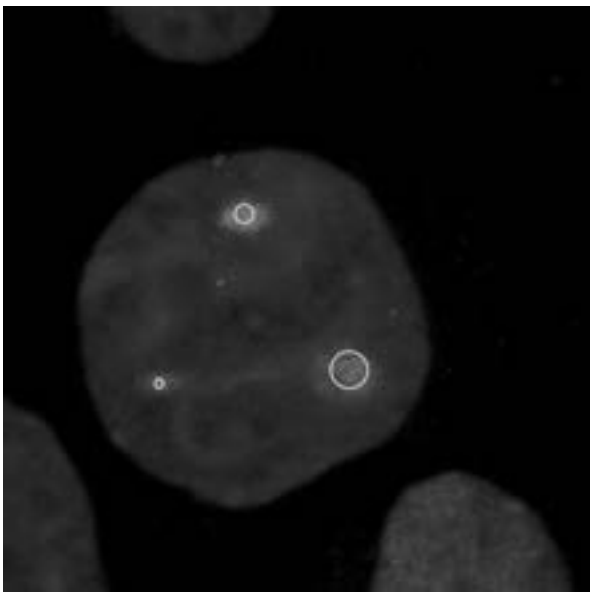

Figure 1. LaserTAG is used to "select" three small circular domains (bright areas) within a cell nucleus (light gray).

translation in the dendrites of neurons, because this "local translation" has been implicated in learning and memory.

Genomic and Epigenetic Changes Occurring during Carcinogenesis: A Fly Perspective

D. Fagegaltier

How cells become transformed during carcinogenesis, which pathways are involved in reaching a transformed state, and whether each cell takes the same route to reach such a state are questions not fully understood. Using the *Drosophila* model, we are studying the basic mechanisms by which genomes coordinate their genetic and epigenetic responses toward a transformed state activated by specific oncogenes or tumor suppressors.

We have completed our first aim of this STARR consortium project: 40 cell lines with cancer-relevant genotypes have been generated that express an oncogene or are depleted for a tumor suppressor. Such cell lines derive from *Drosophila* embryos expressing in specific cell types the oncogene-activated Ras or depleted for tumor suppressors (PTEN, warts), using a number of different Gal4 drivers (ubiquitous, actin5C-Gal4; blood cells, serpent-Gal4; muscle, D-mef2-Gal4). We determined the time to confluence, the ploidy of the cell lines, the ability of the cell line to establish tumor in vivo, and the phenotype of the cells (fibroblast-like, mesodermal-like, and neuronal-like) as well as their adherent properties. The efficiency of the establishment of cell lines measured by recording cellular heterogeneity, passage time, and transplantation in vivo has been recorded to evaluate the potency of various oncogenes and loss of tumor suppressor genes to drive cells toward transformation. Different drivers gave different efficiency: Although D-mef2-Gal4 has proved inefficient, generating cell lines using serpent-Gal4 was effective at producing cell lines that resemble blood cells, and actin5C-Gal4 was very effective. The potency to generate cell lines further depends on the nature of oncogene or tumor suppressor: Activated Ras (RasV12) giving rise to diploid cells, the tumor suppressor background PTEN, and the combination of RasV12;wts-RNAi that leads to epithelial cells represent the most effective backgrounds at generating tumorigenic cell lines within a few months. Our second aim is to assess the transcriptional and epigenetic changes that occur during the establishment of cancerous cell lines and to characterize the transcriptome of the transformed cell lines. We have completed the latter: We generated RNA-Seq libraries and sequenced using next-generation sequencing the transcriptomes of a representative set of 14 endpoint cell lines covering five isolates of RasV12 cell lines, three isolates of cells depleted for PTEN, two isolates of PTEN −/− ;RasV12 cell lines, one RasV12;warts RNAi cell line, and three RasV12;Pc −/− cell lines. Our downstream analyses reveal that cell lines expressing the same oncogene or depleted of the same TS show a very similar fate, demonstrating the robustness of such technique in establishing transformed cell lines. The transcriptional programs of primary cancerous cells correlate best between cell lines expressing the same oncogene, or lacking the same tumor suppressor, compared to other cancer-relevant cell lines in this data set. This is exemplified by the dominant epithelial nature of Ras-expressing cell lines. Cultures are not necessarily pure, however, as cells of different type can survive (namely, muscle, lymph, blood, or neuronal cells). We have identified for each primary cell line, markers and tissue-specific transcription factors relevant to each of the one to five cell types they retain. We are currently validating these markers in vivo.

Tissue formation during development requires that constant yet sequential transcriptional changes occur that define cell fate. Signaling pathways are crucial to orchestrate such programs. As primary cell lines become tumorous, they also undergo transcriptional reprogramming and cell-fate decisions. We have identified for each cell line the active and inactive sets of ligands and receptors, transcription factors, and noncoding RNAs (ncRNAs) whose expression is specific or common to individual or sets of cell lines. Using next-generation sequencing, we are dissecting further the transcriptional changes (alterations in small and long noncoding RNAs, and mRNAs) and alterations in signaling pathways as they occur during the establishment of the cell lines. We have generated libraries and sequenced the time courses of three different RasV12 cell lines at 9–12 different time points, complementing our pilot microarray experiment on time courses on two Ras-expressing cell lines. The computational analyses are currently ongoing. The comprehensive collection of transcriptomes generated above will serve as a base to formulate hypotheses as to how

deficient or stimulated signaling pathways appear and how transcription factors, ncRNAs and other pathways have a role in cell fate during transformation that can be tested experimentally in vivo. In particular, we will perform functional tests to discriminate driver from passenger alterations. We will determine whether the cells have become "addicted" to the initiating oncogene or loss of tumor suppressor genes and which other pathways are required for the cells to proliferate and maintain a transformed state. We will test the role of candidate genes, with a focus on chromatin-remodeling genes, in tumorigenesis. Finally, our transcriptomic studies took another exciting turn as they revealed that several cell lines express components of the piRNA processing machinery, including piwi and aubergine—whose functions in guarding the genome against the invasion of repeated selfish elements are the subject of extensive studies in the germline. We have validated this finding by immunofluorescence: piwi protein is found in the nucleus, its natural location in somatic follicle cells, whereas aubergine aggregates at the nuclear periphery as well as unusually large cytoplasmic foci. To better characterize the role of such proteins outside the germline, we are currently cloning the small RNAs they sequester. Assessing the function of PIWI proteins in the context of cancerous cell lines prone to genomic rearrangements promises to uncover novel functions of the piRNA machinery. These studies hence not only serve as a rich source of information, but also will represent invaluable community resources to study cancer, cell fate, and small RNA functions.

Decoding ENCODE: An Integrated Analysis of Epigenetic States Reveals Functional Elements in the Genome

E. Hodges [in collaboration with F. Schlessinger and T. Gingeras, Cold Spring Harbor Laboratory]

Our efforts during the last year have focused on the in-depth analysis of genome-wide DNA methylation data produced from primary human blood cells. We have been specifically interested in characterizing intergenic, cell-type-specific hypomethylated domains that we hypothesize to be novel *cis*-regulatory elements. To accomplish this, we have integrated publicly available sequencing resources from ENCODE with our own data sets to identify active enhancers. Our approach allows us to classify these noncoding domains into functional subsets based on their chromatin signatures. This work has resulted in several new and interesting observations, including the identification of a subclass of noncoding enhancer RNAs associated with lymphocyte enhancer activity.

Molecular Determinants of Breast Cancer Metastasis

E. Wagenblast

Breast cancer is one of the most common malignant diseases in women, in which the metastatic disease is the main cause of death. Metastatic disease progression requires the acquisition of altered gene expression. However, current cancer therapies only target the hyperproliferative nature of the tumor and neglect to inhibit metastasis formation. Therefore, the aim of this study was to identify and validate metastatic regulators using cell lines and mouse models. For this, we isolated primary, circulating, and metastatic tumor cells of mice that were transplanted with murine cell lines, which differ in their metastatic potential. After FACS (fluorescence-activated cell sorting) of these subpopulations, we conducted in-depth transcriptome analysis using RNA-Seq. This has allowed us to generate gene signatures between the primary tumor and circulating/metastatic cells within each cell line. Taking a systematic approach, we interrogated the metastatic potential of our top differentially expressed genes using RNAi. Using in vitro invasion assays and in vivo metastasis mouse models, we screened our candidate genes individually and in a pooled fashion, respectively. Current efforts are addressing the biological mechanisms of our candidate genes.

Patterns of Epigenetic Change during Tumorigenesis in Mice and Humans

F. Rollins [in collaboration with A. Smith, University of Southern California; R. Sordella, Cold Spring Harbor Laboratory]

In the past year, I have been working on understanding the patterns of DNA methylation in non-small-cell lung cancer. We have been using cancer cell lines, and through the combination of bisulfite conversion and high-throughput, whole-genome sequencing, we

are unraveling the epigenetic patterns of cancer cells. We have identified a set of characteristics that not only are specific to cancer cells, but could also underlay some of the most basic phenotypes of cancer cells, including increased rates of mutation and genomic instability. These insights give further understanding to how cancer cells are different from normal cells and will provide a foundation for future cancer research.

Role of an X-linked miRNA Cluster in Mouse Spermatogenesis

I. Falciatori

We identified an X-linked cluster of miRNAs highly and specifically expressed in the male germline. We established that this cluster is specifically expressed in testis in several stages of development, but it is not detectable in other organs. We therefore became interested in determining the function of this miRNA cluster during spermatogenesis. Toward this end, we decided to ablate this cluster at different stages during spermatogenesis in mice and analyze their phenotype. We prepared a modified bacterial artificial chromosome (BAC) in which this miR cluster is flanked by LoxP sites, and we are in the process of generating the targeted embryonic stem cells (ESCs). Meanwhile, we produced transgenic Mili-Cre or Miwi-Cre mice in which the Cre recombinase is expressed in germ cells starting at embryonic stages or only after meiosis, respectively. We have also purchased Stra8-Cre mice in which Cre is expressed postnatally in the differentiating spermatogonia. By crossing the different Cre-deleter strains with the miR-floxed mice, we will generate mice in which this cluster could be selectively ablated at different stages of spermatogenesis. We will then assess their phenotype to infer the function of the cluster. Because miRNAs exert their function by down-regulating their target genes, we have produced a modified BAC in which we added a TRE promoter to drive the expression of the cluster in embryonic stem cells under the control of tetracycline. We produced transgenic ESC lines carrying this BAC. We are currently screening the clones that have the highest difference in miR expression in the presence or absence of tetracycline. We will then compare their mRNA profiles (by RNA-Seq) in the presence or absence of tetracycline to discover potential miRNA targets. These targets will be then validated in spermatogonial stem cells (SSCs).

RNAi as a Path to Target Discovery for Breast Cancer Treatment

K. Chang, S. Knott

Genome-Scale Screening Approach. Breast cancer is a heterogeneous disease that can be clinically categorized into three therapeutic subgroups: estrogen receptor (ER)-positive, HER2-amplified, and triple-negative (basal-like). The ER-positive (most diverse and numerous) and the Her2-amplified subgroups account for 80%–85% of all human breast cancers, whereas patients diagnosed with the triple-negative subtype have the most aggressive form of the disease and often have poor prognosis. Our goal is to apply genome-wide, loss-of-function RNAi screens in cell culture to uncover vulnerabilities of breast cancer cells in all subtypes and discover genes and pathways that modify responses to targeted therapies (Lapatinib, Trastuzumab, Tamoxifen, and Estrogen-deprivation) for de novo and acquired resistance. Our group has been using both established and novel preclinical models from collaborators (including Dennis Slamon, University of California, Los Angeles, and Rachel Schiff, Baylor College of Medicine) to conduct RNAi screens in order to identify novel therapeutic targets/drugs that can be translated to the clinic. This study is funded by the Stand Up to Cancer (SU2C) foundation. We have completed 32 genome-wide screens representing all three therapeutic treatment classes through a collaborative effort with Stephen Elledge's Lab (Harvard Medical School). To date, half of the RNAi screens have been deconvoluted (deep sequencing or microarray analysis), and sequencing of the remaining screens is ongoing. Candidate validations will be performed in the coming year.

Focused RNAi Screens Using an In Vivo Approach. Three-dimensional culture systems mimicking the spherical organization of breast epithelial cells recapitulate much of the morphogenetic programs of mammary development and thus are useful tools to address how breast cancer cells survive in a more physiological context than in two-dimensional cell culture. To further understand the determinants of tumor survival, we aim to study the behavior of breast cancer cells in a more natural environment than

three-dimensional in vitro systems, and to this end, we are developing RNAi screening in vivo using cells derived from human primary tumors engrafted into immunocompromised mice. We have acquired patient tumor-derived xenografts (PDXs) of different breast cancer subtypes from Alana Welm (University of Utah) and are expanding these PDXs in vivo to obtain sufficient quantities of tumor cells for in vivo screening. One of the challenges of regulated short hairpin RNA (shRNA) expression in vivo is the lack of uninduced (T0 or OFF state) or control cells within each tumor upon transgene induction, necessitating comparison of ON and OFF states between different tumors. To overcome this deficiency, we have designed regulatable shRNA expression vectors that enable the creation of equal populations of shRNA-ON and shRNA-OFF cells within the same tumor. We are currently validating these vectors for background expression and level of shRNA inducibility. Our goal is to enable RNAi screening in vivo (orthotopic or subcutaneously) on PDX tumors for all subtypes of breast cancer.

Fourth-Generation shRNA Library Construction. We have developed a computational algorithm (trained on the most comprehensive shRNA sequence/potency database available) that is capable of predicting, for any RNA target, potent shRNAs with high confidence. We have combined this tool with additional algorithms to reduce off-target effects and to ensure that the maximal number of each gene's transcripts are targeted by the predicted shRNA molecules. With these tools, we have predicted a novel shRNA library for each of the human, mouse, and rat genomes. These libraries have been licensed to a vendor and are now available to the scientific community.

Macrostomum lignano: Building a Molecular Toolkit for a New Model Organism

K. Wasik

Macrostomum lignano is a cross-fertilizing hermaphrodite flatworm with impressive regenerative potential. It can regenerate every part of its body (also gonads) except the nervous system. The stem cells that are responsible for this regenerative potential are called neoblasts and are present throughout the worm's body. Interestingly, these cells seem to express PIWI proteins, which, in other species, repress transposable elements and prevent their mobilization in the germline. Studying PIWIs in *Macrostomum* will uncover PIWI function in a stem-cell-specific context. To take advantage of *Macrostomum* initially for the study of PIWI biology, we have started by making a set of basic tools to transform this flatworm into a model organism.

We are currently assembling *Macrostomum*'s genome in collaboration with Michael Schatz (CSHL) and Eugene Berezikov (Hubrecht Institute). We generated sequencing data using Ilumina and PacBio sequencers. The average contig is currently ~5 kb long, but the assembly is improving constantly with new data. We have additionally generated transcriptome data (long and small RNAs) and are using de novo assembly algorithms to help with the genome assembly and to characterize transcribed regions and their promoters.

To genetically manipulate the organism more easily, we have designed a transgenesis protocol and obtained our first transgenic worms. We are currently working on making transgenesis more efficient.

Additionally, we have developed a protocol to sort neoblasts and are characterizing their transcriptome. We are also testing different anti-PIWI antibodies to perform small RNA pull-downs and immunofluorescence experiments.

Finally, we are making a *Macrostomum* BAC library consisting of 60,000 BACs with 20-kb inserts that will cover most genes and 40,000 BACs with 60-kb inserts that will cover bigger genes. This library will be partially sequenced to facilitate the genome assembly and will also serve as a tool to manipulate the genetic content of *M. lignano*.

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

L. Sabin, M.J. Delas

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

regulation of gene expression, and they may act by physically recruiting epigenetic modifiers and other protein complexes to target genomic loci. One developmental process that requires coordinated regulation of gene expression is hematopoiesis. During hematopoiesis, hematopoietic stem cells (HSCs) differentiate to generate both the myeloid and lymphoid lineages of blood cells. Importantly, the malignant transformation of various hematopoietic lineages leads to the development of several forms of leukemia and lymphoma, and the disruption or misregulation of epigenetic modifications is a common feature of cancer cells. Therefore, we are studying the function of lncRNAs in the murine hematopoietic system, with the aim of identifying lncRNAs that have critical roles in the epigenetic control of gene regulation. In particular, we hope to uncover lncRNAs responsible for regulating the processes of tumorigenesis, stem cell self-renewal, and differentiation. To this end, we have used high-throughput sequencing of various cell types within the murine hematopoietic lineage. In total, we have performed transcriptome sequencing of 26 samples, which include HSCs, as well as normal and transformed cells from both myeloid and lymphoid lineages. Despite having been studied for decades, the low abundance and complex physiological niche of HSCs in animals make these stem cells a challenging experimental model. We have established in the lab a unique in vitro culture system that allows us to expand HSCs for several weeks by coculturing them with endothelial cells, one of the essential cell types present in their physiological niche. These HSCs are able to fully reconstitute hematopoiesis when injected into lethally irradiated mice, which provides ultimate proof of their functionality. We can use this coculture system to study the role of lncRNAs in HSC self-renewal and differentiation using an in vitro assay, which reduces the experimental time. The candidate lncRNAs that will be tested in this loss-of-function assay are currently being selected using the transcriptome sequencing data, and we are testing several shRNA delivery vectors to obtain optimal lncRNA knockdown in HSCs. To further characterize the functionality of promising candidates, we can quickly transition to the in vivo model using bone marrow reconstitution assays. We hope to understand how these molecules participate in the regulation of gene expression during hematopoietic regeneration.

To identify lncRNAs important in the maintenance or progression of leukemia, we are using a mouse model of acute myeloid leukemia (AML), which is driven by the oncogene MLL-AF9, a common fusion protein found in human AML. MLL-AF9 leukemia cells can be manipulated and studied in culture, but they can also be injected into recipient animals to induce leukemogenesis. Therefore, we are using shRNA knockdown technology to determine which candidate lncRNAs have important biological roles in leukemia both in vitro and in vivo. To test whether lncRNAs can be depleted by shRNA-mediated RNAi, we have chosen a small pilot set of candidates expressed in MLL-AF9 leukemia cells and have measured the efficiency of RNAi-mediated knockdown of these candidates by quantitative polymerase chain reaction (Q-PCR). Similar to protein-coding genes, we found that some shRNAs potently deplete candidate lncRNAs, whereas others are ineffective. We therefore will use at least three shRNAs per candidate when performing RNAi screens in order to minimize the effects of inefficient shRNAs. We have also developed and piloted competitive proliferation assays with MLL-AF9 leukemia cells in vitro and in vivo. These assays are amenable to large-scale screening efforts. Therefore, we have compiled a catalog of the lncRNAs that are expressed most abundantly in MLL-AF9 leukemia cells (859 candidates) and are building an shRNA library that individually targets each of these candidates. We aim to identify lncRNAs that are important in leukemogenesis using our in vivo competitive proliferation assay and will validate hits both in vitro and in vivo. We hope that this work will provide important insights into lncRNA function in the epigenetic control of gene expression during normal hematopoiesis and malignant transformation.

The Role of miRNAs in Mouse Embryonic Stem Cell Differentiation

M. Kudla

miRNAs take part in the posttranscriptional regulation of gene expression. In the most well-studied example, they inhibit translation of a set of transcripts, thus forming an additional layer of control with respect to the regulation of transcription. The mechanism of miRNA-mediated inhibition has been relatively well described. The miRNA binds its inhibition target by the classic nucleic acid hybridization principle. In plants, the rules for complementarity between miRNA and its

target are strictly followed, and thus, finding target sites for miRNAs is a relatively easy task. However, in mammals, the rules for miRNA:transcript complementarity are relaxed, and thus, determining the target site purely on the basis of sequence information is challenging. It has been the target of multiple bioinformatics initiatives; however, the results of these efforts leave a lot to be desired. Specifically, the high false-positive ratio is the reason that the experimental method for finding target sites is necessary. The RNA cross-linking and immunoprecipitation method has been developed to answer this need. In our work, we are using the HITS-CLIP technique to obtain information on the impact of miRNA on the embryonic stem cell differentiation process. Using sophisticated statistical analysis and modern motif-finding algorithms, I hope to unravel this additional layer of regulation of the differentiation process.

Multiple Roles for Piwi in Silencing *Drosophila* Transposons

N.V. Rozhkov

Silencing of transposons in the *Drosophila* ovary relies on three Piwi-family proteins, Piwi, Aubergine (Aub), and Ago3, acting in concert with their small RNA guides, the piRNAs. Aub and Ago3 are found in the germ cell cytoplasm, where they function in the ping-pong cycle to consume transposon mRNAs. The nuclear Piwi protein is required for transposon silencing in both germ and somatic follicle cells, yet the precise mechanisms by which Piwi acts remain largely unclear. We investigated the role of Piwi by combining cell-type-specific knockdowns with measurements of steady-state transposon mRNA levels, nascent RNA synthesis, chromatin state, and small RNA abundance. In somatic cells, Piwi loss leads to concerted effects on nascent transcripts and transposon mRNAs, indicating that Piwi acts through transcriptional gene silencing (TGS). In germ cells, Piwi loss showed disproportionate impact on steady-state RNA levels, indicating that it also exerts an effect on posttranscriptional gene silencing (PTGS). Piwi knockdown affected levels of germ cell piRNAs presumably bound to Aub and Ago3, perhaps explaining its post-transcriptional impacts. Overall, our results indicate that Piwi has multiple roles in the piRNA pathway, in part enforcing transposon repression through effects on local chromatin states and transcription but also participating in germ cell piRNA biogenesis.

Exploiting RNAi to Identify a Putative Target for Pancreatic Cancer Treatment

S. Fenoglio, M. Hemann, G. Hannon

The technology of RNAi is a powerful tool used to discover the factors necessary for tumor growth by specifically knocking down one protein per cell, revealing the phenotypic consequences of the loss of gene function. The development of shRNA libraries further extended the potential of RNAi as many genes can be interrogated at once in a high-throughput fashion. The Hannon laboratory pioneered the technology and is now pushing it to the limit by embracing a genome-wide approach. At the same time, improved versions of shRNA libraries are designed and validated in the laboratory. We are interested in performing in vivo screening to identify new putative targets for the treatment of pancreatic cancer by means of shRNA libraries. One of us (S.F.) recently transferred to the Massachusetts Institute of Technology to work in the laboratory of Professor Michael Hemann to establish a transplantable model system for pancreatic ductal adenocarcinoma. The advantage of this system is the efficiency of the in vivo growth after transplant in immunocompetent mice, which calls for a good engraftment ability of the cell line, fundamental for the success of the screening. Combining this approach with the newly optimized libraries designed to improve the efficacy of the knock down dramatically increases the chances of successful and comprehensive screening results.

Pancreatic cancer occurs at low incidence in the United States; nevertheless, it is currently the fourth leading cause of cancer-related death in the country. The high mortality rate is the result of poor diagnostic tools and ineffective therapeutic options; in fact, most of the patients present at diagnosis with an advanced and metastatic disease. For this reason, resection of the tumor mass is impractical in the majority of cases, leaving chemotherapy and radiotherapy as the only options, which are applied with very low success rate. Currently, the elective treatment is based on gemcitabine, which being a cytosine analog is an unspecific cytotoxic drug. Because of the lethality of pancreatic cancer, it is crucial to achieve

a better understanding of the disease in order to improve treatment efficacy.

We will perform the same screening experiment in an in vivo and in vitro setting. We believe that it is important to include the tumor microenvironment as a variable in the experiments, as the interactions with the environment shape the behavior of cancer cells in response to treatment, here replicated by RNAi-mediated knockdown of genes.

From our efforts, we expect to find both obvious and nonobvious candidates for pancreatic cancer treatment, and we will continue by validating the best scoring hits of our screening, in particular those that are specific for the in vivo setting, to increase the chances of a relevant clinical impact. Moreover, from our data, we will gain relevant insights into the biology of the disease, with potential for new discoveries about mechanisms of cancer development and clinical application to diagnosis.

The Final Frontier of shRNA Libraries

S. Knott

This past year, my work has mainly involved optimizing the RNAi tools we use in large-scale shRNA screens. First, I have developed a computational algorithm (trained on the most comprehensive shRNA sequence/potency database available) that is capable of predicting, for any RNA target, potent shRNAs with high confidence. We have combined this tool along with additional algorithms to reduce target effects and to ensure that the maximal number of each gene's transcripts are targeted by the predicted shRNA molecules. With these tools, we have predicted a novel shRNA library for each of the human, mouse, and rat genomes. These libraries have been licensed to a vendor and are now available to the scientific community. Second, I have designed vectors to harbor pairs of hairpins, with the intent of simultaneously silencing two genes. These vectors have been designed to maximize hairpin processing and to allow the hairpin pairings within each cell of a complex population to be extracted with next-generation sequencing. With these vectors, we are set to begin performing RNAi screens where pairs of genes are interrogated for their combined therapeutic benefit. Our current goal is to interrogate the full pairwise set of druggable genes. This gene set was chosen because it shows the most promise for a prompt transition from preclinical to clinical trials. Finally, we have performed matched +/− gemcitabine (a chemotherapeutic agent) genome-wide RNAi screens on six pancreatic cancer cell lines with the aim of identifying targets that sensitize the cells to chemotherapy. These screens have been analyzed and ~150 putative hits are now being validated.

Optimizing shRNA Expression to Enable Discovery of Targets for Melanoma Therapy

S. Shaw

Building on our existing in vitro screening technologies and in collaboration with Dr. Meenhard Herlyn of the Wistar Institute, we are establishing in vivo RNAi screening in biopsied human melanoma tumor cells as patient-derived xenograft (PDX) mouse models. Using this system, our goal is to interrogate cells of different melanoma subtypes to identify genes upon which these cells depend for their survival, as well as targets that modify their drug sensitivity or resistance. Essential to our efforts to conduct screens in vivo is the ability to drive expression of our libraries in different primary tumor cells. We continue to expand our repertoire of promoters in order to facilitate optimal transcription and processing of shRNA transcripts in any cell type and to reduce variation in expression levels from different viral integration sites. We are also identifying elements that can be used in our vectors to prevent promoter silencing.

Argonautes in Gene Regulation

F. Karginov

Argonaute proteins are guided by their miRNA partners to mRNA targets, leading to regulation of the mRNA in a spatially and temporally controlled manner. In recent years, it has become more evident that the interactions between Ago/miRNAs and mRNAs are further affected by ancillary RNA-binding proteins, creating a combinatory system of regulation akin to transcription factors. We have continued to use transcriptome-wide technologies (CLIP) to identify the sites of interaction between Argonaute and mRNAs, to dissect the Ago regulatory networks and their integration with other RNA-binding proteins.

Recently, I have completed a study examining changes in Argonaute occupancy as a result of acute cellular stress to assess the participation of Ago in this process. Ago2 responds to stress by rearranging its set of interactions with mRNAs, in a fashion that is not simply a result of translation inhibition or stress granule assembly. The majority of Ago2-mRNA interactions are strengthened; however, a smaller subset of transcripts is released upon stress. Profiling of transcript abundance along polysome gradients, taken as a measure of their translation status, shows a correlation between their translatability and Ago2 association, suggesting the participation of Ago2 in modulating mRNA translation as part of the stress response.

CG5508 Is a Critical Component in Primary piRNA Biogenesis

V. Vagin, Y. Yang, Y. Luo

Piwi proteins and their associated small RNAs are essential for fertility in animals. This is due, in part, to their roles in guarding germ cell genomes against the activity of mobile genetic elements. piRNA populations direct Piwi proteins to silence transposon targets and, as such, form a molecular code that discriminates transposons from endogenous genes. Information ultimately carried by piRNAs is encoded within genomic loci termed piRNA clusters. These give rise to long, single-stranded, primary transcripts that are processed into piRNAs. Despite the biological importance of this pathway, neither the characteristics that define a locus as a source of piRNAs nor the mechanisms that catalyze primary piRNA biogenesis are well understood. We searched an ethylmethane sulfonate (EMS)-mutant collection annotated for fertility phenotypes for genes involved in the piRNA pathway. Twenty-seven homozygous-sterile strains showed transposon-silencing defects. One of these, which strongly impacted primary piRNA biogenesis, harbored a causal mutation in CG5508, a member of the *Drosophila* glycerol-3-phosphate *O*-acetyltransferase (GPAT) family. These enzymes catalyze the first acylation step on the path to the production of phosphatidic acid (PA). Although this pointed strongly to a function for phospholipid signaling in the piRNA pathway, a mutant form of CG5508, which lacks the GPAT active site, still functions in piRNA biogenesis. Interestingly, the mutation in CG5508 causes truncation of the predicted RNA binding motif carboxy-terminal end without perturbing GPAT activity. CG5508 colocalizes on the outer mitochondrial membrane together with another key primary piRNA factor, endoribonuclease Zucchini, and is required for its proper cellular localization. PAR-CLIP analysis in the cell culture revealed Zucchini and CG5508 directly interact with the same set of transcripts, including primary piRNA precursor *flamenco*, but the CG5508 truncation version without the RNA-binding motif does not bind any transcripts. We proposed that primary piRNA transcripts are bound by CG5508 in order to be processed by Zucchini into mature piRNAs.

Quantitative Genome-Wide RNAi Screen Identifies Novel Factors That Affect Specific Aspects of *C. elegans* piRNA Biogenesis

W.S. Goh

piRNAs are germline-specific small RNAs that load onto piwi proteins, and their loss leads to sterility. In the nematode *Caenorhabditis elegans*, piRNAs, or 21Us are loaded onto the piwi protein PRG-1. piRNAs have a 1U bias, 5′ monophosphate and modified 3′ ribose. Unlike their 25–31-nucleotide nonnematode counterparts, worm piRNAs are all 21 nucleotides long and are mostly located in the intergenic or intronic regions of two large clusters on chromosome IV. Previous work in the field has implicated these piRNAs in silencing transposons and pseudogenes, and triggering transgenerational germline nuclear RNAi. Recently, ~26-nucleotide capped piRNA precursors (pre-21Us) have been identified. Because each piRNA is located downstream from a proximal DNA motif, this suggests a biogenesis model where each DNA motif acts as a promoter that controls expression of pre-21Us that are then processed into mature piRNAs. Detailed mechanisms for piRNA biogenesis are still unknown because no regulating factors have ever been identified.

In the past year, I utilized a quantitative PCR-based, genome-wide RNAi screen and identified 22 Twenty-one-u Obviously Fouled Up (TOFU) factors involved in the biogenesis of mature piRNAs. Unexpectedly, seven suppressors were also identified that, when knocked down, increased both piRNA and

PRG-1 mRNA levels. To characterize the top seven TOFUs in detail, I sequenced both piRNAs and pre-21Us. Although perturbations of individual TOFUs all led to global reductions in piRNA profiles, this could be accompanied by either an accumulation or reduction of pre-21Us. Together, my data suggest that different TOFUs affect specific aspects of the worm piRNA biogenesis pathway, including transcription of pre-21Us and their conversion to piRNAs.

Exploring Biological Function of Prokaryotic Argonaute Protein in Bacteria

X. Zhou

Argonaute proteins are key components of RNAi pathways in the eukaryotic kingdom. Despite the wide existence of Argonaute proteins in prokaryotes, such as bacteria and archaea, the biological functions of prokaryotic Argonaute remain unknown. Therefore, we aim to reveal the roles of Argonaute in a model microorganism, *Thermus thermophilus*. Previous studies reported that *T. thermophilus* Ago, which is structurally highly conserved to eukaryotic Ago, is able to cleave RNA using a single-stranded DNA guide strand in vitro, indicating biological functions in vivo. Overexpression of His-tagged Ago and immunoprecipitation in *T. thermophilus* showed binding of small nucleic acids of ~30 nucleotides. Moreover, an increased level of Ago seems to be toxic to cells, indicating a potential gene-silencing pathway mediated by Ago. In summary, Argonaute protein is likely to mediate a novel gene-silencing pathway in *T. thermophilus*. Future work includes a functional study in Argonaute knockout/knockin mutants and discovery of other components of the Ago-mediated pathway.

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REPRESENTATION METHYLATION SEQUENCING ANALYSIS

R. Lucito N. Dimitrova K. Wrzeszczynski
M. Vigliotti

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

We have now sequenced eight tumors and four normals. We have sequenced two of the samples on two different formats of the Illumina sequencers and have determined the coverage of each and switched to the newest platform. We have developed sequencing pipelines for the analysis of the data and have determined the number of reads necessary to account for 85% of the CpG sequences in the genomic island sequences. We are presently further analyzing the data with the goal of identifying new regions that may serve as markers for diagnosis and also as a discovery tool to identify gene candidates that are involved in therapy response.

Genomic Analysis of Ovarian Cancer

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

In the United States, there will be ~22,000 new cases of ovarian cancer in 2012. Of these, ~14,000 will succumb to the disease. To better treat these women, and improve survival, our goal is to determine the molecular changes that have occurred in the patients' tumors, and to be able to interpret the effects these changes have on the growth and development of the tumor. This aberrant growth is a result of chromosomal abnormalities and epigenetic variations. In addition, generally low rates of somatic nucleotide mutation in ovarian cancer as compared to other solid tumors suggest an increased significance of copy-number and epigenetic aberrations. This type of regulation has been shown to affect the many tumor suppressors and oncogenes discovered in ovarian cancer. The identification of genetic and epigenetic alterations from primary tumor cells has become a common means of identifying genes critical to the development and progression of cancer. We have performed a bioinformatics analysis of copy-number variation and DNA methylation covering the genetic landscape of ovarian cancer tumor cells. We individually examined the copy-number variation and DNA methylation for 42 primary serous ovarian cancer samples using our MOMA-ROMA technology and 379 tumor samples analyzed by the Cancer Genome Atlas. We have identified 346 genes with significant deletions or amplifications among the tumor samples. Using associated gene expression data, we predict 156 genes with significantly altered copy-number and correlated changes in expression. Among these genes, *CCNE1, POP4, UQCRB, PHF20L1*, and *C19orf2* were identified within both data sets. We were specifically interested in copy-number variation as our base genomic property in the prediction of tumor suppressors and oncogenes in the altered ovarian tumor. We therefore identified changes in DNA methylation and expression for all amplified and deleted genes. We predicted 615 potential oncogenes and tumor suppressor candidates by integrating these multiple genomic and epigenetic data types. Genes with a strong correlation for methylation-dependent expression exhibited at varying copy-number aberrations include *CDCA8, ATAD2, CDKN2A, RAB25, AURKA, BOP,* and *EIF2C3.* We provide copy-number variation and

DNA methylation analysis for more than 11,500 individual genes covering the genetic landscape of ovarian cancer tumors. We show the extent of genomic and epigenetic alterations for known tumor suppressors and oncogenes and also use these defined features to identify potential ovarian cancer gene candidates.

Resistance Mechanisms in Ovarian Cancer

N. Cutter, M. Vigliotti, K. Wrzeszczynski, G. Fan
[in collaboration with N. Tonks, Cold Spring Harbor Laboratory]

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

Genomic Alterations of Phosphatases

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

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

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

We first generated small hairpin RNAs (shRNAs) to suppress expression of the *MTSS1* gene in cell culture assays, which would mimic the transcriptional repression caused by promoter methylation. Because this gene was selected based only on the comparison of tumor to normal, we did not have a selection for assay development. We surmised that because MTSS1 possessed an actin-binding domain, the protein might have a role in cytoskeletal rearrangement, cell movement, and invasion. Therefore, we performed an invasion assay with MCF10A mammary epithelial cells, which are used typically as a normal line. We observed that very few cells migrate without the addition of the attractant epidermal growth factor (EGF). Furthermore, RNA interference (RNAi)-mediated suppression of MTSS1 enhanced migration in the presence of EGF. Evidence in the literature shows that in ERBB2-positive breast cancer, there is loss of *MTSS1* transcription. To determine whether ERBB2 overexpression synergizes with loss of MTSS1, we performed an invasion assay in MCF10AN cells. These cells express a chimeric ERBB2 gene in which the activity of the cytoplasmic PTK domain of ERBB2 is acutely regulated by a small molecule dimerizing agent. We noted that the level of invasion is highest in the cells in which MTSS1 was suppressed and ERBB2 activated, demonstrating cooperation between these two gene products. Our current data indicate that these effects are mediated via changes in tyrosine phosphorylation regulated by RPTPδ.

MAMMALIAN FUNCTIONAL GENOMICS

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

Our research is focused on elucidating the genetic basis of human diseases such as cancer and autism. We have discovered genes controlling these diseases, revealing insight into how the encoded proteins normally work and what goes wrong in the disease state. These findings have had a major impact in the scientific community and have affected how clinicians analyze and treat these genetic diseases.

Major discoveries:

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

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

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

My group also discovered that *p63* affects aging. *p63* is needed for stem cells to replenish the tissues of our body; when *p63* is depleted, rapid aging ensues. These features of aging include curvature of the spine, hair loss, and severe skin lesions, and they indicate that *p63* maintains youthfulness. However, there is a fine balance, as *p63* must be controlled at just the right level. Indeed, we recently discovered that too much of a certain p63 protein (ΔNp63α) leads to carcinoma—the most prevalent type of human cancer (Paul et al., in press). Pathologists throughout the world currently use ΔNp63α as a marker for diagnosing human cancers of the breast, skin, and prostate. In contrast to the tumor-causing ΔNp63α mentioned above, we discovered that other classes of p63 proteins (TAp63 proteins) function in the opposite way: They *prevent* cancer. We showed that TAp63 proteins are able to shut down tumor growth, causing the tumor to shrink dramatically. Importantly, TAp63 is able to halt tumor growth even when its sister protein, p53, is completely absent. This work stunned the p53 cancer community, as it had always been assumed that having ineffective p53 was a virtual death sentence that would render any anticancer therapy ineffective. Our work shows that this is not the case, as p63 can step in and do the job. We are currently working on strategies to activate *p63* that might be feasible in the clinic.

Discovering *CHD5* as a New Cancer-Preventing Gene

Another major breakthrough in my laboratory was the discovery of *CHD5* as a tumor suppressor mapping to human 1p36—a region of our genomes that is often sabotaged with deletions in cancer cells. 1p36 deletions occur in many different types of human cancers, including those of the epithelia and brain, as well as those of the blood. Although the cancer community had searched for more than 3 decades for this tumor suppressor, identifying this gene presented a major challenge. My laboratory discovered *CHD5* as a 1p36 tumor suppressor. By generating mice with gain and loss of the genomic region corresponding to 1p36 using chromosome engineering technology—a

strategy that allows us to generate precise chromosome rearrangements in the mouse—we pinpointed a region of the genome with potent tumor suppressive activity. Using a series of genetic and molecular approaches, we identified *CHD5* as the tumor suppressor gene in the region and found that its product worked as a "circuit breaker" for a cancer-preventing network. In addition, we discovered that *CHD5* was frequently deleted in human glioma. This year, we discovered that *CHD5* uses its plant homeodomains to bind histone 3, an interaction that is essential for it to function as a tumor suppressor (Paul et al., in press). Our work identifying *CHD5* as a tumor suppressor has had a major impact in the cancer field, as it is now known that *CHD5* is mutated in human cancers of the breast, ovary, and prostate, as well as in melanoma, glioma, and neuroblastoma. Furthermore, recent reports indicate that *CHD5* status is a strong predictor of whether anticancer therapy will be effective; indeed, patients with high levels of *CHD5* have much better overall survival than those with low levels.

Revealing the Genetic Basis of Autism

Our recent findings revealed that one of the most common genetic alterations in autism—deletion of a 27-gene cluster on chromosome 16—causes autism-like features (for review, see Horev et al., *Proc Natl Acad Sci 108:* 17076 [2011]). By generating mouse models of autism using chromosome engineering (the same technology that we had used for cancer gene discovery, discussed above), we provided the first functional evidence that inheriting fewer copies of these genes leads to features resembling those used to diagnose children with autism. First, "autism" mice had behaviors such as higher activity, resistance to change in environment, sleeping problems, and repetitive/restrictive behavior; each of these features is similar to clinical criteria used to diagnose autism in humans. Second, these mice had distinctive architectural changes in the brain that were detectable by magnetic resonance imaging (MRI). Our work provides functional evidence for the genetic basis of autism that was not previously appreciated. We believe that these models will be invaluable for pinpointing the genes responsible for autism. They could also be used for designing ways to diagnose children with autism before they develop the full-blown syndrome, as well as for designing clinical interventions.

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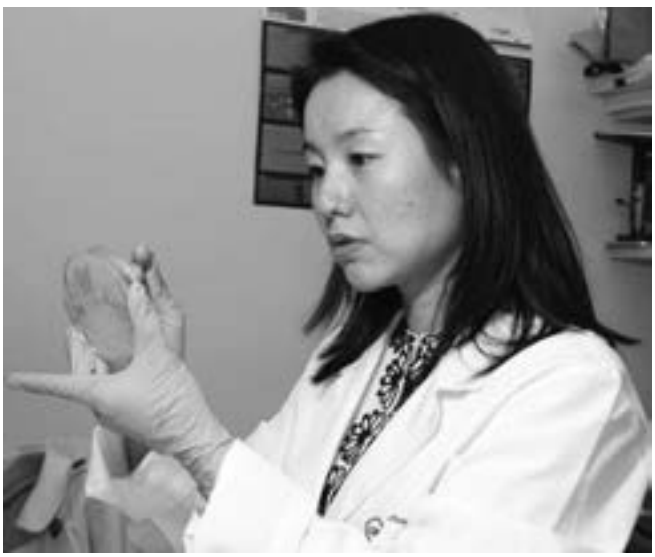

Yon Chang

CANCER GENES

S. Powers C. Eifert J. Li M. Rajaram
B. Gerdes A. Mofunanya Y. Wu

Cold Spring Harbor Laboratory is synonymous with the village-like atmosphere created by its picturesque research buildings. Five miles away in Woodbury, the Genome Center campus has a more industrial ambience imparted by its plentiful next-generation sequencing apparatuses. Here, we use these sequencing machines and other cancer genomic technologies to ask questions about cancer and develop new treatment strategies. For more than 15 years, the main focus of our research has been to utilize genomic analysis of human cancer to discover underlying driver genes—genes that when altered by point mutation or DNA copy-number changes contribute to the selective advantage of the evolving cancer cell.

Driver Genes

A. Mofunanya, J. Li, R. Gerdes, Y. Wu

Our driver gene-screening efforts are funded by the National Cancer Institute (NCI)-sponsored Cancer Target Discovery and Development Consortium (www.ctd2.nci.nih.gov). The Cancer Target Discovery and Development (CTD2) network is a coordinated effort to integrate cancer genome characterization with molecular target discovery and validation. There are seven different funded centers, including one at CSHL, that work in complementary ways to accelerate the translation of the genomic discoveries into new cancer treatments. The network centers each have a primary technology platform, be it bioinformatics, genome-wide loss-of-function screening, a specific cancer-type focus, small-molecule inhibitors, proteomics, or in our case, cancer-genome-focused screening. We are currently performing cancer-genome-focused screens in ovarian cancer (in collaboration with two other CTD2 centers) and more recently breast cancer. In breast cancer, we utilized genomic analysis of the the Cancer Genome Atlas (TCGA) breast cancer data set to identify 100 novel candidate oncogenic driver genes, which are currently being screened using pooled approaches for their ability to induce cancer when overexpressed in a normal mammary cell, as well as their ability to inhibit cancer when their expression is selectively silenced in human breast cancer cell lines.

RNAi Screen for Sensitizers to PI3K Inhibition

C. Eifert

Human breast cancer cells with activating mutations in the gene for phosphoinositol-3 kinase show a stronger dependency upon PI3K/mTOR (mammalian target of rapamycin) signaling than do other breast cancers. However, it is not clear whether this selective oncogene dependency can form the basis of clinically effective treatments. Through a pooled short hairpin RNA (shRNA) screen, we showed that knockdown of two genes upstream in the PI3-biosynthetic pathway, PI4KB and PIP5K1A, specifically enhanced cell death induced by PI3K and dual PI3K/mTOR inhibitors in PIK3CA-mutant breast cancer cells.

Multiple Signaling Interactions between Cancer Cells and Associated Fibroblasts Are Involved in Promoting Tumorigenicity

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

Many fibroblast-secreted proteins can promote tumorigenicity, and several mechanisms by which cancer cells induce these proteins have been proposed. Whether there is a single dominant pathway underlying these processes that could be targeted therapeutically is unclear. Here, we identified both new and previously recognized fibroblast-secreted factors induced by breast cancer cells by comparative genomics (Fig. 1). Three of five tested fibroblast-secreted factors had equally major roles in promoting tumorigenicity. In particular, fibroblast-secreted amphiregulin promoted breast cancer cell survival, whereas the chemokines CCL2

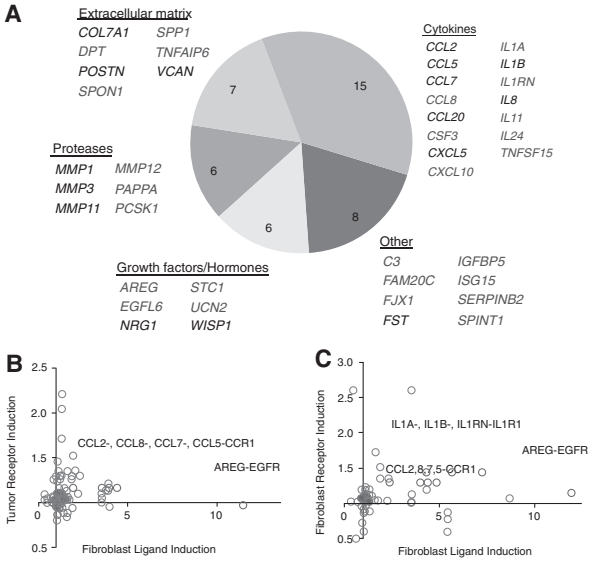

Figure 1. Functional classes of selectively secreted proteins and autocrine/paracrine induction of ligand-receptor pairs. (*A*) Diagram showing the functional classification of the 42 secreted proteins that are up-regulated in both tumor-supportive fibroblasts and primary breast cancer stroma. Most of these proteins had not previously been shown to have a tumor-supportive role as fibroblast-secreted proteins, although others such as IL-1B have. (*B*) Paracrine up-regulation of the expression of ligand-receptor pairs upon coculture of tumor-supportive fibroblasts with basal breast cancer cells. The fold-change in ligand expression in tumor-supportive fibroblasts (*x* axis) is plotted against the fold-change in receptor expression in breast cancer cells (*y* axis). (*C*) Autocrine up-regulation of ligand-receptor pairs upon coculture of tumor-supportive fibroblasts with basal breast cancer cells. The fold-change in tumor-supportive fibroblast ligand expression (*x* axis) is plotted against the corresponding fold-change in receptor expression (*y* axis).

and CCL7 stimulated tumor cell proliferation, innate immune cell infiltration, and angiogenesis. Importantly, the whole repertoire of functionally important fibroblast-secreted factors could not be induced by stimulating fibroblasts with a single factor. Simultaneous targeting of fibroblast-secreted amphiregulin and of CCR1, the CCL7 receptor, on cancer cells in mice was more efficacious than blocking either alone. Thus, our data strongly suggest that multiple tumor–stroma interactions should be targeted.

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In Press

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CANCER AND HUMAN GENETICS

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

The Genetics of Autism

We are examining the genetic basis of autism spectrum disorders (ASDs) by studying de novo mutations occurring in affected individuals and comparing rates of different types of mutations in the unaffected siblings. The publication of Iossifov et al. (2012) summarizes findings after studying nearly 350 simplex families (only one affected child, and in our study, at least one unaffected sibling) drawn from the Simons Simplex Collection (SSC). The major conclusions of that work are strengthened by ongoing work and parallel studies of the same collection being carried out in other laboratories. In particular, there is statistically significant increased incidence of de novo mutations of functional consequence (copy-number changes, nonsense mutations, splice site mutations, and frameshifts) in affected children compared to siblings, whereas de novo mutations that are likely without effect (synonymous mutation) show no increase. Mutation incidence is higher in affected girls and increases with the age of the parents. Target analysis indicates strong overlap of autism candidates with genes expressing mRNAs that are associated with the fragile X mental retardation protein FMRP (in collaboration with Robert Darnell at the Rockefeller University). Recurrence analysis suggests that there are on the order of 500 target genes. The overall incidence of new mutation, observed and projected, is consistent with new mutation being the major source of autism risk in sporadic autism, especially for females.

De Novo Mutation in Sporadic Human Disease

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

Congenital heart disease (CHD) is one of the most common malformations in humans—found in nearly 1% of live births—and is largely sporadic, with evidence of a significant genetic component. Utilizing the same approach as in our autism and cancer studies, we studied 213 families in collaboration with Dr. Dorothy Warburton at Columbia University Medical Center. As in the other studies, we found strong evidence that de novo CNVs may cause CHD, with greater than 10% of affected children having a germline variant—a rate five times higher than was seen in a control population. These studies reveal that rare de novo mutation contributes to many types of sporadic disorders in humans.

Algorithms for Genomic Analysis

We develop tools for genome analysis by refining existing methods and creating novel algorithms when "off-the-shelf" software is inadequate. Insertions and deletions (indels), as well as other genome rearrangements, contribute significantly to genetic variation and disease. Many small indels can be detected by existing software, but in some genomic contexts—such as within microsatellites—indels can be difficult

to discern. To address this need, we have developed our own software. Large-scale rearrangements, such as sequence inversions, and intermediate-scale indels are also not handled well by openly available code, and we are in the process of creating computational tools to accurately detect these classes of variation. Our methods are being applied to exome sequence data from families of the SSC. As concrete results, we have identified ~150 de novo mutations in 104 families at 90,000 microsatellite loci, and we have found examples of de novo pseudogene formation in ~500 families. We are also analyzing large-scale indels (also known as copy-number variants) in the SSC, for which we have helped to develop much of the existing software, including methods based on principal component analysis (Lee et al. 2012).

Molecular Methods

We have continuously worked on developing methods to perform measurements with greater resolution, or more efficiently, or to make new types of measurements. In the area of genomics, this includes algorithm development as described above, but also bench methods. In this regard, we have efforts in three projects. First, we are developing copy-number measurements based on sequencing and driving down experimental costs using sample barcodes, pooled samples, and genome fragmentation. Second, we are applying similar strategies for single-cell DNA methods. Finally, we are developing methods for single-cell RNA profiling. The latter incorporate the idea of varietal tagging to reduce the distortion caused by polymerase chain reaction (PCR) amplification.

Single-Cell Analysis of Mouse Brain Neurons

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

Genomic Landscape of Prostate Cancer

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

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

Single-Cell Viewer

We are developing interactive visualization software to view whole-genome copy-number data from single-cell sequence data. Data from hundreds of cells comprising many regions within a tumor (in which the ploidy of nuclei has been sampled by fluorescence-activated cell sorting [FACS]) can be assessed together. The

cells are clustered by their copy-number profiles, highlighting clonal differences and where those clones are physically located within a tumor. The entire collection of cells can be analyzed in a combined tree and heatmap, cells can be subdivided by tumor region to profile individual sectors within a tumor, and each cell can be evaluated individually. It is possible to zoom in to any region within any of these figures to get a detailed view of a subset of samples or a specific genomic region.

Collaborators

In addition to our external collaborators, cited above, virtually all of our work is performed and planned with collaborators at CSHL: Jim Hicks and Alex Krasnitz in cancer; Ivan Iossifov, Dan Levy, and Michael Schatz in genetics; and Josh Huang and Pavel Osten in neurobiology. Within the Wigler lab, Michael Ronemus has been largely responsible for the exome sequence data production pipeline; Jude Kendall, Boris Yamrom, Yoon-ha Lee, Mitch Bekritsky, Peter Andrews, and Ewa Grabowska for algorithm development, processing, and analysis of single-cell and genome sequence data; Chris Yoon for analysis of copy-number and genetic transmission; Anthony Leotta and Steve Marks for building and maintaining and querying databases; Ravi Kandasamy, Zihua Wang, and Zhu Zhu for studies of somatic mutation, monoallelic-specific expression, validating sequence discovery, and developing copy-number and single-cell bench methods; and Joan Alexander for the cancer landscape projects.

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CANCER: SIGNAL TRANSDUCTION

Mikala Egeblad and colleagues study tumors and, in particular, the contributions of the microenvironment in which the cancer cells arise and live. Solid tumors are abnormally organized tissues that contain not only cancer cells, but also various stromal cell types and the extracellular matrix, and these latter components constitute the microenvironment. Communications between the different components of the tumor influence its growth, its response to therapy, and its ability to metastasize. Among the tumor-associated stromal cells, the lab's main focus is on myeloid-derived immune cells, a diverse group of cells that can enhance angiogenesis and metastasis and suppress the cytotoxic immune response against tumors. Egeblad is interested in how different types of myeloid cells are recruited to tumors and how their behaviors, for example, their physical interactions with cancer cells and other immune cells, influence cancer progression, including metastasis. The lab studies the importance of the myeloid cells using mouse models of breast and pancreatic cancer and real-time imaging of cells in tumors in live mice. This enables them to follow the behaviors of and the interactions between cancer and myeloid cells in tumors during progression or treatment. This technique was instrumental when the lab recently showed that cancer drug therapy can be boosted by altering components of the tumor microenvironments, specifically reducing either matrix metalloproteinases (enzymes secreted by myeloid cells) or chemokine receptors (signal receptors on myeloid cells).

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

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

Despite their large variety of genetic abnormalities, cancer cells have been found to be extremely sensitive to the reversal of certain mutations. **Raffaella Sordella** and colleagues study why cells in

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

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

Lloyd Trotman's recent research path traces to his discovery some years ago that the loss of a single copy of a master tumor suppressing gene called PTEN is sufficient to permit tumors to develop in animal models of prostate cancer. His team later found that complete loss of PTEN paradoxically triggers senescence, an arrested state that delays or blocks cancer development in affected cells. These findings explained why many patients only display partial loss of this tumor suppressor when diagnosed with prostate cancer. Now the team is researching ways to restore the PTEN protein levels in these patients. This therapy approach could slow disease progression and thus greatly reduce the need for surgical removal of the prostate or of similar drastic interventions that carry the risks of incontinence and impotence. Their second approach to combat prostate cancer is to model the lethal metastatic disease in genetically engineered mice. They are developing a novel approach that allows for quick generation and visualization of metastatic disease. The efficacy of existing and novel late-stage therapies, such as antihormonal therapy, can then be tested and optimized in these animals. At the same time, the Trotman lab is exploring the genetic alterations associated with metastatic disease and with resistance to therapy. To this end, they use single-cell genome sequencing techniques developed at CSHL by Drs. Wigler and Hicks.

David Tuveson's lab uses mouse and tissue models of neoplasia to explore the fundamental biology of these diseases and thereby identify new diagnostic and treatment strategies. His team's main focus is pancreatic cancer, a lethal malignancy that has eluded clinical solutions despite intensive study. The lab's approaches at CSHL run the gamut from designing new model systems of disease to inventing new therapeutic and diagnostic platforms for rapid evaluation in preclinical and clinical settings. For example, they have adopted a new method of culturing tissue fragments indefinitely in cell culture, enabling deep analysis with genetic and pharmacological probes. Second, therapeutic experiments in mouse models have revealed an important role of redox metabolism and stromal interactions on influencing therapeutic response. Tuveson's lab also has a strong link to clinical trials locally and internationally, with confirmation in early phase trials the ultimate goal. Collectively, their strategy in the preclinical and clinical arena is codified as the "Cancer Therapeutics Initiative," and this initiative will provide these same approaches to the entire CSHL cancer community.

Linda Van Aelst's lab studies how aberrations in intracellular signaling involving enzymes called small GTPases can result in disease. They are particularly interested in Ras and Rho GTPases, which help control cellular growth, differentiation, and morphogenesis. Alterations affecting Ras and Rho functions are involved in cancer and various neurodevelopmental disorders. Van Aelst's team has extended its prior study of mutations in a Rho-linked gene called *oligophrenin-1* (*OPHN1*), part of an effort to connect the genetic abnormalities associated with mental retardation to biological processes that establish and modify the function of neuronal circuits. In addition to a role for OPHN1 in activity-driven glutamatergic synapse development, lab members have obtained evidence that OPHN1 has a critical role in mediating mGluR-LTD, a form of long-term synaptic plasticity, in CA1 hippocampal neurons. Their findings provide novel insight not only into the mechanism and function of mGluR-dependent LTD, but also into the cellular basis by which mutations in *OPHN1* could contribute to the cognitive deficits observed in patients. Defects in cortical neurogenesis have been associated with cerebral malformations and disorders of cortical organization. The Van Aelst team discovered that interfering with the function of the Rho activator DOCK7 in neuronal progenitors in embryonic cerebral cortices results in an increase in the number of proliferating neuronal progenitors and defects in the genesis of neurons. In an extension of these studies, the Van Aelst team this year showed that DOCK7 has a central regulatory role in the process that determines how and when a radial glial cell progenitor "decides" to either proliferate, i.e., make more progenitor cells like itself, or give rise to cells that will mature, or "differentiate," into pyramidal neurons. These lines of research provide novel insight into mechanisms that coordinate the maintenance of the neural progenitor pool and neurogenesis.

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

THE INFLUENCE OF THE TUMOR MICROENVIRONMENT ON DRUG RESISTANCE AND METASTASIS

M. Egeblad J. Cappellani T. Kees J. Park J. Qiu R. Wysocki
M. Fein E. Nakasone J.-H. Park M. Shields

Interactions between epithelium and stroma are essential for normal organ development. The stroma is the supportive framework of the organs and includes the extracellular matrix (ECM), stromal cells such as fibroblasts and adipocytes, cells of the vascular system, and immune cells. Like organs, solid tumors are composed of cancer cells and stroma, which is also known as the tumor microenvironment. As tumors develop and progress, dramatic changes occur in both the cancer cells and the microenvironment.

We study how the tumor microenvironment influences cancer cells in the context of drug resistance and metastasis. We use mouse models of breast and pancreatic cancer together with real-time spinning-disk confocal microscopy of tumor–stroma interactions in living mice. This allows us to study how cancer cell proliferation, migration, and survival are influenced by vascular changes or myeloid cell infiltration in real time.

Effects of the Tumor Microenvironment on Response to Cancer Therapy

E. Nakasone, M. Fein, T. Kees, J.-H. Park, J. Park, J. Qiu, R. Wysocki

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

We are using in vivo spinning-disk confocal imaging to study responses to anticancer drugs, such as the chemotherapeutic drug doxorubicin, in the context of evolving tumor microenvironments. Imaging revealed that cancer cell death started ~24 h after doxorubicin treatment, and the cells died by necrosis and not apoptosis. We further revealed that the microenvironment of different tumor stages participated in regulating the drug response. Early-stage and very late-stage lesions responded poorly compared to intermediate-sized, early-carcinoma-stage tumors. However, in vitro, sensitivity to doxorubicin was similar for cancer cells from different stages. Live imaging of doxorubicin penetration further revealed that drug penetration was influenced by the tumor stage. Thus, the acute cell death response to doxorubicin correlated with the infiltration of the drug (Nakasone et al. 2012). Matrix metalloproteinase (MMP) 9 is an ECM-degrading enzyme that regulates vascular permeability. We determined that the lack of MMP9 activity increases phosphorylation of vascular endothelial (VE)-cadherin, leading to loose cell-cell junctions between endothelial cells. We determined that both mouse mammary tumor virus (MMTV)-Neu and MMTV-PyMT tumors responded significantly better to treatment with doxorubicin in mice that lacked MMP9 activity (Nakasone et al. 2012).

Doxorubicin treatment also led to a new microenvironment, with recruitment of new myeloid-derived cells. Imaging revealed that myeloid cells were recruited to tumors ~30 h after chemotherapeutic treatment with doxorubicin and after cancer cell death was observed. We determined that activation of the chemokine receptor CCR2 on monocytes, caused by the CCL2 chemokine that was secreted from stromal cells, led to recruitment of the cells to the tumors. We further determined that the infiltration of these CCR2-positive monocytes contributed to chemoresistance, as the effect of doxorubicin and cisplatin on tumors transplanted to mice lacking CCR2 was significantly better than that of tumors in normal mice (Nakasone et al. 2012). Furthermore, when tumors recurred in mice lacking CCR2, they were of lower histological grade than tumors relapsing in wild-type mice.

Imaging intact tumors acutely after treatment with chemotherapy thus revealed a complex and evolving relationship between microenvironment and the drug

sensitivity of cancer cells. We are currently working on implementing imaging windows in our analysis, as this technology will allow us to image repeatedly days apart and therefore to study long-term response to therapy, including relapse.

Effects of Myeloid Cells on Breast Cancer Metastasis

M. Fein, J. Park

The prognosis of metastatic breast cancer is poor. More than a century ago, Dr. Stephen Paget observed that metastases develop preferentially in certain organs, suggesting that factors external to cancer cells influence metastasis. Indeed, it is now recognized that growth factors and cytokines secreted by myeloid-derived cells have an important role in the formation of metastasis.

Traditionally, studies on metastasis have relied primarily on measurements made at the endpoint of the process, the establishment of micro- or macrometastases. However, the metastatic process is dynamic and characterized by the ability of cancer cells to move from one part of the body to another: Cells exit the primary tumor, invade the local tissue, enter blood or lymphatic vessels, and are transported to a distant site where they exit the vessels and move into the tissue. Thus, a different level of understanding of metastasis might be achieved using technologies that can follow these dynamic processes in vivo.

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

Using Tumoricidal Activities of Macrophages

T. Kees, M. Shields

Tumor-associated macrophages have tumor-supporting activities and their infiltration is associated with poor patient prognosis. However, macrophages are capable of killing tumor cells if they are activated with interferon-γ (IFN-γ) and agonists of receptors for pathogen-associated molecular patterns. We are using live cell imaging of co-cultures between macrophages and breast and pancreatic cancer cells to understand the mechanisms responsible for the tumoricidal activities. We have determined that such macrophage-mediated cancer cell killing is highly effective and requires direct cell-cell contact between macrophages and cancer cells. A mediator of cancer cell killing has been identified and is under further investigation. Live imaging has also revealed that a small number of cancer cells survive the tumoricidal activities of macrophages. Such macrophage-evading cancer cells would be of great importance for therapeutic translation, and thus we used RNA sequencing to identify candidate genes involved in the evasion. Several candidates are under further investigation.

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

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

localized along tumor margins and engaged in long-lived interactions with tumor-specific T cells. In vitro, these myeloid cells could capture cytotoxic T cells in signaling-competent conjugates. Yet, the interaction did not support full T-cell activation or sustain cytolysis of cancer cells. Thus, these antigen-presenting cells fail to stimulate T cells and may serve as a barrier to an effective T-cell response. This work demonstrated the behavior of tolerized T cells within tumors and identified a target for immunotherapies.

Functional Genomic Approaches to Determine How Cancer Cells and Microenvironment Interact

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

With Scott Powers, we have undertaken a genome-wide approach to identify and test genes in fibroblasts that are both induced upon interaction with basal breast cancer cells in culture and up-regulated in stromal cells from primary human breast cancers. We identified both new and previously recognized fibroblast-secreted factors induced by breast cancer cells. Several of the up-regulated genes encode secreted growth factors or cytokines. Using RNA interference (RNAi) and a co-injection tumorigenicity assay, three of five tested fibroblast-secreted factors had equally major roles in promoting tumorigenicity. Interestingly, the fibroblast-secreted factors had functionally diverse roles in promoting tumorigenicity, such as stimulation of tumor cell proliferation, of innate immune cell infiltration, and of angiogenesis. Rather than a single major mediator, these results indicate multiple points of intervention to prevent fibroblasts from supporting basal breast cancer. Additionally, we show that breast cancer subtypes differ markedly in the expression of these and other stromally secreted proteins. We are currently performing imaging of the development of tumor–fibroblast interactions in live mice to gain insights into how specific factors might be targeted for cancer treatment.

Collagen Architecture in Pancreatic Cancer Progression

M. Shields, J. Cappellani

The interstitial matrix is a major category of the ECM and consists of macromolecules such as fibrillar collagens, fibronectin, and proteoglycans. Type I collagen is the major fibrillar collagen in tissues and forms a scaffold that provides stability. Type I collagen also has signaling functions mediated by integrins and discoidin domain receptors. The synthesis and proteolytic remodeling of the fibrillar type I collagen increases in many tumors, particularly in pancreatic tumors.

Tumors are often discovered as nodules that are harder than the surrounding tissue. This reflects the changes in extracellular matrix stiffening and architecture. An abnormal collagen architectural structure correlates with tumor progression. Whereas collagen fibers are curly and oriented in parallel to normal or hyperplastic epithelium, there is a progressive change in the fibers so that they are straighter and mostly perpendicular to the tumor border in the late stages. This changed architecture may promote cell invasion by enabling cells to migrate along the collagen fibers or by enhancing integrin signaling. Enzymes of the lysyl oxidase family, involved in collagen fiber cross-linking, promote cancer progression and metastasis. In collaboration with Dr. Weaver's lab at University of California, San Francisco, we previously reported that inhibiting collagen cross-linking in mouse models of mammary carcinoma delays tumor onset and slows tumor progression. Pancreatic cancer has a very higsh level of type I collagen, and we are currently investigating the effects of collagen cross-linking in pancreatic carcinoma.

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PROTEOMICS LABORATORY AND PROTEOMICS SHARED RESOURCE

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

Testing the Putative Lipase Activity of Zucchini

This work was done in collaboration with J. Ipsaro, L. Joshua-Tor, A. Haase, and G. Hannon (Cold Spring Harbor Laboratory).

Recent genome-wide screens have identified a number of molecules involved in Piwi-interacting RNA (piRNA) biogenesis and action. One of these proteins, Zucchini, was identified as a key biogenesis factor, but its mechanism of action remained unclear. This ambiguity stemmed from the bioinformatic categorization of Zucchini into the phospholipase D family of phosphodiesterases, which includes both lipases and nucleases. To determine the biochemical function of this protein, a number of in vitro studies were designed. Recombinantly purified protein was incubated with liposomes, and the reaction products were followed by MRM-MS (multiple-reaction monitoring–mass spectrometry). Using this method, Zucchini was not found to possess detectable lipase activity compared to controls. Complementary biochemical and structural studies in the Hannon and Joshua-Tor labs, respectively, have since shown this protein to be a nuclease.

Intact Mass Measurements of Methylation Targets

This work was done in collaboration with J. Ipsaro and L. Joshua-Tor (Cold Spring Harbor Laboratory).

Posttranslational modification of proteins has long been established as a means to regulate protein localization and/or function. In the case of PRMT (protein arginine methyltransferase) targets, arginine residues are able to be methylated, or in some cases dimethylated. These posttranslational marks often complete molecular epitopes that can subsequently be recognized by binding partners of the methylation target. To study the activity of one such methyltransferase, an in vitro methylation assay was performed on an array of arginine-containing substrates. The intact masses of these substrates were then assessed by ESI-MS (electrospray ionization–mass spectrometry) to determine the extent of their methylation. While these studies are ongoing, the robustness of the ESI-MS assay unambiguously resolves the number of methylation events on any given protein target and allows for relatively rapid determination of the methylation profiles of a number of targets.

Mapping the Long Noncoding RNA Interactome

This work was done in collaboration with J. Bergmann and D. Spector (Cold Spring Harbor Laboratory).

In an RNA sequencing screen, we have identified a series of nuclear-enriched long noncoding RNAs (lncRNAs) expressed at moderate abundance in mouse embryonic stem cells (ESCs). To identify proteins interacting with two of these lncRNAs, we generated biotinylated lncRNAs and control RNA by in vitro transcription. Biotinylated transcripts were purified, folded in RNA structure buffer, and subsequently incubated with nuclear extracts prepared from either mouse AB2.2 ESCs or NIH-3T3 fibroblasts. Ribonucleoprotein (RNP) complexes were pulled down with streptavidin Dynabeads, washed, and eluted into protein sample buffer. Proteins were separated by SDS-PAGE and visualized by silver staining. Protein bands specifically present or markedly enriched in lncRNA pull-downs versus controls were excised and processed for in-gel tryptic digest and analysis by mass spectrometry. Several proteins of interest were initially identified, and we have decided to stop further in vitro pull-down analysis in favor of alternative approaches aimed at yielding a more comprehensive lncRNA

interactome. To this end, we are in the process of generating ESC stably expressing lncRNA candidates tagged with a bacteriophage MS2 stem-loop cassette. MS2-lncRNPs will be pulled down via the MS2 coat protein fused to enhanced yellow fluorescent protein (EYFP). We plan to use UV cross-linking followed by pull-down of the MS2 RNP, stringent washes, and subsequent two-dimensional multidimensional protein-identification technology (MudPIT) proteomics analysis to describe the "complete" in vivo interactome of our lncRNA candidates.

Role of Phosphoinositide Lipids in Signal Transduction

This work was done in collaboration with A.M. Naguib and L. Trotman (Cold Spring Harbor Laboratory).

Phosphoinositide lipids comprise a small fraction of total cellular lipids but perform essential roles in signal transduction. Among many other functions, these include propagating signals through a variety of signaling axes. We have recently developed a mass spectrometry (MS) technique for the identification and quantification of these important molecules. This high-throughput, automated system for analysis of cellular lipid preparations is capable of analyzing and quantifying the entire "phosphoinositide lipidome" from cells. This technique allows the unambiguous identification of phosphoinositide (PI), phosphoinositol phosphate (PIP), and phosphoinositide bisphosphate (PIP2) species and assessment of their relative abundance. Automation of this method allows interrogation of tens to hundreds of samples in a single run. Phospholipid composition (fatty-acid content, double-bond content, phosphorylation status) has direct implications for membrane thermodynamics and signaling pathways. Wide-scale assessment of these parameters allows understanding of these vital molecules for the first time and can be used to follow the dynamic alterations in their state following a variety of cellular treatments. This includes (but is not restricted to) clinical drug application and changes in nutrient abundance. Presently, we are assessing the phosphoinositide lipidome of a panel of cell lines under a variety of conditions, for the first time generating both a catalog of this lipid species and an understanding of their changing composition.

Proteomic Analysis of Exosomes

This work was done in collaboration with S. Chakrabortty and T. Gingeras (Cold Spring Harbor Laboratory).

Intercellular transference of RNA through exosomes has recently emerged as a possible novel mode of communication between cells and tissues of multicellular organisms. Exosomes are small 40–100-nm vesicles of endocytic origin that are secreted into the extracellular milieu upon fusion of multivesicular bodies (MVBs) to the plasma membrane. Not only are exosomes secreted by many cultured mammalian cells, they also appear to be abundantly present in various body fluids such as serum, plasma, urine, saliva, and cerebrospinal fluid. Because of their endocytic origin, exosomes also contain many proteins involved in membrane transport and fusion (e.g., Rab proteins), multivesicular body biogenesis (e.g., Tsg101 and Alix), integrins, tetraspanins, and heat shock proteins (e.g., hsp70 and hsp90). Exosomes are also known to be enriched in raft-lipids such as cholesterol, sphingolipids, and glycerophospholipids. RNA-Seq analysis of exosomes pointed to the presence of both mRNA and several classes of small regulatory RNA, including microRNA (miRNA). Although several annotated classes of short RNAs were found to be enriched in exosomes, the regulatory machinery in the cell involved in sorting RNA to these extracellular vesicles is unknown. We speculate that specific RNA-binding proteins may be involved in this enrichment process. To address this question, mass spectrometric analysis of purified K562 exosomes was performed. A total of 462 proteins were reproducibly detected between the replicates, of which 101 were characterized as having RNA-binding properties along with other functional associations. These proteins constitute an interesting collection of candidates for further analyses to determine their possible role in RNA enrichment in exosomes, or as convenient biomarkers for the extracellular vesicles.

Phosphorylation of Ago2 in Senescence

This work was done in collaboration with M. Yang and N. Tonks (Cold Spring Harbor Laboratory).

We are looking at the tyrosine phosphorylation change in Ago2 in human primary cells during the process of oncogene-induced senescence. Previous studies with

mutant Ago2 transfected into 293 cells suggested that a specific tyrosine residue (Y393) might be responsive to oncogenic Ras stimulation and regulated by PTP1B (the phosphatase we are also interested in studying). We are attempting to determine if endogenous Ago2 undergoes similar phosphorylation during Ras-induced senescence in human cell lines. To test this, we have immunoprecipitated Ago2 using a specific antibody and are using MRM methods to look at changes in specific phosphorylation of Y393. One technical challenge we are facing is that there are multiple S/T residues on the same tryptic peptide as Y393, which also seem to undergo phosphorylation (as suggested by MS data). This significantly increases the complexity of our MRM transitions and measurements. We are presently trying to confirm that the multiple phosphorylation is indeed present in the immunoprecipitation (IP). To do this, we first immunoprecipitate Ago2 and then perform on-bead digestion with trypsin. The tryptic peptides are then treated with PTP1B (dephosphorylating only pY) or γ-phosphatase (general phosphatase) to see if the treatment causes a corresponding decrease in the signal intensity of the MRM transition masses derived from potential multiple phosphorylated peptides. When this work is completed, we aim to have a rapid MRM method that can be used to follow changes in Y393 phosphorylation as cells move toward senescence.

Characterization of Mouse Models of Vascular Inflammation

This work was done in collaboration with L. Li and N. Tonks (Cold Spring Harbor Laboratory).

In a mouse model of vascular inflammation, we have found that wild-type mice develop skin injury after challenge with lipopolysaccharide–tumor necrosis factor-α (LPS-TNF-α). Interestingly, phosphatase Jsp1 knockout mice were apparently free of skin lesions following insult. Subsequent studies have identified neutrophils as the cells that are responsible for the vascular lesion phenotype, and functional assays have suggested that the adhesion step is Jsp1-dependent, possibly through integrin signaling. To identify the targets of JSP1 in integrin signaling, we have prepared samples of mouse bone-marrow-derived neutrophils, with and without JSP1 and with or without integrin activation, for proteomic analysis. We are particularly interested in the phosphorylation status of Hck and Syk (two major kinase candidates in the integrin signaling in neutrophils). MRM assays were devised to monitor two phosphorylation sites in each of these kinases, one in the activation loop that needs to be phosphorylated to be fully functional and the second in a negative phosphorylation site that needs to be dephosphorylated to activate the kinase. These assays will be used to test the hypothesis that Jsp1 dephosphorylates the negative phosphorylation site to activate the kinase.

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

This work was done in collaboration with R. Sordella and I. Tas (Cold Spring Harbor Laboratory) and B. Stiles and O. Elemento (Cornell University).

More than 220,000 patients are diagnosed each year with lung cancer in the United States, with an overall 5-year survival of ≤15%. This is due to the advanced stage at diagnosis and to difficulties in detecting early tumors. As in the case of prostate-specific antigen (PSA) for prostate cancer, a reliable and easily accessible biomarker would prove invaluable in the early identification of patients with non-small-cell lung cancer (NSCLC). We are implementing a novel strategy to identify biomarkers from the serum of NSCLC patients by mass spectroscopy using iTRAQ (isobaric tagging for relative and absolute quantitation) technology. Unlike previous efforts aimed at identifying serum biomarkers using peripheral blood from NSCLC patients, in our studies, the peripheral blood is being used only as reference, whereas the blood draining directly from the tumor (pulmonary vein) will instead be used for biomarker identification. Because of its proximity to the cancer, the pulmonary venous blood (PVB) is highly enriched for tumor proteins, potentially allowing for greater sensitivity of detection of relevant proteins. In addition, each patient will serve as his/her own control, eliminating many of the sources of heterogeneity that have plagued the serum proteomic field and hampered biomarker discovery. The proteomic signature elaborated from the PVB will then be validated in the peripheral blood using more sensitive targeted techniques such as single- and multiple-reaction monitoring and the clinical applicability tested in an independent set of blood

from patients screened for lung cancer. We have now analyzed pulmonary venous blood, nontumor, and peripheral blood samples from 16 patients using the 8-plex iTRAQ reagent and 17-step MudPIT LC-MS. Preliminary data analysis is being performed now, and we anticipate collection of data from at least 20 additional patients over the next 12 months.

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

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There is a broad consensus that, in essence, every tumor contains distinct cell subpopulations characterized by different morphologies, proliferation rates, levels of antigen expression, and metastatic potentials. This intratumor heterogeneity has direct clinical implications for disease classification and prognosis as well as for treatment efficacy and the development of resistance to cancer therapies. Hence, obtaining a more detailed understanding of the origin of tumor clonal heterogeneity and of molecular features of distinct tumor cell populations is of fundamental relevance and could facilitate the development of more effective therapies. Large-scale analyses of solid tumors have revealed that by the time a cancer is diagnosed, it is composed of billions of cells carrying a large number of genetic mutations. Single-tumor high-resolution genomic maps indicate that these genetic lesions are not uniformly distributed but that within a tumor, discrete populations carry distinct mutations. This suggests that the evolution of genetically diverse populations underpins the clonal diversity observed in tumors.

In addition to this theory, it has been recently proposed that intratumor heterogeneity could be explained by the capability of phenotypically distinct cell populations to transit between conditional interchangeable cell states (i.e., degeneracy). Although the molecular mechanisms regulating degeneracy in tumors are still largely unknown, the degeneracy theory differs from previous models based on the accumulation of genetic aberrations as degeneracy is more rapid and is reversible. This implies that tumor cells can swiftly evolve and easily adapt to an ever-changing environment. To study tumor degeneracy, we used as a paradigm the ontogeny of a tumor cell subpopulation characterized by increased expression of the surface glycoprotein CD44 and the decreased expression of CD24. $CD44^{high}CD24^{low}$ cells have been described in multiple types of tumors including non-small cell lung carcinomas (NSCLCs), glioblastomas, and leiomyosarcomas and tumors of the breast, colon, prostate, and ovaries. These cells are characterized by increased seeding capacity and invasiveness and high self-renewal and metastatic potentials. Interestingly, in the context of cells harboring epidermal growth factor receptor (EGFR) oncogenic mutations, $CD44^{high}CD24^{low}$ cells also have increased resistance to EGFR inhibitors.

An Epigenetic Switch Modulates Cancer Cell-State Plasticity by Regulating miR-335 Expression

Among the many possible regulators of cell degeneracy, we sought to investigate how microRNAs (miRNAs), an important class of posttranscriptional regulators, might be involved in regulating the ontogeny of $CD44^{high}CD24^{low}$ cells. miRNAs have been proposed to limit stochastic fluctuation of biological systems, making them attractive candidates for the regulation of cell-state transitions and maintenance. miRNAs are small (~22 nucleotides) noncoding RNAs constituting a novel class of gene regulators that posttranscriptionally repress gene expression by initiating the degradation or blocking translation of target mRNAs. More than 1000 unique mature miRNAs have been identified in the human genome and each may regulate up to 200 mRNAs. It is estimated that ~30% of all human gene transcripts are targeted by miRNAs, involving these small RNAs in the regulation of virtually all cellular processes (Bartel [2004]; Griffiths-Jones [2006]).

We found that miR-335 has a critical role in the ontogeny of $CD44^{high}CD24^{low}$ cells. Down-regulation of miR-335 was sufficient to induce phenotypic and molecular features characteristic of $CD44^{high}CD24^{low}$ cells. In particular, miR-335 repressed a molecular

circuitry that, when activated, leads to the epithelial-to-mesenchymal transition (EMT) and increased motility and invasion as previously reported; it also results in a switch in immunotype, in cell vulnerabilities, and, in the case of breast cancer cells, in a transition from a luminal to a basal-like subtype. Importantly, miR335 regulates the expression of genes such as *TGF-β*, *WNT5a*, and *IL-6*, which were previously shown to mediate important aspects of the $CD44^{high}CD24^{low}$ cell state. Interestingly, in all $CD44^{high}CD24^{low}$ cells examined, we observed that miR-335 and its host gene *mesoderm specific transcript* (*MEST*) are epigenetically coregulated. Chromatin immunoprecipitation revealed an increased binding of the transcriptional repressor neuron-restrictive silencing factor (NRSF) to the *MEST* promoter region or knockdown of NRSF decreased DNA methylation and induced reexpression of *MEST* isoform 2 and miR-335 in $CD44^{high}$/$CD24^{low}$ cells. These findings suggest that heterochromatin-mediated silencing of *MEST* is sufficient to modulate miR-335 expression and $CD44^{high}/CD24^{low}$ cell-state plasticity. In summary, our studies define a novel mechanism that couples epigenetic inheritance to degeneracy of tumor cell populations. In principle, this could provide a molecular basis to explain the adaptability of tumor cells to their ever-changing environment and to drug treatment.

p53Ψ Is a Neomorphic p53 Isoform That Reprograms Cells toward the Acquisition of Metastatic-Like Features Despite Lacking Transcriptional Activity

p53 is a highly evolutionarily conserved transcription factor whose origin can be traced back to early metazoans and predates the cnidarian-bilaterian divergence, ~700 million years ago. Somatic *p53* mutations occur in almost every type of cancer, at rates from 38% to 50% depending on the tumor type. Mutations are more frequent in advanced-stage cancer or in cancer subtypes that are highly metastatic. In cancers with low mutation rates, p53 is often inactivated by alternative mechanisms.

In response to strong cellular stresses such as DNA damage or oncogenic signals, p53 regulates the expression of a large cohort of genes that affect cell cycle arrest, senescence, and apoptosis. Recent findings have uncovered additional roles of p53 under basal physiological conditions, including regulation of development, reproduction, metabolism, and self-renewal capacity. In this regard, of particular interest is the recent publication indicating that p53 represses at steady state the surface *trans*-membrane glycoprotein CD44.

CD44 is a *trans*-membrane glycoprotein essential for the motility of bone-marrow-derived cells, fibroblasts, and certain cancer cells. In addition, CD44 modulates cell growth by regulating the availability of growth factors and by altering cell metabolism. Hence, it is not surprising that CD44 has a critical role during tissue remodeling, fibrosis, and metastatic dissemination.

The observation that upon injury, CD44 is increasingly expressed in tissues that are wild type for p53 led us to investigate the regulation of p53 in these cells. To probe a possible regulation of p53 in $CD44^{high}$ cells, we used a murine lung injury paradigm based on intraperitoneal naphthalene administration. Administration of naphthalene causes rapid necrotic change within Clara cells of the terminal and respiratory bronchioles due to conversion of the drug into a toxic form by the cell-specific microsomal enzyme Cyp2F2. Consistent with a decreased activation of p53 in $CD44^{high}$ cells, expression analysis of known p53-regulated genes revealed decreased expression of *Triap1, Sens1, Sens2, Pgam1, Pgam2, Sco1, Sco2, Tigar,* and *Gpx1* in $CD44^{high}/CD24^{low}$ cells.

Prompted by this observation, we next determined the expression of p53 in these cells. Reverse transcriptase–polymerase chain reaction (RT-PCR) analysis using primers localized in exons 6 and 8 indicated a less intense and slower migrating band in the $CD44^{high}CD24^{low}$ cells. Sequence analysis revealed the existence of a novel *p53* mRNA variant generated by the usage of an alternative 3′ splice acceptor site within intron 6 that is highly conserved across species. We referred to this p53 variant as *p53Ψ*. We confirmed this observation by RT-PCR analysis using primers that are specific for this isoform in multiple lung tissue samples upon injury.

p53 is mutated in more than 50% of cancers; hence, we undertook to determine whether *p53Ψ* represents a novel mode of regulation of p53 in human tumors. Notably, ~30% of early-stage NSCLC tumors are characterized by a $CD44^{high}CD24^{low}$ immunotype.

To this end, we assessed expression of *p53Ψ* and *p53FL* by an RNA fluorescence in situ hybridization (FISH) technique in a tissue microarray composed of

NSCLC tissues from 233 patient cases. The majority of these tumors are early-stage adenocarcinomas. p53Ψ- and p53FL-specific probes were designed and validated in tumor-derived cell lines. When lung tumor samples were stained for *p53Ψ,* we observed significant heterogeneity of staining. Overall, we found that although certain tumors were clearly enriched for *p53Ψ* (22.3%), others were devoid of *p53Ψ* expression. Interestingly, tumor cores positive for *p53Ψ* tended to be enriched for $CD44^{high}/CD24^{low}$ cells.

As described above, although with different representation, cells characterized by a $CD44^{high}CD24^{low}$ immunotype have been described in virtually all tumor-derived cell lines. Because in principle these $CD44^{high}CD24^{low}$ cells are genetically identical to the cells of all other immunotypes that are present in the main population, we undertook to determine whether p53Ψ was also in these cells regulated by alternative splicing. RT-PCR analysis of fluorescence-activated cell sorting (FACS) from multiple tumor-derived cell lines that are wild type for p53 revealed the presence of *p53Ψ* expression exclusively in $CD44^{high}CD24^{low}$ cells.

In summary, altogether, these observations indicated the existence of a novel p53 isoform generated through an alternative splicing event that is conserved across species. At the molecular level, the use of an alternative 3′ splice site in intron 6 generates a previously uncharacterized p53 isoform that is incapable of sequence-specific DNA binding and *trans*-activation of canonical p53 target genes. Yet, expression of this isoform, p53Ψ, attenuated the expression of E-cadherin but induced expression of markers associated with EMT and enhanced the invasive and prometastatic capacity of cells through a novel mechanism involving the activation of ROS by CpD. Although it was previously shown that p53 controls ROS (reactive oxygen species) levels through multiple mechanisms, the fact that ectopic expression of p53Ψ in p53FL cells was unable to modify the expression of *Tigar, Sens1, Sens2, Gpx1, Bax, and Puma* strongly supports the existence of a novel transcription-independent mechanism of ROS production by p53Ψ. Consistent with these observations, we found that increased expression of p53Ψ in tumors correlated with poor prognosis and that its expression was increased in $CD44^{high}/CD24^{low}$ cells both in tumors and in injured tissues. In these cells, the expression of p53Ψ at the expense of p53FL, although antagonizing p53 tumor suppressor activities, may act to facilitate proliferation and invasion into damaged tissue to facilitate wound repair. The observation that p53Ψ is expressed both in highly metastatic cells and as part of tissue injury/repair mechanisms supports previous findings suggesting tumors retain physiological responses of normal tissues and that the metastatic program is part of an abnormal activation of tissue injury/repair mechanisms. In this regard, fascinatingly, tumors have been referred to as "wounds that never heal." The remarkably similar activities of p53Ψ with certain p53 "gain-of-function" mutants, which also reduce E-cadherin expression and facilitate invasion, also suggests that these p53 mutations "highjack" a regulated and reversible program that in normal tissues coordinates the tissue damage response and contributes to the biology of p53 mutations during tumorigenesis. Hence, in principle, it suggests a physiological origin for p53 "gain-of-function" mutants.

PROTEIN TYROSINE PHOSPHATASES AND THE CONTROL OF SIGNAL TRANSDUCTION

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

During the last year, Ben Boivin left to take up a position as Assistant Professor in the Montreal Heart Institute and Ulla Schwertassek went back to Germany to a position in the Fraunhofer Institute of Cell Therapy and Immunology in Leipzig.

Identification and Characterization of Small-Molecule Inhibitors of PTP1B

A major focus of research in the lab has been the investigation of novel approaches to therapeutic inhibition of PTP1B, in particular for application to HER2-positive breast cancer. The *PTP1B* gene is located at chromosome 20q13, which is a region that is frequently amplified in breast cancer and associated with poor prognosis. It has also been reported that PTP1B is overexpressed in breast tumors together with the oncoprotein tyrosine kinase HER2. Mice expressing activated alleles of HER2 in mammary glands develop multiple mammary tumors and frequent metastases to the lung; however, when such mice were crossed with *PTP1B*-knockout mice, tumor development was delayed and the incidence of lung metastases was decreased. Conversely, targeted overexpression of PTP1B alone was sufficient to drive mammary tumorigenesis in mice. These observations illustrate that PTP1B plays a *positive* role in promoting signaling events associated with breast tumorigenesis, which suggests that inhibition of PTP1B function may represent a novel therapeutic strategy to address HER2-induced mammary tumorigenesis and malignancy.

Several potent, small-molecule inhibitors of PTP1B have been developed in industry and academia; however, these inhibitors, which target the active site of the enzyme, are highly charged and consequently have poor bioavailability and limited drug development potential. Therefore, there is a pressing need for an alternative approach to generate inhibitors against PTP1B that do not involve the active site of the protein. We have identified a novel allosteric inhibitor of PTP1B, a small-molecule natural product that binds to the noncatalytic, carboxy-terminal portion of the phosphatase. We have defined a unique mechanism for inhibition of PTP1B by this molecule (Fig. 1), and, in collaboration with Wolfgang Peti (Brown University), we have used nuclear magnetic resonance (NMR) to solve the first ensemble structure of PTP1B containing this intrinsically disordered carboxy-terminal segment. We have demonstrated that this PTP1B inhibitor antagonizes migration preferentially in HER2-positive cells. In addition, we showed that the formation of hyperplastic

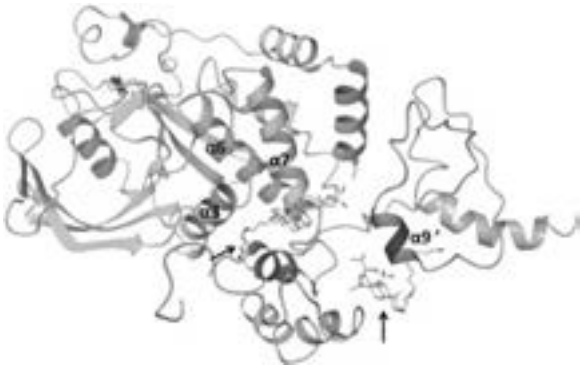

Figure 1. Structure model for PTP1B (residues 1–405) with two molecules of the allosteric inhibitor MSI-1436 (marked with arrows) docked to the two putative binding sites. Important structural features within PTP1B are highlighted.

structures in three-dimensional culture following activation of HER2 in MCF-10A cells was attenuated by treatment of the cells with the PTP1B inhibitor. Most strikingly, we have also demonstrated that the inhibitor attenuates HER2 signaling in animal models. In tumor xenografts, in which BT474 cells were injected orthotopically into the fat pad of beige SCID mice, inhibitor treatment (at 5 mg/kg) attenuated tumorigenesis when administered either at the time the cells were deposited in the mammary fat pad or following the appearance of palpable tumors. Finally, in mouse mammary tumor virus (MMTV)-NDL2 transgenic mice, which express an activated mutant form of HER2 and develop mammary tumors and lung metastases that display features of human breast cancer, treatment with the inhibitor resulted in extensive inhibition of tumor growth, and, perhaps most importantly, metastasis to the lung was essentially abrogated. These data reinforce the therapeutic potential of inhibiting PTP1B by this allosteric mechanism to antagonize HER2-induced signaling and tumorigenesis. The molecule has already been tested in a Phase-1 trial and is extremely well tolerated; the effects observed in mice are achieved at ~20% of the maximum dose that has been administered to patients. Currently, we are working with scientists at the Monter Cancer Center at North Shore LIJ to take this inhibitor into clinical trials for HER2-positive cancer. Although it is an exciting therapeutic candidate, it is a natural product for which synthesis is not straightforward. Consequently, more tractable, novel inhibitors of PTP1B that act with the same mechanism may be of benefit. We have developed an assay of PTP1B that can be applied to high-throughput screening of libraries of small molecules. In collaboration with Fraser Glickman at The Rockefeller University, we have completed a screen of their library of 180,000 compounds and have identified 350 "hits" from the screen. Of these, 28 show properties similar to those of the natural product, but their structures are distinct. These are being validated further for their activity in vitro, in cells, and in animal models of HER2-positive cancer.

Role of PTP1B in Oncogenic RAS-Induced Senescence

Oncogene expression in normal cells can cause an irreversible growth arrest called oncogene-induced senescence, which serves as a barrier to cancer initiation. Elevated reactive oxygen species have been detected in the senescent cells, and they are believed to have a second messenger function in transducing signals. We demonstrated that PTP1B underwent redox regulation during oncogenic RAS-induced senescence in IMR90 cells. Inhibition of PTP1B function was important for oncogene-induced senescence, and overexpressing PTP1B in RAS-expressing cells induced senescence bypass. We found that AGO2, an Argonaute protein that is required for RISC (RNA-induced silencing complex) function in the RNA interference (RNAi) pathway, was a substrate of PTP1B in senescent cells. Reversible oxidation of PTP1B during senescence caused hyperphosphorylation of AGO2 on its Y393 site and lowered the binding capacity of AGO2 for microRNAs (miRNAs). These data suggest that PTP1B is involved in the control of p21 expression, a key regulator of oncogene-induced senescence, in a manner mediated by AGO2 tyrosine phosphorylation.

PTP Function in Breast Cancer Models

These projects are part of a long-standing collaboration with Senthil Muthuswamy. Progress has been made on several projects, including identifying a role for receptor protein tyrosine phosphatase δ (RPTPδ) in metastasis induced by loss of the *Missing in Metastasis* gene, defining the role of PTPD2/PTPN14 as a positive regulator of HER signaling and the development

of mouse models to explore further a tumor suppressor function of PTPN23. In addition, this year we published a study revealing an important tumor suppressor role for PTPRO (protein tyrosine phosphatase receptor type O) in breast cancer. We noted that when PTPRO, which is up-regulated in mature MCF-10A mammary epithelial three-dimensional structures, was suppressed by RNAi, the proliferation arrest that occurs during acinar morphogenesis was inhibited. Furthermore, we demonstrated that the receptor tyrosine kinase HER2 is a direct substrate of PTPRO and that loss of PTPRO increased HER2-induced cell proliferation and transformation, together with tyrosine phosphorylation of HER2. Moreover, in patients with HER2-positive breast tumors, low PTPRO expression correlated with poor clinical prognosis compared to HER2-positive patients with high levels of PTPRO. Thus, these data illustrate that PTPRO is a novel regulator of HER2 signaling and a novel prognostic marker for patients with HER2-positive breast cancers.

Tyrosine Phosphorylation-Dependent Signaling in Ovarian Cancer Cells

Ovarian cancer, which is the leading cause of death from gynecological malignancies, is a heterogeneous disease known to be associated with disruption of multiple signaling pathways. Nevertheless, little is known regarding the role of protein phosphatases in the signaling events that underlie the disease; such knowledge will be essential to gain a complete understanding of the etiology of the disease and how to treat it. We have demonstrated that PTP1B was underexpressed in a panel of ovarian-carcinoma-derived cell lines, compared to immortalized human ovarian surface epithelial cell lines. Stable restoration of PTP1B in those cancer cell lines substantially decreased cell proliferation, migration, and invasion, as well as anchorage-independent survival. Mechanistically, the pro-survival insulin-like growth factor-1 receptor (IGF-1R) signaling pathway was attenuated upon ectopic expression of PTP1B. This was due to dephosphorylation by PTP1B of IGF-1R β-subunit and BRK/PTK6, a SRC-like protein tyrosine kinase that physically and functionally interacts with the IGF-1R β-subunit. Restoration of PTP1B expression led to enhanced activation of BAD, one of the major pro-death members of the BCL-2 family, which triggered cell death through apoptosis. Conversely, inhibition of PTP1B with a small-molecular inhibitor, MSI-1436, increased proliferation and migration of immortalized HOSE (human ovarian surface epithelial) cell lines. These data reveal an important role for PTP1B as a negative regulator of BRK and IGF-1Rβ signaling in ovarian cancer cells.

Characterization of Novel Kinase Inhibitors

In collaboration with Drs. Darryl Pappin and Jim Watson here at CSHL and Mate Hidvegi and his colleagues from Budapest, this lab led a project to identify the active components of Avemar®, a proprietary fermented wheat germ extract (FWGE) nutraceutical that has been shown to display significant antiproliferative effects and to trigger tumor cell death through apoptosis. Clinical data reveal significant benefits to patients from treatment with FWGE, including in combination with existing cancer therapies. Through this collaboration, a novel small-molecule protein kinase inhibitor (CSH-4044) has now been isolated from FWGE and characterized. This inhibitor has both a unique structure and a unique specificity for PIM (proviral integration site for Moloney murine leukemia virus) and DYRK (dual specificity tyrosine-regulated kinase) when assayed against a panel of 140 distinct protein kinases. Current efforts are focused on testing this inhibitor in appropriate cancer models and optimizing its structure, both to enhance potency, specificity, and bioavailability, as well as to improve drug-like characteristics. In collaboration with Dave Tuveson, CSH-4044 is being tested in cell and animal models of pancreatic cancer, using inhibition of PIM and DYRK as a new approach to inhibiting signaling downstream of KRAS. In addition, it is being tested in collaboration with Chris Vakoc in his leukemia models. It is anticipated that this study will validate a novel therapeutic candidate for treatment of these cancers.

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UNDERSTANDING LETHAL PROSTATE CANCER

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We focus on the fundamental understanding of the molecular mechanisms behind the transition from indolent to lethal metastatic prostate cancer. Our aim is to understand the principles of prostate cancer progression and to use this information to recreate lethal disease in faithful mouse models that can be studied and used for preclinical trials that help us develop better therapies. PTEN is a haploinsufficient tumor suppressor: Partial loss or degradation results in growth advantage, and in contrast, we found that complete loss triggers senescence arrest. These findings raised questions about whether and when senescence plays a role in human prostate cancer, because it was assumed that senescence is a feature of precancerous lesions. We now have answered this question thanks to the past 3 years of research and revealed that the senescence response is a barrier to metastasis. Thus, we have established a biological principle that underlies the distinction between indolent (senescence-competent) and lethal (postsenescent) disease.

Through a combination of mouse and human genetics, we formulated molecular guidelines for each disease stage: (1) PTEN haploinsufficiency or protein degradation triggers prostate cancer formation through partial AKT pathway activation. (2) In contrast, lethal *PTEN* mutant disease acquires strong pathway activity through, for example, deletion of *PTEN* and *PHLPP1* tumor suppressors and inactivation of the senescence response genes *p53* and *Rb1.* On the basis of these insights, we now analyze patient samples in order to separate them into the two categories. At the same time, we develop novel types of animal models that allow us to better model the disease.

Molecular and Genetic Analysis of Prostate Cancer Evolution

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

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

Comprehensive analyses of changes in transcription, proteome, and secretome have led to identification of signature responses, which are being validated in human prostate cancer specimens (in collaboration with Dr. Tewari).

To study the genetic evolution of prostate cancer, we collaborate with the teams of Drs. Wigler and Hicks, who have developed copy-number analysis at the single-cell level. This approach allows us to identify the genetic makeup and to infer migration of clones of cells within a surgically removed prostate cancer sample. Through this approach, we are able to learn at what stage of cancer progression key criteria for breaking the senescence response are fulfilled.

To validate alterations and key genetic events that are found in the human samples, we are developing more flexible mouse modeling techniques. RNA interference (RNAi) has been shown to recapitulate key features of gene knockout models, especially given the fact that the majority of tumor suppressors are found in hemizygous deletions in cancer. RNAi-based models of Pten/p53 loss have been generated and are being followed up for disease initiation and progression characteristics. These will serve as a platform to study effects of gene alterations derived from human.

Living with Lethal Genes

M. Chen, A. Naguib, T. Herzka, W. Zheng [in collaboration with S.-S. Tan and J. Howitt, The University of Melbourne, Australia]

PTEN is the major negative regulator of PI3K signaling with cell-specific functions that go beyond tumor

suppression. In fact, it is surprising to find a gene that prevents cells from growing and can cause them to die, yet is constitutively expressed in most healthy tissues. This raises the fundamental question of how the *PTEN* gene is suppressed to allow for normal growth, tissue repair, and development. We are interested in this question because many cancer types reveal degradation of PTEN protein while the gene remains intact. Our hypothesis is that such cancers indirectly target PTEN by loss of genes that are essential for PTEN maintenance. Thus, understanding the normal processes behind PTEN regulation may unearth critical cancer genes.

Ischemic injury results from insufficient blood flow to organs or tissues, examples of which include stroke and myocardial infarction. PTEN, under normal physiological conditions, acts to antagonize PI3K/AKT-mediated signaling and thereby promotes growth arrest and can trigger apoptosis. However, subsequent to ischemic injury, limiting the ability of wounded tissue to renew, or promoting cell death, would be detrimental to the healing effort. To our surprise, we learned that nuclear seclusion of PTEN is one mechanism to temporarily suppress its function: In collaboration with experts in mouse models for stroke, we have demonstrated that cytoplasmic Pten is translocated into the nucleus of neurons following cerebral ischemia. Critically, this transport event is dependent on the surge in the Ndfip1 protein, as neurons in Ndfip1-deficient mice fail to import Pten. Ndfip1 binds Pten, resulting in enhanced ubiquitination by Nedd4 E3 ligases, an event that we previously showed to control import. In vitro, Ndfip1 overexpression increases the rate of Pten nuclear import detected by photobleaching experiments, whereas *Ndfip1*$^{-/-}$ fibroblasts show very poor import rates. In vivo, *Ndfip1*-mutant mice suffer larger infarct sizes associated with suppressed pAkt activation. These findings have unraveled the first physiological stimulus to trigger shuttling of Pten into nuclei. Moreover, they suggest that getting PTEN out of the cytoplasm and into the nucleus for a limited time after stroke is critical for neuronal survival and prevents irreversible brain damage. These findings may thus open novel avenues for therapeutic intervention after stroke, centered around ensuring proper trafficking of PTEN.

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CANCER MEDICINE LABORATORY/CANCER THERAPEUTICS INITIATIVE

D. Tuveson I.C. Chio, B. Creighton, B. Delcuze, D. Engle, C. Hwang, S. Moran, E.D. Olund, Y. Park, M. Spector, H. Tiriac, K. Wright, K. Yu

Our laboratory uses mouse and tissue models of neoplasia to explore the fundamental biology of these diseases and thereby identify new diagnostic and treatment strategies. Our main focus is pancreatic cancer, a lethal malignancy that has eluded clinical solutions despite intensive study. Our approaches at CSHL run the gamut from designing new model systems of disease to developing new therapeutic and diagnostic platforms for evaluation in preclinical and clinical settings. For example, we have adopted a new method of culturing tissue fragments indefinitely in cell culture, enabling deep analysis with genetic and pharmacological probes. Second, therapeutic experiments in mouse models have revealed an important role of redox metabolism and stromal interactions on influencing therapeutic response. We also have a strong link to clinical trials locally and internationally, with confirmation in early-phase trials our immediate goal. Collectively, our strategy in the preclinical and clinical arena is codified as the "Cancer Therapeutics Initiative," and this initiative will provide these same approaches to the entire CSHL cancer community.

Cancer Diagnostics

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

Cancer Therapeutics

This work was done in collaboration with K. Yu, Cold Spring Harbor Laboratory/Memorial Sloan-Kettering Cancer Center; A. Krainer and G. Hannon, Cold Spring Harbor Laboratory. Novel therapies and drug delivery methods are being explored for pancreatic cancer patients.

Development of Methods to Improve Drug Delivery in Pancreatic Ductal Adenocarcinoma

Pancreatic cancer is refractory to conventional and targeted agents. We and other investigators had found

that the drug resistance demonstrated by pancreatic ductal adenocarcinomas (PDACs) is caused by both biochemical signaling pathways in neoplastic cells and the biophysical compression of the tumor vasculature to limit angiogenesis and tumor perfusion. A sensitive method was developed to measure the intracellular active metabolite of gemcitabine, gemcitabine triphosphate (dFdCTP), and chemotherapy delivery was found to be limited to the stroma-rich tumor tissue due to a deficient and compressed vasculature. Hedgehog inhibition and stromal digestion with pegylated hyaluronidase (PEG-PH20) led to both increased perfusion and chemotherapy delivery in PDAC tumor tissues and transient increases in GEMM survival. These findings became the basis of a clinical trial using the hedgehog inhibitor IPI926 in combination with gemcitabine for patients with metastatic PDAC, but unfortunately, the first randomized trial was negative and the analysis is ongoing. Early-phase clinical trials with gemcitabine in combination with PEG-PH20 are under way, with interim results expected shortly.

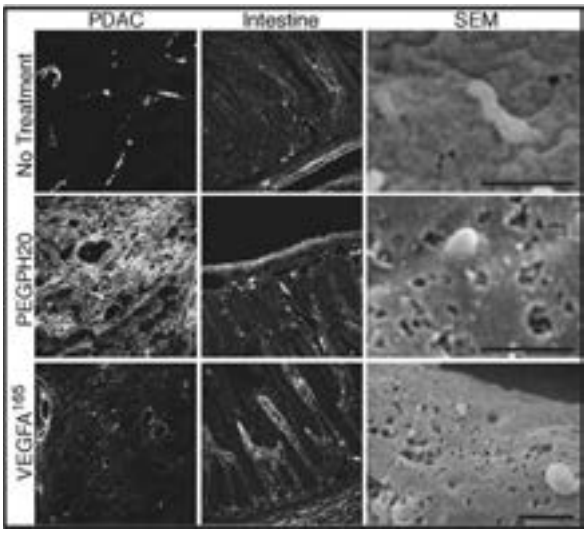

Figure 1. 2-MDa lectin (yellow) is specifically delivered to PDAC tissue following PEG-PH20 treatment, accompanied by endothelial fenestrae. VEGF-A also induces fenestrae and nonspecific delivery to intestinal villi. (Scale bar, 0.5 µm.)

Interestingly, PEG-PH20 treatment also specifically increased the delivery of high-molecular-weight agents including 2 MDa dextran to PDAC tissue. Scanning and transmission electron microscopy revealed that intratumoral endothelial cells had a quiescent morphology in untreated mice, and became markedly fenestrated following PEG-PH20 treatment, reminiscent of the effects of vascular endothelial growth factor (VEGF)(Fig. 1; Jacobetz et al. 2012). This observation allows us the opportunity to evaluate novel high-molecular-weight therapeutic agents that are otherwise difficult to deliver to PDAC tissues, including antibodies and nucleic acids. Dr. Ken Yu, working with our laboratory as the CSHL clinical fellow and an attending medical oncologist at MSKCC, has written a proof-of-concept clinical trial to assess whether PEG-PH20 and related stromal disruption approaches will increase the effectiveness of biological therapeutics in pancreatic cancer patients. His trial will be conducted to establish whether PEG-PH20 can increase the delivery of an FDA-approved anti-epidermal growth factor receptor (EGFR) antibody (cetuximab) in patients scheduled to undergo surgery for resectable pancreatic cancer. The design will administer PEG-PH20 2 days before surgery, treat with therapeutic antibody 24 h later, and then surgery following 24 h later. Comparisons will be made to patients who do not receive PEG-PH20, and resected tumors will be assessed for the delivery of the antibody and any biochemical alterations by immunohistochemistry (IHC). Cetuximab is the first antibody to be considered because it binds to EGFR that is expressed by the majority of PDAC cells, and it can be easily detected through the chimeric murine portion of the antibody. Cetuximab was not shown to alter the effects of gemcitabine treatment in PDAC patients in a recent SWOG trial, potentially because it is not delivered optimally. Positive findings will motivate the assessment of additional agents in more advanced preclinical and clinical trials, including neoadjuvant trials in locally advanced pancreatic cancer patients and mice to improve resectability and survival. Besides the limitations in drug delivery observed in pancreatic tumors, we also lack effective methods that inhibit the *K-RAS* oncogene responsible for cancer initiation and maintenance. In the cancer therapeutics initiative, we are seeking new methods that will neutralize the effects of oncogenic *K-RAS* in pancreatic cancer by evaluating unusual nucleic-acid-based payloads targeting *K-RAS*. These approaches will comprise antisense oligonucleotides and short interfering RNAs (shRNAs) that were developed with Adrian Krainer's and Greg Hannon's lab, respectively.

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

This work was done in collaboration with H. Clevers, Hubrecht Institute, Utrecht, the Netherlands. We established a method to culture matched normal and malignant cells from the same murine tissue in three-dimensional "organoid" cultures. This approach was based on the pioneering work of Dr. Hans Clevers, who demonstrated that intestinal villi could be indefinitely propagated in three-dimensional culture when forming functional organoids. The Clevers and Tuveson laboratories collaborated to develop a method of preparing and propagating pancreatic ductal organoid structures from normal and malignant mouse pancreata (Fig. 2), and we are in the process of establishing conditions for growing human pancreas organoids from matched normal and disease tissue. These cultures will enable the less costly, high-throughput assessment of small-molecule and shRNA screens in cell culture, prior to characterization in vivo. Additionally, they can be directly characterized at the genomic, transcriptomic, and proteomic levels to determine the molecular correlates of therapeutic response. This approach will be compared to routine two-dimensional tissue culture systems, and in parallel, they will be compared to the traditional patient-derived xenograft (PDX) models. If organoids offer advantages to PDX models, we will determine how to extend this to other organ types and how to best utilize this technology for patient benefit. For example, serial organoids prepared from patients under therapy may reveal a model system for interrogating drug resistance and identifying new targets of drug sensitivity. We also have found that cancer cell lines readily adapt to growth in organoid conditions, suggesting that this approach may work for circulating tumor cells.

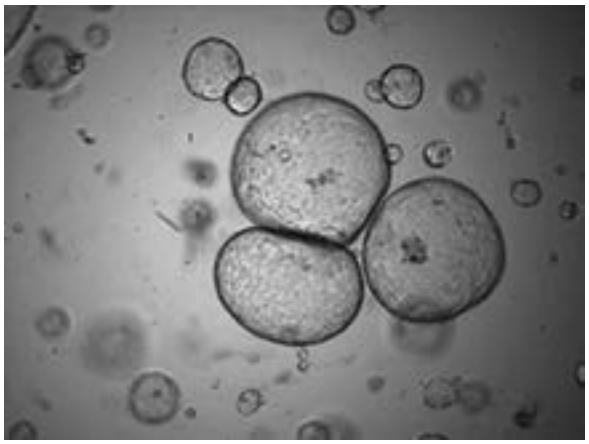

Figure 2. Organoids prepared from mouse pancreas tissue (4×).

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RAS AND RHO GTPases AND THE CONTROL OF SIGNAL TRANSDUCTION

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

DOCK7 Interacts with TACC3 to Regulate Interkinetic Nuclear Migration and Cortical Neurogenesis

DOCK7 is a member of the evolutionarily conserved DOCK180-related protein superfamily, which we initially identified as a novel activator of Rac GTPases. Significantly, sequence variations in DOCK7 have been reported in schizophrenia patients; however, the role(s) of DOCK7 in neuronal development and/or function has remained largely elusive. We had found that DOCK7 is highly expressed in major regions of the brain, including hippocampus and cortex, during early stages of development, and, importantly, that the protein is asymmetrically distributed in unpolarized hippocampal neurons and becomes selectively expressed in the axon. We then obtained evidence for a key role of DOCK7 in controlling early steps of axon development in cultured hippocampal neurons. Knockdown of DOCK7 prevents axon formation, whereas overexpression induces the formation of multiple axons. We further demonstrated that DOCK7 and Rac activation leads to phosphorylation and inactivation of the microtubule destabilizing protein stathmin/Op18 in the nascent axon and that this event is important for axon development. Thus, our findings unveiled a novel pathway linking the Rac activator DOCK7 to a microtubule regulatory protein and highlight the contribution of microtubule dynamics to axon development.

More recently, we extended our studies to the characterization of DOCK7 function in the development of cortical pyramidal neurons in the mouse embryonic neocortex, which interestingly led to the discovery of a key role for DOCK7 in cortical neurogenesis. Cortical pyramidal neurons primarily derive from radial glial progenitor cells (RGCs) located in the ventricular zone (VZ) of the dorsal telencephalon, and they migrate radially along RGC processes from the VZ, through the intermediate zone (IZ), to the cortical plate (CP) to form the six-layered neocortex. Notably, the genesis of neurons in the developing neocortex critically relies on the ability of RGCs to switch from proliferative to differentiative neuron-generating divisions; the molecular mechanisms that control this switch in a correct temporal manner are, however, not well understood. We found that DOCK7 has a key role in the regulation of RGC proliferation versus differentiation. In particular, we found that silencing of DOCK7 in RGCs of developing mouse embryos impedes neuronal differentiation and maintains cells as cycling progenitors. In contrast, DOCK7 overexpression promotes RGC differentiation to basal progenitors and neurons.

We further obtained evidence that DOCK7 influences the mode of RGC division and thereby neurogenesis by controlling a unique property of RGCs, namely, interkinetic nuclear migration (INM)—a process where RGC nuclei undergo a cell-cycle-dependent change in position along the apical-basal axis of the VZ, with proliferative signals apparently being high at the apical (ap) side and neurogenic signals

high at the basal (bl) side. Knockdown of DOCK7 in RGCs accelerates bl-to-ap INM, leading to extended apical residence of RGC nuclei and apical mitoses that produce two RGCs. Ectopic DOCK7 expression, on the other hand, impedes bl-to-ap INM, causing an extended basal residence of RGC nuclei and ectopic mitoses that produce two neurons. Intriguingly, DOCK7's role in INM and neurogenesis does not involve its GEF (guanine exchange factor) activity, but instead requires its interaction with the centrosome- and mitochondrial (MT)-associated protein TACC3. We found that DOCK7 exerts its effects by modulating the MT growth-promoting function of TACC3. Thus, DOCK7 interacts with TACC3 to control INM, thereby governing RGC proliferation versus differentiation and genesis of neurons during cortical development, processes associated with numerous neurodevelopmental disorders.

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

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

We have begun to unveil the function of OPHN1 both at the pre- and postsynaptic site of the hippocampal CA3-CA1 synapse. We previously described that during early development, presynaptic OPHN1 is important for efficient retrieval of synaptic vesicles, whereas postsynaptic OPHN1 has a key role in activity-dependent maturation and plasticity of excitatory synapses, suggesting the involvement of OPHN1 in normal activity-driven glutamatergic synapse development. Interestingly, we more recently found that postsynaptic OPHN1 also has a critical role in mediating a form of plasticity (mGluR-LTD) that relies on the activation of group I metabotropic glutamate receptors, which consist of mGluR1 and mGluR5 in CA1 hippocampal neurons. Alterations in this form of plasticity have been linked to drug addiction and cognitive disorders. A key characteristic of mGluR-LTD is its dependence on rapid protein synthesis; however, the identities of the proteins mediating LTD have remained largely elusive. We obtained evidence that OPHN1 expression is translationally induced in dendrites of CA1 neurons within 10 min of mGluR activation and that this response is essential for mGluR-dependent LTD. Specifically, acute blockade of new OPHN1 synthesis impedes mGluR-LTD and the associated long-term decreases in surface AMPARs. Interestingly, the rapid induction of OPHN1 expression is primarily dependent on mGluR1 activation and is independent of fragile-X mental retardation protein (FMRP).

Importantly, we further demonstrated that OPHN1's role in mediating mGluR-LTD is dissociable from its role in basal synaptic transmission. mGluR-LTD and the associated long-term decreases in surface α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs) are dependent on OPHN1's interaction with endophilin A2/3, whereas regulation of basal synaptic strength requires OPHN1's Rho-GAP activity and association with Homer 1b/c proteins. As to how OPHN1 could mediate the strengthening of synapses via interactions with Homer 1b/c and RhoA, we previously showed that OPHN1 becomes enriched in spines in response to synaptic activity and *N*-methyl-D-aspartate receptor (NMDAR) activation, where by locally modulating RhoA/Rho kinase activities (i.e., in the proximity of AMPARs), it contributes to the stabilization of AMPARs, thereby facilitating synaptic maturation and plasticity. Homer 1b/c proteins, on the other hand, were shown to serve a critical role in the positioning of the endocytic zone (EZ) near the postsynaptic density (PSD). The close juxtaposition of EZ and PSD enables a local cycle of endocytosis and recycling in response to synaptic activity that maintains synaptic AMPARs at postsynaptic sites. Interestingly, we recently found that OPHN1, via its interaction with Homer 1b/c, contributes to the positioning of the EZ near the PSD and hence to the recycling of AMPARs to the PSD. Thus, a conceivable scenario is that OPHN1, via its interactions with Homer 1b/c and RhoA, regulates the recycling and stabilization of AMPARs at the synapse, thereby controlling activity-dependent

maturation and strengthening of synapses. We are currently further investigating this.

Together, these findings point to a multifunctional role for OPHN1 at CA1 synapses. Independent of its role in activity-driven glutamatergic synapse development, regulated OPHN1 synthesis has a critical role in mGluR-dependent LTD. Thus, it is conceivable that, on one hand, OPHN1 might have an important role in synapse maturation and circuit wiring during early development; on the other hand, the regulated OPHN1 synthesis could operate during adulthood to weaken synapses in response to behaviorally relevant stimuli.

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

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

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

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REGULATION OF MALIGNANT GLIOMA HETEROGENEITY AND LINEAGE DEVELOPMENT

H. Zheng B. Guo A. Vaseva
S. Klingler L. Zhang

Malignant glioma is the most common type of brain tumor, and they are currently incurable. The lack of effective treatments highlights the urgent need for new therapeutic approaches and targets. The long-term goal of our research is to define the complex biology of malignant glioma pathogenesis with the aim of translating this knowledge into patient benefits. Along this line, our research has been focused around two major areas: (1) We develop various genetically engineered animal models to recapitulate the process of human glioma pathogenesis and utilize the animal models as tools to investigate in vivo tumor initiation and progression, as well as their response to various therapeutic treatments; and (2) we apply an integrated approach combining model system, stem cell biology, and RNA interference (RNAi) to identify the molecular and developmental pathways relevant to glioma pathogenesis and treatment. We are particularly interested in the genetic and epigenetic programs involved in regulation of normal neural progenitor and glioma cell-fate determination. We believe that an improved understanding of the developmental programs governing the self-renewal and differentiation processes along the neural progenitor-glial axis and, by extension, the glioma initiation cells-progeny axis will be instrumental in guiding future development of efficient treatments targeting this dreadful disease.

Characterizing EGFR-Targeted Therapeutic Resistance

As a signature genetic event of malignant gliomas, epidermal growth factor receptor (EGFR) amplification/mutation is present in ~40%–50% of human patient samples, making it a compelling candidate for targeting therapy. Surprisingly, the clinically approved small-molecule EGFR inhibitors for treating other types of cancers have so far shown little therapeutic efficacy to malignant glioma patients in dozens of clinical trials. The missing concordance between clinical response and *EGFR* amplification/mutation status in malignant glioma patients has raised uncertainties about EGFR as a viable therapeutic target for malignant gliomas. To address the questions, we developed a novel maligant glioma mouse model driven by the tetracycline-induced overexpression of a malignant glioma-specific mutant EGFR* with concurrent brain-specific inactivation of *Ink4a/Arf* and *Pten*. The tightly tetracycline-controlled mutant EGFR expression in this case ensures a faithful model system to mirror EGFR targeting therapy and therefore provides us an opportunity to dissect the resistance mechanism(s) under in vivo settings. Using both genetic and pharmacologic approaches, we demonstrated that a large population of glioma cells, particularly EGFR-overexpressing cells, remains dependent on aberrant EGFR signaling. Inhibition of *EGFR** induction in the tumor cells by doxycycline treatment effectuated significant tumor regression, validating EGFR as an important therapeutic target for malignant gliomas. The profound response to doxycycline-mediated *EGFR** inactivation is in stark contrast to the unimpressive outcome of EGFR kinase inhibitor treatments that marginally decelerated glioma growth without causing any visible tumor regression. This finding suggests that lack of response to EGFR kinase inhibitors in the clinic is likely due to the inability of the inhibitors to sufficiently suppress aberrant EGFR signaling in malignant gliomas. Moreover, our data indicate that EGFR tyrosine phosphorylation is not the absolute indicative of overall EGFR oncogenic functions. Instead, low levels of EGFR kinase activity underdetected by phosphospecific EGFR antibody might be sufficient to sustain the survival of EGFR-dependent glioma cells. Additionally, kinase-independent EGFR functions could also contribute to the refractoriness to EGFR kinase inhibitors in malignant gliomas. Likewise, next-generation inhibitors will need to consider EGFR-kinase-independent

activities in order to achieve optimal efficacy against malignant gliomas carrying *EGFR* amplification and/or mutations.

EGFR gene amplification in human malignant glioma occurs invariably as extrachromosomal double-minute fragments. When devoid of selective pressure, the amplified *EGFR* diminishes rapidly upon cycles of cell division. As a result, heterogeneous intratumoral *EGFR* amplification and gene expression are commonly observed in human malignant gliomas. This heterogeneous EGFR expression pattern is also notably recapitulated in our inducible EGFR mouse gliomas, often with the high EGFR expression cells present in the peripheral invasive edge of the tumors compared to low EGFR expression subpopulations in the relatively solid tumor center. Although inhibition of *EGFR** transgene induction by doxycycline in our system elicits rapid cell death and initial tumor regression, gliomas have not regressed fully upon *EGFR** transgene inactivation, consistent with the mosaic *EGFR* gene expression pattern observed in the developed tumors. The finding that only a subpopulation of tumor cells persisted under acute EGFR* inactivation suggests that EGFR*-dependence in *iEIP* tumors is likely cell-type-dependent. Indeed, we observed that the Gfap-positive *iEIP* glioma cell population was eradicated completely in the relapsed tumors, suggesting that the astrocyte lineage tumor cells are likely cell-type-sensitive to *EGFR** inhibition. These findings support a model directly linking the glioma plasticity with therapeutic resistance. Considering the ability of tumor cells to shift lineage marker expression during treatment, we reason that the sensitive and resistant tumor cells may simply represent alternate differentiation states of the disease that rely on different developmental survival signaling, although further work will still be necessary to exclude the potential contributions from the emergence of genetic mutation(s).

Epigenetic Regulation during Glioma Pathogenesis

Biologically, malignant glioma is known for its explicit inter- and intratumoral genetic and histological heterogeneity, hence the moniker of glioblastoma "multiforme." The glioma cells within individual tumors often exist in distinct phenotypic states that differ in their differentiation as well as functional attributes, with generally less differentiated tumor cells possessing higher propagation potential. The recent identification of glioma-initiating cells with stem-cell-like properties provided an important conceptual advance and indicated targeting developmental programs as an avenue for therapeutic development. We and other investigators demonstrated that this "differentiation arrest" feature of glioma cells has a general role in sustaining their long-term self-renewal, further supporting the idea of targeting of cell fate decision pathways for glioma treatment. Because epigenetic regulation as a core mechanism has been implicated in controlling cellular lineage differentiation of the central nervous system, we set up a program in collaboration with Scott Lowe and Chris Vakoc's groups here at CSHL to identify the epigenetic networks essential for maintaining the glioma cell differentiation state. In a focused primary epigenetic screen of a customized small hairpin RNA (shRNA) library targeting epigenetic regulators, we identified 10 genes essential for primary mouse glioma cell self-renewal. Starting from this small panel of chromatin modulators, we are using various assays to narrow down candidates into those whose suppression sensitizes the glioma cell to differentiation. Upon completion of this goal, we will develop a spectrum of differentiation-based chromatin modulators as therapeutic targets for future drug discovery and development.

Another aspect of the project is to probe the same epigenetic RNAi library for chromatin modulators whose suppression may compromise the neural progenitor cell's differentiation capacity and therefore act as tumor suppressor genes to prevent malignant transformation. We have identified several interesting candidates including α-thalassemia/mental retardation syndrome X-linked (ATRX), which has been recently found frequently mutated in human malignant gliomas, as well as pancreatic neuroendocrine tumors. Our data suggested that ATRX is involved in controlling gene expression along neural stem cell lineage differentiation. Mutations of ATRX have been shown to cause diverse changes in the pattern of DNA methylation. Given that it belongs to the SWI/SNF family of chromatin remodeling proteins, it stands to reason that ATRX might provide a link between chromatin remodeling, DNA methylation, and gene expression in neural stem cell lineage differentiation. We are currently investigating the molecular functional targets responsible for its roles in neural stem cell-fate determination and glioma pathogenesis.

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Ling Zhang and Stefan Klingler

NEUROSCIENCE

How does the brain encode stimuli from the outside world, within and across sensory modalities, to generate specific perceptions that, in turn, trigger complex behaviors? How is the brain shaped by sensory experience and what modifications occur in neuronal circuits that allow us to learn and remember? These are the questions guiding the work of **Florin Albeanu,** who is using the olfactory bulb and olfactory cortex of mice as the subject of his current studies. Airborne chemicals, translated into neuronal signals by specific receptors in the nose, are sent directly to the olfactory bulb. Advances in optical imaging and optogenetics combined with electrophysiological recordings enable Albeanu and colleagues to monitor and/or alter patterns of activity at unprecedented synaptic and millisecond resolution, in real time, as animals are engaged in various behaviors. For survival, rodents need to identify the smells of objects of interest such as food, mates, and predators, across their recurring appearances in the surround, despite apparent variations in their features. Furthermore, animals aptly extract relevant information about environment across different sensory modalities, combining olfactory, visual, or auditory cues. By recording neuronal activity in the input and output layers of the olfactory bulb, as well as feedback from olfactory cortical areas and neuromodulatory signals, Albeanu and his team aim to understand computations the bulb performs and how this information is decoded deeper in the brain. They have recently published evidence suggesting that the mouse olfactory bulb is not merely a relay station between the nose and cortex, as many have supposed. Using optogenetic tools and a novel patterned illumination technique, they discovered that there are many more information output channels leaving the olfactory bulb for the cortex than the number of inputs received from the nose. They are currently investigating how this diversity of bulb outputs is generated, as well as how downstream areas, such as the piriform and parietal cortex, make use of such information during behaviors.

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

Joshua Dubnau and colleagues use the fruit fly to investigate two different questions. First, Dubnau and his team are investigating mechanisms of memory. Since biological mechanisms of memory are highly conserved through evolution, many features of human memory are observed in simpler organisms such as fruit flies. The lab's efforts on memory focus on understanding the gene pathways that are required as well as the neural circuits in the fly brain. They have recently shown that short-term memory forms in one set of neurons but that long-term memories form in a different brain area. A second area of research in the Dubnau group is focused on uncovering mechanisms of neurodegeneration that underlie amyotrophic lateral sclerosis (ALS) and frontotemporal

lobar degeneration (FTLD). Using the fruit fly as an experimental system, together with analyses of genomic data sets from mice, rats, and humans, the Dubnau group has developed a novel hypothesis to explain several different neurodegenerative disorders. This year, they showed that the awakening of dormant transposons in the genome of some brain cells might be responsible for causing cell death. Ongoing work in flies and mice will be used to investigate this hypothesis with the goal of developing avenues for therapeutic intervention.

Grigori Enikolopov and colleagues study stem cells in the adult brain. They have generated several models to account for how stem cells give rise to progenitors and, ultimately, to neurons, and they are using these models to determine the targets of antidepressant therapies, identify signaling pathways that control generation of new neurons, and search for neuronal and neuroendocrine circuits involved in mood regulation. Recent experiments have suggested to the team a new model of how stem cells are regulated in the adult brain, with a focus on stem cells' decision on whether to divide—and embark on a path of differentiation—or remain quiescent. This model explains why the number of new neurons decreases with advancing age and may lead to impairments in memory and depressed mood. It also explains why multiple brain trauma and prolonged neurodegenerative disease may lead to accelerated decrease of cognitive abilities. In other research, the team has identified stem cell targets of various therapies used for treating depression and developed a general platform to determine the effect of drugs and therapies and predict their action. The team is now focusing on the signaling landscape of neural stem cells and on their interaction with the surrounding niche. Enikolopov's group is also part of a team studying biomarkers that permit neuronal progenitor cells to be tracked, noninvasively, in the brains of living humans and animals. The lab is now using these discoveries to reveal how neurogenesis is related to the course of diseases such as depression and Alzheimer's and how stem cells may contribute to complex forms of behavior.

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

To better understand neuronal circuits, **Josh Huang** and colleagues have developed novel means of visualizing the structure and connectivity of different cell types at high resolution in living animals and of manipulating the function of specific cell types with remarkable precision. Huang is particularly interested in circuits that use GABA, the brain's primary inhibitory neurotransmitter. The lab's work has direct implications in neurological and psychiatric illness such as autism and schizophrenia, which involve altered development and function of GABAergic circuits. Huang's team also previously developed 20 different mouse lines, each of which is engineered to express markers of specific cell types, and demonstrated the exquisite specificity of this technology by

imaging GABA cells. This has been used to shed new light on synapse validation, which is at the heart of the process by which neural circuits self-assemble and is directly implicated in neurodevelopmental pathologies. Huang's team looked closely at neurexins, proteins that interact with neuroligins to form the "zipper" that holds synapses together. They discovered that α and β neurexins respond in different ways to neural activity—the α molecules searching out compatible connection partners and the β molecules securing preliminary connections that prove to be strong. The team's most recent observations show that GABA also regulates the process by which synapses are pruned after they have been formed. The lab has also made good progress in studying perturbations in the developing GABAergic system in a mouse model of Rett syndrome, one of the autism spectrum disorders. In a recent landmark study, the team also made a major breakthrough in determining the origin of and the genetic mechanisms that specify the cortex's powerful and enigmatic chandelier cells. This is a critical class of inhibitory brain cells, and the team showed that chandelier cells are born in a previously unrecognized portion of the embryonic brain, which they have named the VGZ (ventral germinal zone).

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

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

Dysfunction of synapse in the brain is believed to have an important role in the pathogenesis of major psychiatric disorders, including depression, anxiety, and schizophrenia. But what are the causes? Where in the brain does the dysfunction occur? How does it result in the behavioral symptoms of illness? To address these issues, **Bo Li** and colleagues are studying, in animals, normal synaptic plasticity underlying adaptive behaviors, and synaptic aberrations responsible for maladaptive behaviors that are related to depression, anxiety, and schizophrenia. Their long-term

goal is to develop methods allowing the manipulation of activity in specific brain circuits in order to change disease-related behaviors. Li's group uses a variety of methodologies, including patch-clamp recording and calcium imaging of labeled neurons, two-photon imaging of spine morphology and tagged receptors, in vivo stereotaxic virus injection; RNAi-based gene silencing, activation of specific axon terminals using light-gated cation channels, activation or silencing of specific brain regions using transgenes, and assessment of the behavioral consequences of certain manipulations. A project focusing initially on a gene called *ErbB4* seeks to determine the genetic causes of attentional deficit, a cognitive impairment that is consistently observed in schizophrenia. This past year, Li and his lab members demonstrated that neurons in a tiny area of the mammalian brain called the central amygdala encode fear memory and control fear expression. These findings laid the foundation for future work aimed at understanding the circuit mechanisms of anxiety disorders, in particular post-traumatic stress disorder (PTSD).

Partha Mitra seeks to develop an integrative picture of brain function, incorporating theory, informatics, and experimental work. In the ongoing Mouse Brain Architecture Project, Mitra and colleagues are well on their way to generating the first-ever brainwide mesoscopic-scale circuit map of the entire mammalian brain. This year, they released the first set of gigapixel (1 billion pixel) images from the project (http://mouse.brainarchitecture.org). The current data set is close to 1 petabyte in size (uncompressed). This is a first step toward mapping vertebrate brain architecture across species and in mouse models of human disease, including knockout models of autism spectrum disorders. These maps will be cross-referenced to the Allen Brain Atlas of gene expression, a complementary landmark data set on which the Mitra lab has innovated multiple analyses. Related collaborative efforts include characterization of different types of GABAergic neurons in the mouse brain, optogenetic studies of the rodent brain, and connectivity mapping in marmoset and zebra finch brains. These individual projects fall under the new and growing research area of whole-brain digital/computational neuroanatomy, which the Mitra laboratory is helping to develop.

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

Stephen Shea's lab studies the neural circuitry underlying social communication and decisions. He uses natural social communication behavior in mice as a model to understand circuits and processes that are evolutionarily conserved and therefore shared broadly across species, likely contributing to disorders such as autism. Shea and colleagues have examined how emotion and arousal enable mice, via their olfactory systems, to store memories of other individuals and of related social signals. The team has exploited the intimate relationship between memory and emotion to effectively create memories in anesthetized mice, allowing them unprecedented access to neurobiological processes that typically only occur during behavior. The lab has been making a detailed

analysis of the changes in neural connections that underlie odor memory. The team is particularly focused on an enigmatic cell type (granule cells or GCs) that has long been hypothesized to be crucial for memories, but has resisted direct study. They have developed methods for recording that are giving them the first glimpse of the dynamics of these cells while the animal is learning an odor. The results show unexpectedly complex population dynamics among the GCs that were independently predicted by a model of odor learning developed in Alexei Koulakov's lab. The two labs are collaborating to discern how GC population activity gets integrated by olfactory bulb output neurons and to pinpoint the synaptic circuit that underlies this form of learning. In parallel, another member of the lab is using imaging techniques to determine how memories are stored among broad neuronal ensembles, at a different level of the system. This year, the lab made a key breakthrough, developing the ability to record from GCs in awake animals and discovering that their activity is dramatically modulated by state of consciousness. Finally, the Shea lab completed a series of studies of a different form of social recognition: auditory recognition of pup vocalizations by their mothers. Through this research, they have shown that a mouse model of Rett syndrome exhibits deficits in communication and learning not unlike those in human patients. Grants from the Simons and Whitehall Foundations are allowing the lab to extend this work by directly linking these deficits to the action of the gene *MeCP2* in the auditory cortex.

What is a memory? When we learn an association, information from two different sensory streams somehow becomes linked together. What is this link in terms of neural activity? For example, after a few bad experiences, we learn that the "green" smell of an unripe banana predicts its starchy taste. How has the neural response to that green smell changed so it becomes linked to that taste? What are the underlying mechanisms—what synapses change strength, what ion channel properties change? These are the questions that drive research in **Glenn Turner**'s laboratory. His team addresses these questions by monitoring neural activity using a combination of different techniques. Using electrophysiological methods, they can examine individual neurons with very high resolution, monitoring synaptic strength and spiking output. They have also developed functional imaging techniques to monitor the activity of the entire set of cells in the learning and memory center of the fly brain. This comprehensive view of neural activity patterns enables them to actually predict the accuracy of memory formation in separate behavioral experiments. By examining the effects of learning-related genes on this process, they can connect their network-level view of memory formation to the underlying molecular mechanisms that govern the basic cellular and synaptic changes that drive learning.

Anthony Zador and colleagues study how brain circuitry gives rise to complex behavior. Work in the lab is focused on two main areas. First, they ask how the cortex processes sound, how that processing is modulated by attention, and how it is disrupted in neuropsychiatric disorders such as autism. The lab has shown the influence of expectation on neuronal activity in rat sensory cortex, suggesting that improvements in auditory perception that result from valid temporal expectation arise from changes in sensory representations as early as the first stages of cortical processing. This past year, they published new research in which the loss of a gene commonly mutated in autism, PTEN, is associated with hyperconnectivity in the brain's auditory cortex. Importantly, these effects can be blocked by the immunosuppressant rapamycin. Second, they are developing new methods for determining the complete wiring instructions of the mouse brain at single-neuron resolution, which they term the "Connectome." In contrast to previous methods, which make use of microscopy, these methods exploit high-throughput DNA sequencing. Because the costs of DNA sequencing are plummeting so rapidly, these methods have the potential to yield the complete wiring diagram of an entire brain for just thousands of dollars.

Yi Zhong's lab studies the neural basis of learning and memory. The team works with fruit fly models to study genes involved in human cognitive disorders, including neurofibromatosis,

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

UNDERSTANDING NEURONAL CIRCUITS IN THE MAMMALIAN OLFACTORY BULB

F. Albeanu F. Anselmi H.G. Chae M. Davis M. Koh G. Otazu
A. Banerjee P.G. da Silva P. Gupta F. Marbach H. Patel

The focus of our research group is to understand how neuronal circuits encode and interpret inputs from the environment and lead to meaningful behaviors. Toward this end, we use optogenetic methods (such as fast multiphoton laser-scanning imaging of genetically encoded neuronal activity reporters or patterned illumination of light-gated neuronal activity switches) coupled with electrophysiological measurements (extracellular and intracellular recordings). We want to understand (1) how inputs become processed at different synapses of the underlying neuronal circuits, (2) how these representations change with the state of the system and its circuits (awake vs. anesthetized, naïve vs. learning), and (3) what changes in the activity patterns of well-defined neuronal circuits contribute to specific behaviors. The broad scope of this effort is observing how perceptions arise.

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

Characterizing the Input-Output Transform of the Olfactory Bulb

In the OB, sensory neurons expressing the same type of olfactory receptor converge in tight focus, forming ~2000 clusters of synapses called glomeruli. The layout of glomeruli on the OB is highly reproducible across individuals, with a precision of 1 part in 1000. However, nearby glomeruli are as diverse in their responses to odors as distant ones, lacking an apparent chemotopic arrangement (Soucy et al., *Nat Neurosci 12:* 2 [2009]). From each glomerulus, a few dozen mitral cells (principal output neurons of the OB) carry the output farther to the olfactory cortex and several other brain areas. Such "sister" mitral cells typically have only one primary dendrite that projects to a single glomerulus, but they can sample inputs on their primary and secondary dendrites from functionally diverse glomeruli via several types of interneurons (Fig. 1). Using optogenetic manipulations and digital micromirror device (DMD)-based patterned photostimulation (Fig. 2), we found that sister cells carry different information to the cortex: Average activity represents shared glomerular input, and phase-specific information refines odor representations and is substantially independent across sister cells (Dhawale et al., *Nat Neurosci 13:* 1404 [2010]).

Activity in the OB is a rich mix of excitation and inhibition, via both direct inputs and feedback connections. Many different classes of interneurons interact with the sensory afferents and/or the output neurons via both short- and long-range connections. Their connectivity patterns and roles in olfactory processing, however, remain largely unknown. We use a Cre/loxP approach to express reporters (i.e., synaptopHluorin, GCaMP6) and light-gated switches of neuronal activity (ChR2, Halorhodopsin, Arch) in different bulbar neuronal types. We monitor the inputs (glomerular activity patterns) and the outputs (mitral cell firing) in response to numerous odorants across a wide range of concentrations (five orders of magnitude) as we systematically perturb the activity of the input nodes (glomeruli) and of different interneuron types. Patterned photostimulation allows us to bypass odor stimulation and gain precise spatiotemporal control over the inputs by directly activating glomerular patterns of choice. We record bulbar outputs via multitetrode recordings and patch clamp or use optical imaging readouts via multiphoton microscopy in vivo to understand what computations the OB performs.

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

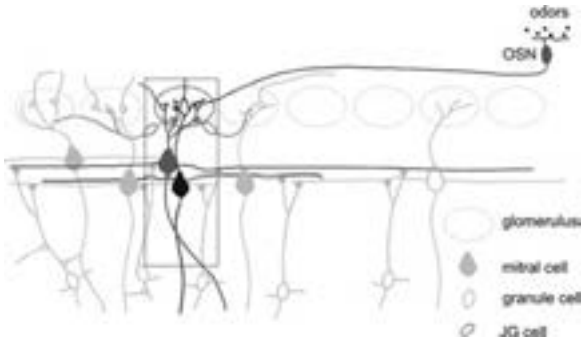

Figure 1. Main olfactory bulb wiring schematics.

Short Axon Cells Implement Gain Control in the Olfactory Bulb

SA cells, in the glomerular layer, receive inputs from olfactory sensory neurons and/or external tufted (ET) cells and release both GABA and dopamine, synapsing onto juxtaglomerular cells as far as tens of glomeruli away (Kiyokage et al., *J Neurosci 30:* 1185 [2010]). Computational models (Cleland et al., *Trends Neurosci 33:* 130 [2007]) have suggested that SA cells may be involved in long-range normalization of bulb outputs, but to date, their function in the intact brain has not been investigated. We imaged GCaMP3 responses by wide-field microscopy to odor stimulation across a wide range of concentrations. Odorants induced transient yet widespread SA responses, in contrast to focal glomerular patterns observed via intrinsic optical imaging.

To understand the roles played by the SA network on the bulb output dynamics, we recorded extracellularly from mitral/tufted (M/T) cells using tetrodes in anesthetized mice. In conjunction, we selectively activated/inactivated SA cells by shining blue/yellow light either throughout the dorsal bulb surface or in specific spatial patterns in mice expressing ChR2 and Halorhodopsin, respectively. In parallel, we developed complementary tools to silence SA cells pharmacogenetically using inhibitory DREADDs and to monitor mitral cell activity at the population level by retrograde expression of GCaMP3. Strikingly, pairing odor presentation

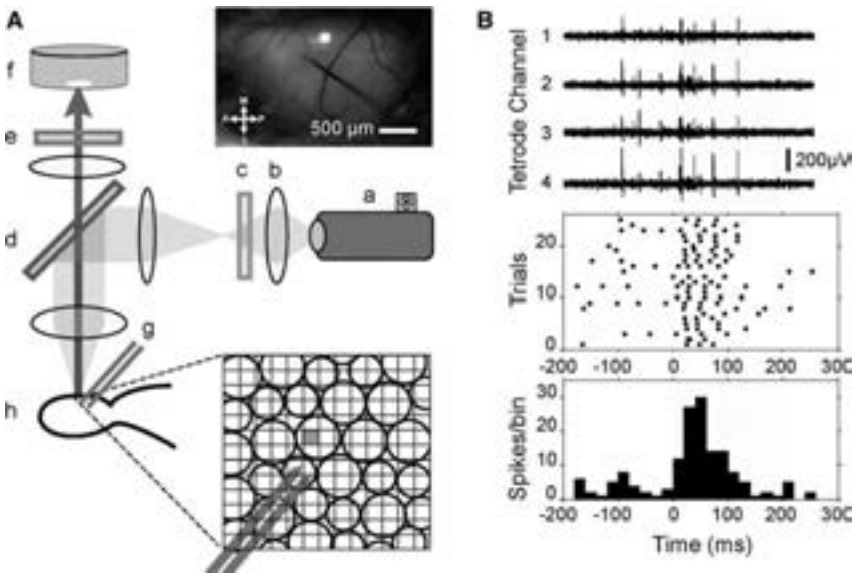

Figure 2. DLP patterned illumination in ORC mice maps the parent glomeruli of mitral cells in vivo. (*A Left*) A DLP projector coupled to a system of three lenses delivered light patterns to activate individual glomeruli on the dorsal surface of the bulb. A CCD camera was used to visualize the bulb surface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

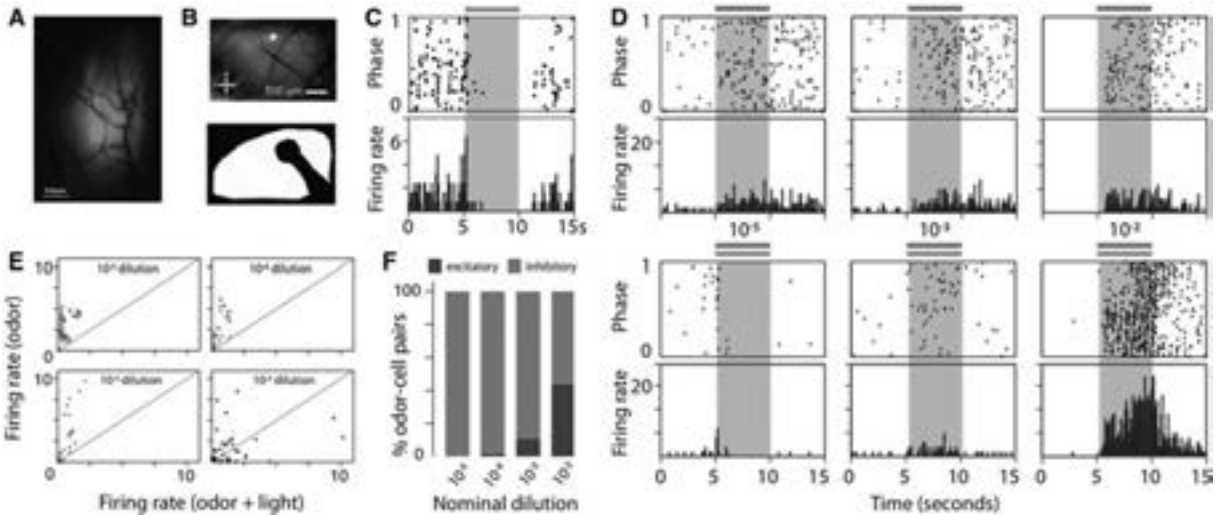

Figure 3. Optogenetic suppression of SA cells reveals both excitatory and inhibitory drives on M/T cell output. (*A*) Homogeneous expression of DIO-Halorhodopsin AAV construct injected in DAT-Cre mice. (*B*) Example of full-field yellow light mask designed to avoid the recording electrode. (*C*) Spike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

at various concentrations with light indicates that SA cells provide both excitatory and inhibitory drive to the M/T cells in a stimulus-specific fashion (Fig. 3). At low odorant concentrations, for weak odor stimuli, SA cells provide excitatory input, whereas in response to stronger stimuli, both excitatory and inhibitory drives are present. Pharmacological manipulations indicate that SA cells both excite and inhibit individual mitral cells in different contexts through a diverse machinery of electrical and chemical synapses, using multiple neurotransmitters. Synaptic blockade experiments in acute bulb slices and in vivo revealed a widespread excitatory drive of SA cells that is implemented by a distributed network of gap junctions in the glomerular layer and D1 receptors. This unique morphological and chemical organization of SA cells facilitates potentiation of responses to weak inputs and conversely suppression of strong ones, thereby implementing intensity-dependent bidirectional control of the dynamic range of bulb outputs.

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

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

Computational models and behavioral studies have suggested critical roles for these cells in olfactory processing and learning. However, very little is known about their response properties in vivo, owing to technical difficulties in electrophysiological recordings from these small-sized neurons. To begin characterizing the odor response properties of GCs, we express a genetically encoded calcium indicator (GCaMP3) in the GCs (CST-Cre × Ai38) and use multiphoton imaging to monitor the odor-evoked responses of GCs in awake head-fixed mice. GCs show robust spontaneous activity and are sparsely activated upon odor presentation, displaying a diverse range of enhanced and suppressed, ON, OFF, and ON-OFF responses. We observe odor responses in slightly more than half of the GCs imaged. Strikingly, to our stimulus panel (up to 20 odors, 100-fold mineral oil dilution), 25% of GCs displayed purely enhanced responses, 26% of GCs showed suppression, and only a small fraction

(5%) showed both enhancement and suppression. We find that enhanced responses are as common as suppressed responses. Furthermore, a significant fraction (~25%) of GCs exhibited characteristic enhanced OFF responses, independent of the stimulus duration. Pairwise analysis of GCs monitored simultaneously show low, but significant, positive noise correlations and odor response spectra correlations. Importantly, neighboring GCs are as diverse in their odor responses as pairs of distant cells. No spatial clustering of similarly responding GCs is apparent within a 350-μm range.

We are further characterizing how individual GC odor responses, as well as the diversity of responses across the population, change with increasing odor concentration and across larger odor sets. To dissect the contribution of the corticobulbar feedback to GC activity, we are currently using pharmacological and optogenetic manipulations of the cortical input in tandem with multiphoton imaging of GCs and M/Ts. This approach will allow us to directly assay the effects of these perturbations on GC dynamics, as well as on the OB output.

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

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

One way to understand how the circuit makes sense of changes in the glomerular activity patterns to extract relevant information is to systematically alter features of odor maps and study the concurrent changes in neuronal outputs and olfactory behavior. Hitherto, however, this has not been possible, primarily due to the inability to activate and modulate individual glomeruli in a controlled manner using odorants. We are using optogenetic tools to bypass odorant stimulation and simulate odor-like glomerular activity patterns, or alter them, by directly activating/inhibiting glomeruli using light in transgenic mice that express ChR2 or Arch in all olfactory system neurons (OSNs) or in a single type of OSN (Olf151). Furthermore, using patterned illumination, we are activating/inhibiting select subsets of glomeruli with single-glomerulus precision in terms of intensity, onset time, or duration. This approach confers an unprecedented ability to make feature-specific perturbations in a glomerular pattern of choice. We aim to understand what features of an odor map are used by a behaving mouse in naturalistic conditions to identify odors and their attributes such as concentration, temporal fluctuations, or spatial location. Furthermore, we are testing the resolution at which mice can in principle detect variations within specific features of odor maps in a strictly controlled artificial regime. While doing so, we will also monitor neuronal responses at multiple layers in the olfactory system to compare the behavioral and neuronal detection thresholds and to understand the neuronal correlates of olfactory behavior.

To this end, we are training head-fixed and water-restricted mice to identify a target stimulus ("A") as being unique from other nontarget ("not A") stimuli by licking a "Left" versus "Right" water reward port placed on either side of the mouth (Fig. 5H, below). Animals are reinforced with water reward for correct choices and a mild air puff as punishment upon licking of the wrong port (Fig. 5H, below). We are using wide-field imaging (intrinsic, fluorescence) and two-photon microscopy to monitor the glomerular input maps evoked by the target ("A") and the nontarget stimuli ("not A") as mice learn and perform the task. These activity patterns are further used as templates to design photostimulation masks for perturbation of stimulus-induced activity patterns during behavior.

Once the task has been learned to >85% accuracy, we reversibly perturb the stimulus "A" activity pattern and determine the behavioral threshold at which an altered activity pattern is classified as "not A."

Investigating the Roles of Cortical Feedback in Invariant Odor Perception

Invariant perception refers to the identification of a sensory stimulus or an object of interest in a generalized fashion across its variable and recurring presentations. This is a common feature across all sensory modalities and becomes particularly preeminent in olfaction. Natural odor scenes are composed of odor plumes originating from multiple sources and traveling at fluctuating intensities that span several orders of magnitude. Despite the turbulent nature of the stimulus, rodents readily identify odors essential for their survival against varying odor-rich backgrounds and faithfully track a fluctuating odor stream to its source. Several models have attempted to explain invariant perception in different sensory modalities. Some of these models are based on the conventional view of sensory processing that relies solely on feed-forward information flow, whereas others argue that feed-forward mechanisms are insufficient and suggest the involvement of feedback between different neuronal layers. Anatomical studies show massive feedback projections from higher brain areas to the sensory periphery that often outnumber direct sensory inputs. The interplay of feed-forward and feedback signals has been proposed to be fundamental for learning and memory recall. Although rich cortical feedback projections innervate the OB, to date, little is known about their contribution to olfactory processing. Corticobulbar feedback innervates multiple bulb layers, but primarily targets the GCs, which form extensive dendro-dendritic synapses with the M/T cells. We aim to understand the role of corticobulbar feedback (Fig. 4) in the identification of an odor, invariant of fluctuations in its concentration and/or timing and presence of background odors. We are taking advantage of optogenetic tools and patterned illumination techniques recently developed in our group to reversibly manipulate the activity of cortical feedback fibers and their targets in awake head-fixed mice as they learn and perform invariant odor perception tasks. To mimic naturalistic odor conditions, we are making use of custom-designed odor delivery systems that reproducibly deliver arbitrary time-varying patterns of individual odors and mixtures. To understand the underlying neuronal mechanisms, we monitor the activity of feedback inputs and their targets via high-speed scanning multiphoton microscopy and multitetrode recordings.

To date, little is known about the contribution of cortical feedback to olfactory processing in the OB in response to novel or previously encountered odors. To examine how cortical feedback shapes input processing in the OB, we use GCaMP5 to monitor the odor-evoked responses of feedback fibers in awake head-fixed mice. Feedback fibers show rich spontaneous activity and diverse enhanced and suppressed odor responses. Suppression of feedback fibers by odors appears to be sparser and more stimulus-specific compared to the enhanced responses.

Pairwise analysis of simultaneously imaged boutons reveals functional local diversity. An excess of functionally similar boutons occurs within 20-μm separation, likely due to local enrichment in same axon-boutons. However, beyond this small excess, no apparent spatial order is apparent within the imaged field of view (<150 μm).

Interestingly, habituation to select sets of odors in our panel across several days results in a significant increase in the fraction of suppressed versus enhanced responses (1.07 ± 0.06) compared to nonhabituated odors (0.67 ± 0.05). These results suggest that odor experience weakens the olfactory cortical feedback drive to the OB. We are currently investigating how cortical feedback dynamics change with olfactory learning by training head-fixed mice in simple "go–no go" odor discrimination tasks. In addition, we are using pharmacological and optogenetic approaches to modulate the corticobulbar feedback, while simultaneously monitoring the changes induced by these manipulations onto granule cell activity patterns.

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

Cholinergic inputs from the basal forebrain have been shown to enhance encoding and discrimination of visual, auditory, and somatosensory cues by modulating attention and learning processes. Although rich cholinergic projections innervate the mouse olfactory bulb

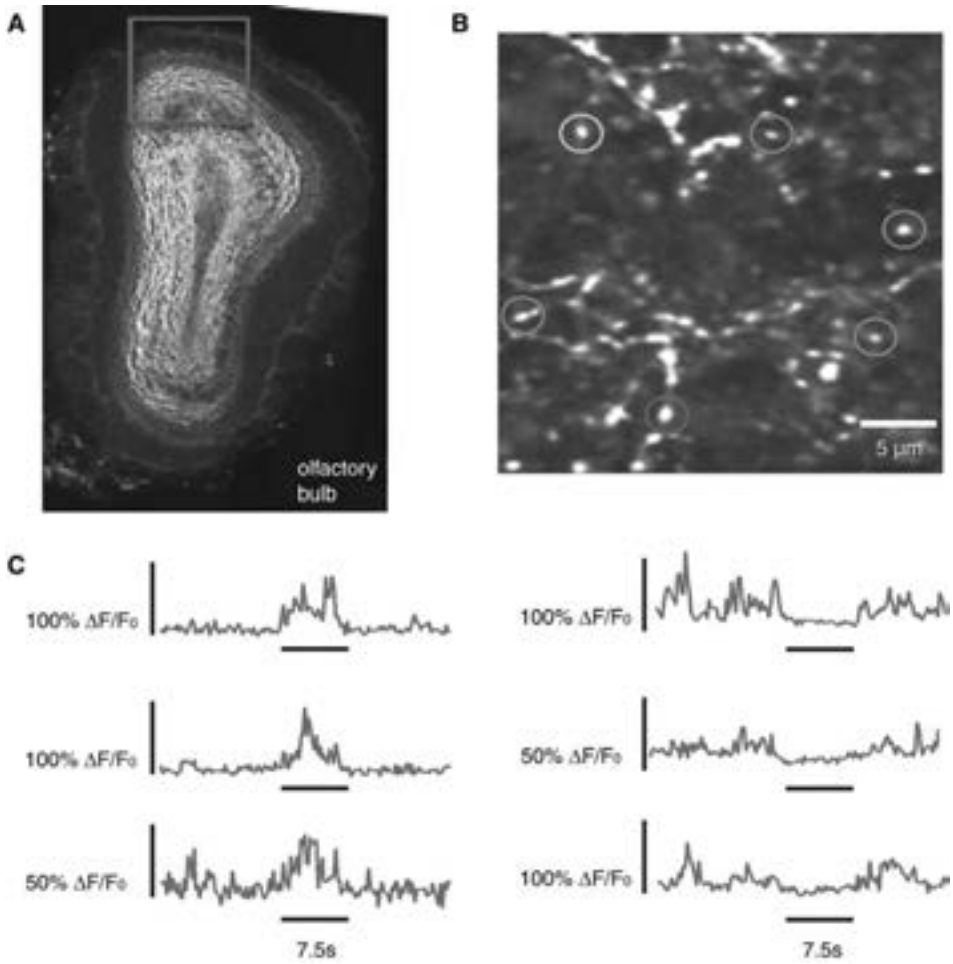

Figure 4. Odor-evoked responses in corticobulbar feedback fibers. (*A*) Corticobulbar feedback fibers labeled with GCaMP5; (*B*) feedback fiber boutons in an optical plane 230 µm below the surface; (*C*) example responses to three different odors (ethyl tiglate, 2-heptanone, and *p*-anis aldehyde) for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

(MOB), to date, little is known about their contribution to olfactory behaviors. Pharmacological blockade of acetylcholine (ACh) action has been shown to impair olfactory behavior, whereas augmenting acetylcholine (ACh) levels improved odor discrimination. A clear understanding of the underlying mechanisms of ACh action and their spatiotemporal statistics, however, has been limited by the low yield of recordings from the basal forebrain, as well as difficulties in ascertaining the downstream targets of the recorded neurons, given the widespread and long-range nature of cholinergic projections.

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

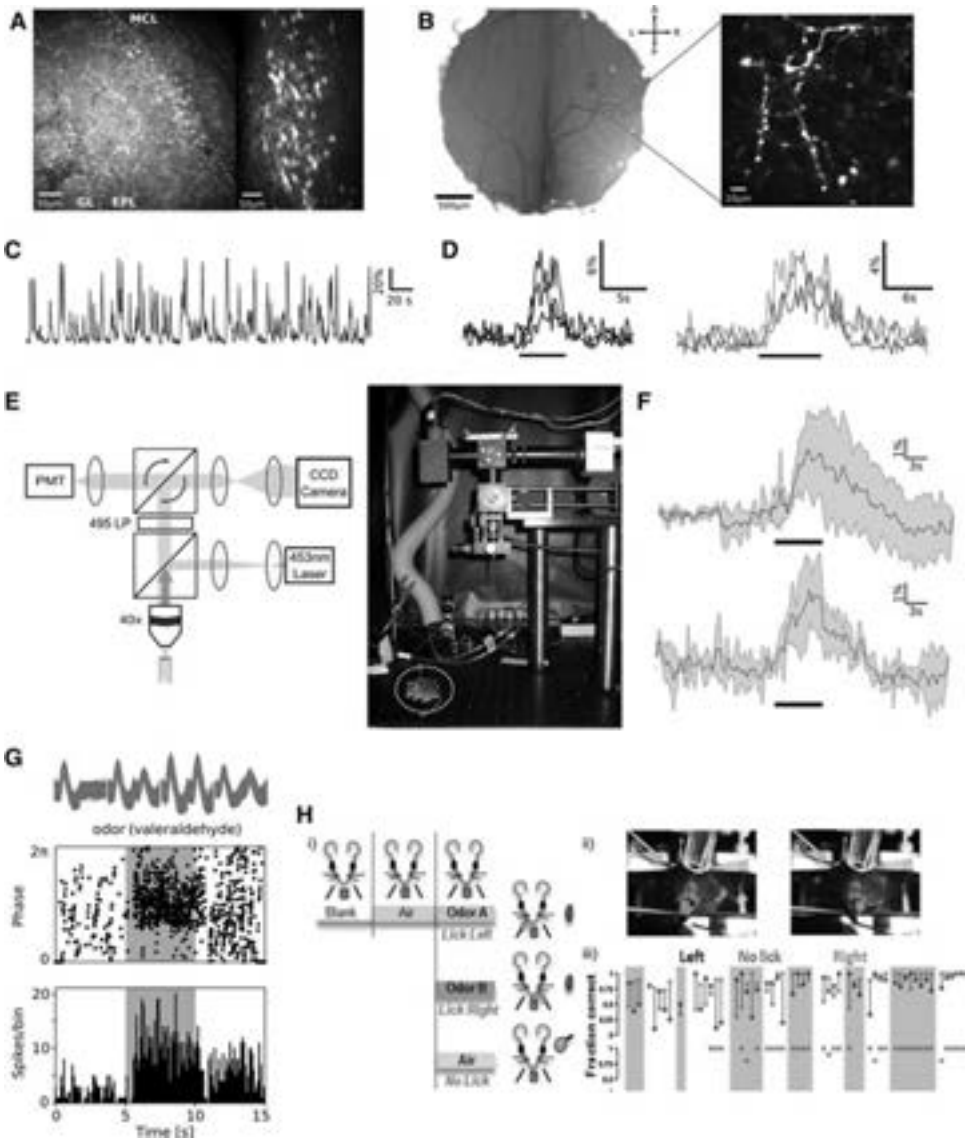

Figure 5. Monitoring of cholinergic feedback and two forced-choice tasks. (*A*) Two-photon images of cholinergic fibers in an MOB sagittal slice (*left*) and the injection site (HDB, *right*). Awake head-fixed imaging: (*B*) 38-day-old cranial window (*left*); 2P field of view of GCaMP5-expressing cholinergic axons (*right*); (*C*) spontaneous activity; (*D*) odor-evoked responses in the MOB to same (*left*) and different odors (*right*); (*E*) schematic of fiber-optic based system and picture of the setup; (*F*) corticobulbar feedback signals recorded using the system in *D* (four repeats, shaded area indicates S.E.M.); (*G*) single-unit recordings of mitral cell odor response with respect to the respiratory cycle; (*H;* i) Schematic of the 2AFC task. Water reward on licking for odor A (*left*) and for odor B (*right*); air puff for licks during air period. (ii) Mouse performing the task and (iii) the corresponding learning curve. (*Top*) Each pair of dark and light gray circles shows the fraction of correct licks on the left (dark gray) and right (light gray) ports. Vertical stripes represent training sessions. (*Bottom*) No-go performance upon air presentation.

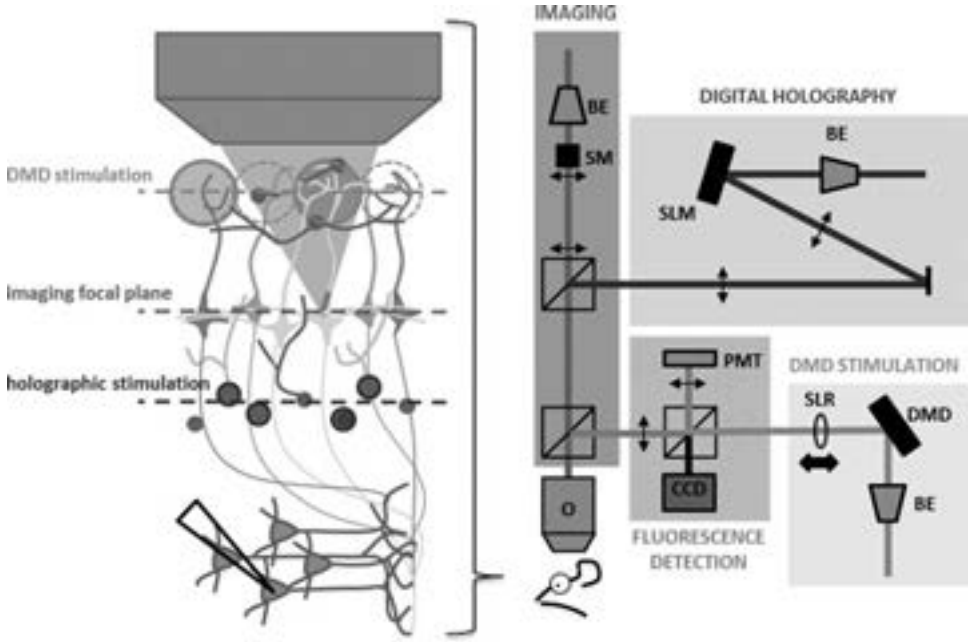

Figure 6. Combined imaging and photostimulation setup. (*Left*) Experimental configuration, combining scanning two-photon imaging, DMD photostimulation, and holographic photostimulation. (*Right*) Schematic of the experimental microscope. DMD stimulation is used to create spatiotemporal light patterns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

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

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

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

Other Collaborative Projects with CSHL Groups

We are collaborating with other fellow CSHL scientists on the following projects: multisensory integration of olfactory and visual information in the mouse

brain (A. Churchland); sequencing the olfactory bulb; bridging the gap between glomerular odor responses and odor receptor sequences by determining the molecular identity of glomeruli (A. Koulakov and G. Hannon); fiber-optic-based approach to monitor neuronal activity in punishment and reward neuronal circuits during behavior (B. Li); developing light-sheet-based approaches for fast optical reconstruction of neuronal circuits (P. Osten); and optical monitoring and manipulation of neuronal activity in genetically and anatomically defined cortical circuits in animal models of cognition (A. Zador).

PUBLICATIONS

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INVESTIGATING NEURAL CIRCUITS FOR SENSORY INTEGRATION AND DECISION MAKING

A. Churchland A. Brown D. Raposo
M. Kaufman M. Ryan
O. Odoemene J. Sheppard

Making use of sensory information requires more than simply relaying incoming signals from the sensory organs. It requires interpreting information, classifying it, drawing inferences, and ultimately using the context of behavioral goals to make a decision about its meaning. A decision is a commitment to a particular choice or proposition at the expense of competing alternatives. In some situations, decisions involve integration of evidence, i.e., they make use of multiple pieces of information from the environment or from memory. These decisions can provide a framework in which to investigate complex cognitive processes and open a window into higher brain function in general (Churchland and Ditterich 2012). Although previous experiments have begun to reveal how neural systems combine evidence to make decisions, they have left a critical gap in our understanding. Specifically, very little is currently known about the neural mechanisms that make it possible to combine information from multiple sensory modalities for decisions. The gap is apparent, although it is clear from behavioral observations that neural systems can combine multisensory information: When parsing speech in a crowded room, for example, the listener makes use of both auditory information (the speaker's vocal sounds) and visual information (the speaker's lip movements). Understanding the neural mechanisms of multisensory integration is critical for two reasons. First, it is essential for a complete understanding of sensory perception because real-world stimuli rarely affect a single sense in isolation. Therefore, understanding how the brain interprets incoming information requires understanding how the brain merges information from different senses. Second, it is likely of clinical importance: Several developmental abnormalities appear to be related to difficulties in integrating sensory information. For example, abnormalities in multisensory processing are a hallmark of subjects with autism spectrum disorder. Impairments in multisensory processing are also observed in subjects with a collection of sensory abnormalities known together as sensory processing disorder and may also be evident in patients with Rett syndrome and dyslexia. Understanding the neural mechanisms of multisensory integration could inform treatment of those conditions. Our long-term goal is to understand how the brain can make decisions that integrate inputs from our multiple senses, stored memories, and innate impulses. Our current projects, described below, are a first step toward this complete understanding of perceptual decision making. In the past year, we have made tremendous strides toward achieving that goal. We have successfully extended our behavioral paradigm for studying decision making in rats and humans to mice (Fig. 1A). Furthermore, we have also continued to record electrophysiological responses from the rats while they are engaged in the task (Fig. 1B). Our first findings with this new paradigm were published this year in the *Journal of Neuroscience* (Raposo et al. 2012b). This year, work in our lab was recognized by several awards. We received foundation awards from the McKnight and Chapman Foundations and were also honored with an award from the Society for Neuroscience (Trubatch Career Development Award). We continue to be grateful for our support from the National Science Foundation, the National Institutes of Health, and the Marie Robertson Fund. This support is critical for ongoing lab projects, listed below.

Role of Parietal Cortex in Multisensory Decision Making

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

The goal of this project is to gain a deeper understanding of the neural mechanisms that enable integration

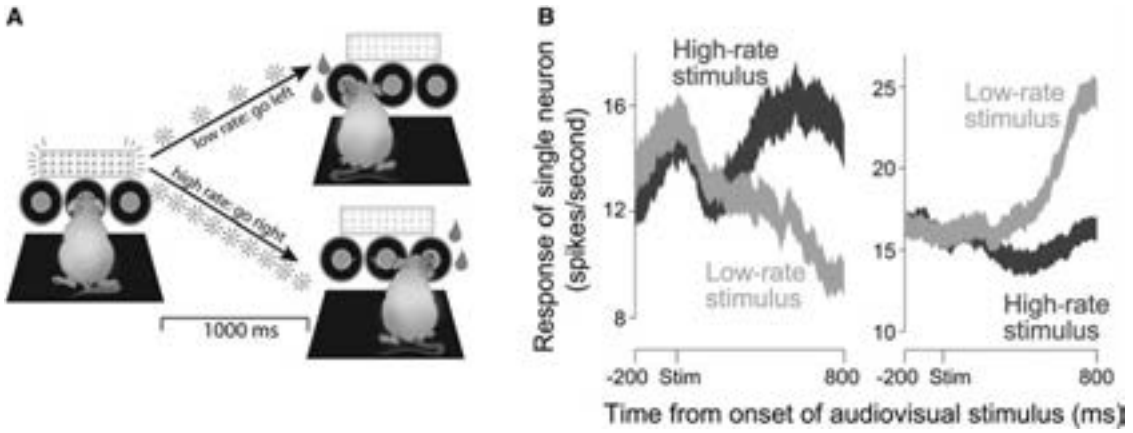

Figure 1. Decision making in rodents (*A*). Schematic drawing of a rat in operant conditioning apparatus. Circles are "ports" where the rat pokes its nose to initiate the presentation of sensory stimuli or to report its decision about the stimuli. The white rectangle is the panel of light-emitting diodes that we use to present visual stimuli. (*Left panel*) Rat in the center port, where he positions himself during presentation of stimuli. (*Right panel*) Rat in the left or right port where decisions are reported and, if correct, are rewarded with a drop of juice or water. (*B*) Responses of two parietal cortex neurons with firing rates that reflect a developing decision. Firing rates (mean ± SEM) for single neurons that are selective for stimulus rate during multisensory decision formation. (Dark blue trace) Responses to high-rate stimuli, (light blue trace) responses to low-rate stimuli. Only correct responses are included. Spike trains were convolved with a half Gaussian (std = 100 ms).

of visual and auditory inputs for decision making. To this end, we record from neurons in a multisensory area of the rat brain, the posterior parietal cortex. Because we record from neurons during multisensory behavior, we can draw connections between the animal's behavior and the underlying neural mechanism. Although the posterior parietal cortex in rats has previously been explored in the context of navigation, our study is the first study to record from this area on a decision-making task. We use a number of theoretical models to formalize our assumptions about how the neural activity we record might give rise to the behavior. In the past year, Dr. Raposo amassed a large body of electrophysiological data (Fig. 1B). He presented this work at the Society for Neuroscience Annual Meeting (Raposo et al. 2012a).

Late in the year, David's efforts were bolstered by the arrival of postdoctoral fellow Matthew Kaufman. Matthew's expertise is in applying dynamical systems analysis to data from the motor cortex. Here, he is bringing that approach to the parietal cortex. His perspective on neural activity has moved our understanding of the neural data forward greatly in the short time he has been in the lab.

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

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

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

we plan to investigate how these small manipulations impact subsequent behavior. In addition to developing the technology to make this experiment possible, we have devoted considerable effort to optimizing our protocols for collecting behavioral data. We anticipate that these findings will be published in the coming year (JP Sheppard et al., in prep.).

Decision Making in Mice

A. Brown [in collaboration with O. Odoemene, Watson School of Biological Sciences]

We have developed a decision-making paradigm using mice that will allow us to take advantage of genetic tools. These tools will allow us to probe the neural circuitry that underlies decision making. In the past year, Amanda Brown built on the foundation we established last year. A new Watson School student, Onyekachi Odoemene, will use the behavioral paradigm that Amanda developed. He will measure behavior along with electrophysiological recordings of several strains of transgenic mice.

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MECHANISMS OF NEURODEGENERATION AND MEMORY FORMATION

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

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

Neurodegenerative Disorders and Endogenous Transposable Elements

N. Chatterjee, L. Krug, W. Li, L. Prazak, J. Dubnau
[in collaboration with M. Hammell and Y. Jin, Cold Spring Harbor Laboratory]

Transposable elements (TEs) are mobile genetic elements that provide a massive reservoir of potential genetic instability and toxicity. We have advanced the novel hypothesis that deregulated TE/ERV (endogenous retrovirus) expression may contribute to TDP-43-mediated neurodegenerative disorders (Li et al. 2013a). We have found that TE transcripts are derepressed during the normal aging process and that this leads to active mobilization of transposons, resulting in new insertions in the genome of neurons (Fig. 1). We used mutations in *Drosophila* Argonaute 2 (dAgo2) to create a situation in which TEs are derepressed in the brain. Such mutants exhibit precocious TE elevation in young animals and exacerbated age-dependent derepression. This is accompanied by rapid age-dependent memory impairment and shortened lifespan. These findings support the hypothesis that protracted loss of TE silencing contributes to age-dependent decline in neuronal physiological function. To investigate the hypothesis that TE derepression contributes to neurodegenerative disorders, we focused (in collaboration with Molly Hammell and Ying Jin) on TDP-43, an RNA-binding protein involved in a broad spectrum of neurodegenerative disorders, including amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). By mining a series of deep sequencing data sets of protein–RNA interactions and of gene expression profiles, we uncovered broad and extensive binding of TE transcripts to TDP-43 (Li et al. 2012). We also find that association between TDP-43 and many of its TE targets is reduced in FTLD patients (Fig. 2). Finally, we discovered that a large fraction of the TEs to which TDP-43 binds become derepressed in mouse TDP-43 disease models. We propose the hypothesis that TE misregulation contributes to TDP-43-related neurodegenerative disease. Current efforts are focused on dissection of the underlying mechanisms of transposon control in the brain and the role of unregulated transposon expression in neurodegenerative disorders.

Neurogenetic Mechanisms of Olfactory Memory

An understanding of memory, indeed of all behavioral phenotypes, will require a multidisciplinary approach to forge conceptual links between the relevant genetic/cell-signaling pathways and neural circuits. Work in genetic model systems such as *Drosophila* can contribute to our understanding in several ways. First, by enabling discovery of genes and genetic pathways underlying normal memory, as well as pathological or degenerative cognitive disorders, model systems provide entry points for dissection of cellular mechanisms that are often conserved. Second, systematic manipulation of gene function within relevant anatomical circuits allows a conceptual integration of findings from cellular, neuroanatomical, and behavioral levels.

A microRNA-Dopamine Receptor Genetic Module in Distinct Neural Circuits for Olfactory Arousal and Olfactory Memory

W. Li, M. Cressy, H. Qin

microRNAs (miRNAs) are ~21–23-nucleotide noncoding RNA transcripts that regulate gene expression

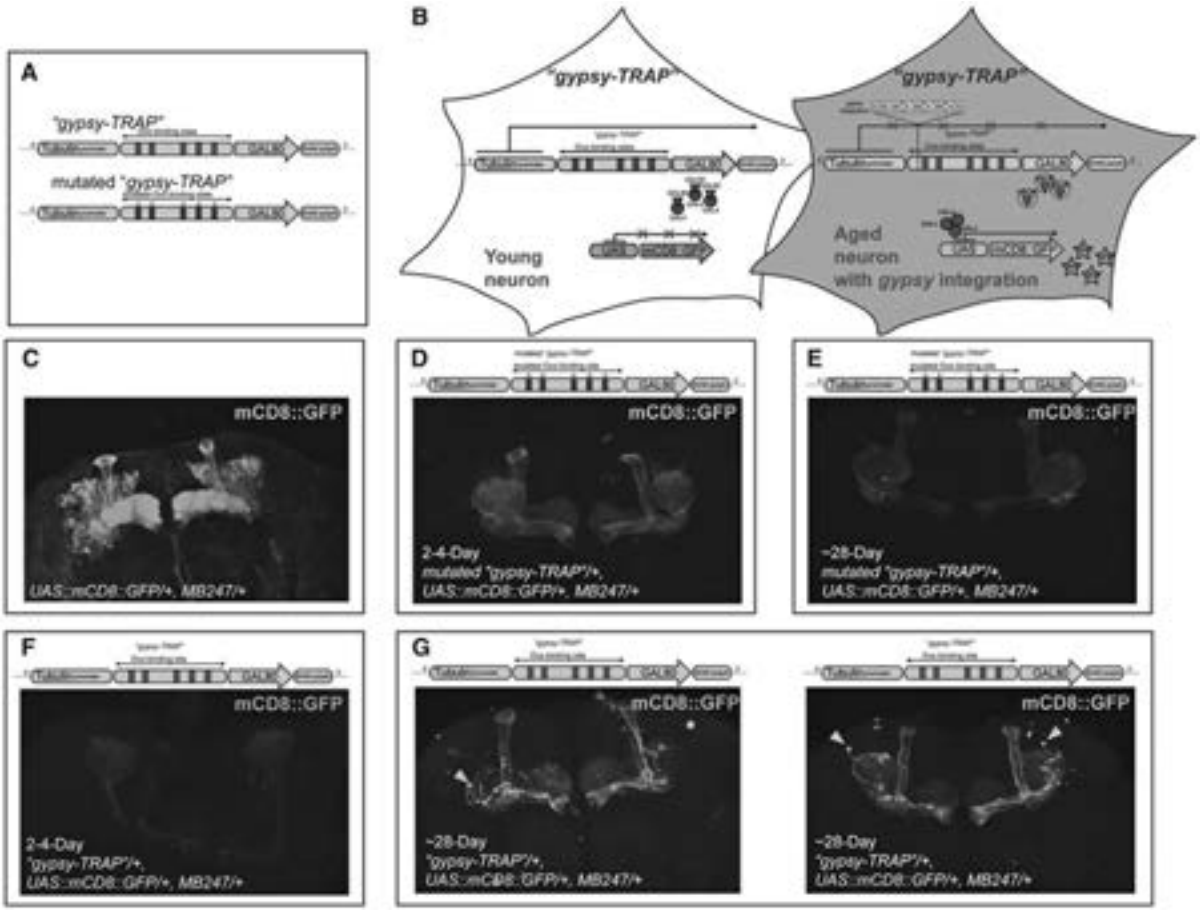

Figure 1. *"Gypsy-TRAP"* reporter detects de novo integration in neurons in aged animals. (*A,B*) Illustration of the design of *"gypsy-TRAP."* A ~500-bp fragment from the *ovo* regulatory region containing five Ovo-binding sites is inserted between the *Tub promoter* and *GAL80* gene (*A*). A mutated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

at the posttranscriptional level. miRNAs regulate gene expression by binding to complementary sequences in the 3′-untranslated regions of target mRNAs. A growing number of studies demonstrate that microRNA biogenesis and function broadly speaking are important for virtually all aspects of cell function, including neuronal function. But there still are relatively few examples where individual miRNA genes have been shown to function acutely in the brain in the context of behavior. We have demonstrated that miR276a expression is required acutely in the *Drosophila melanogaster* brain within two different neural cell types (Li et al. 2013b). In both circuits, the miR appears to target a DA1-type dopamine receptor, but the circuits subserve different aspects of olfactory behavior. This miR276a-dopamine receptor interaction is required in mushroom body (MB) neurons to support long-term olfactory memory and in ellipsoid body R4 neurons

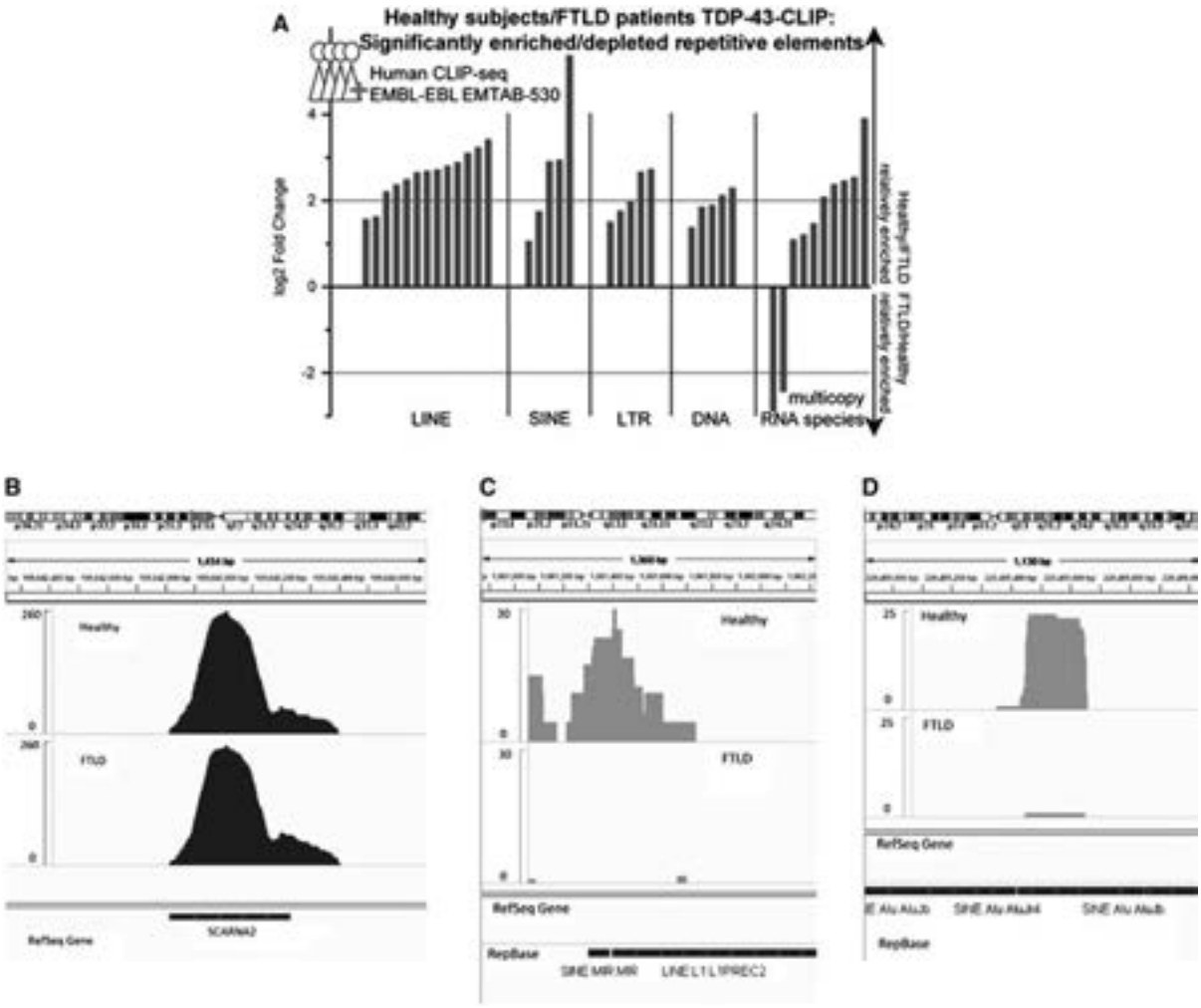

Figure 2. TDP-43 binding to TEs is selectively lost in FTLD patients. (*A*) In the human cross-linking immunoprecipitation-sequencing (CLIP-Seq) data from FTLD versus healthy control, 38 repeat elements showed significant (p-value ≤ 1e-5 and fold changes ≥ 2) differential binding. Log2-fold-binding differences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

in the central complex to modulate olfactory arousal. This may reveal a conserved functional dissection of memory and arousal in MB and central complex.

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

H. Qin, M. Cressy, W. Li, J. Coravos, S. Izzi, J. Dubnau

(MB)-dependent olfactory learning in *Drosophila* provides a powerful model to investigate memory mechanisms. MBs integrate olfactory (CS) inputs with neuromodulatory reinforcement (US), which for aversive learning is thought to rely on dopaminergic (DA) signaling to DopR, a D1-like dopamine receptor that is expressed in MB. A wealth of evidence supports the conclusion that parallel and independent signaling occurs downstream from DopR within two of the three major MB neuron cell types, with each supporting approximately half of memory performance. For instance, expression of the rutabaga adenylyl cyclase (rut) in γ lobe neurons is sufficient to restore learning levels of rut mutants, whereas

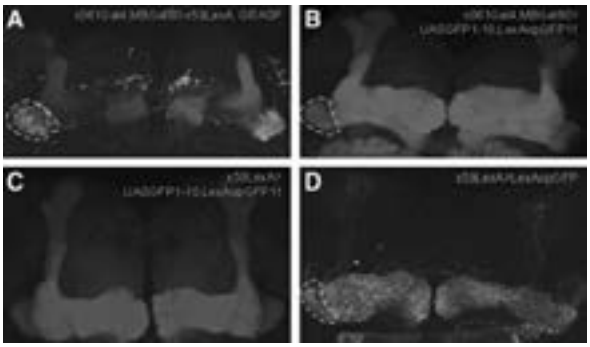

Figure 3. GRASP expressed in MB-MP1 and γ KCs reveals putative synapses in the heel of MB lobes. In each panel, a projection view of a confocal stack from a female fly MB lobe is presented. GRASP signals and dlg-counterstaining are shown. GRASP labeling is observed between MB-MP1 neurons and γ neurons with c061-Gal4;*MB*Gal80 and L5192-LexA (c061-Gal4;*MB*Gal80;*UAS-mCD4::spGFP1-10,LexAop-mCD4::spGFP11*;L5192-*LexA::GAD*). When the two halves of GRASP are expressed this way in MB-MP1 and γ neurons, GFP labeling is observed in the heel (*A*). Dashes outline the MB heel in one hemisphere of each brain. c061-Gal4;*MB*Gal80 was previously reported to express in MB-MP1 dopamine neurons. L5192-LexA drives *LexAop-hrGFP* expression in γ lobes, although weak GFP expression can be detected in α/β and α′/β′ neurons as well (*D*). When either c061-Gal4;*MB*Gal80 (*B*) or L5192-LexA (*C*) alone are used to drive *UAS-mCD4::spGFP1-10* or *LexAop-mCD4::spGFP11,* no detectable GFP signal is seen. These data support the hypothesis that MB-MP1 neurons contact γ neurons at the heel region.

expression of neurofibromatisis I (NFI) in α/β neurons is sufficient to rescue NF1 mutants. Memory consolidation, in contrast, appears to require CREB and rut signaling in α/β neurons. DopR mutations fully eliminate memory performance, consistent with the hypothesis that DopR receives US inputs for both γ lobe and α/β lobe signaling. We have used spatially restricted expression of the DopR gene to map the neuronal cell types that receive dopaminergic US information underlying this learning task. We have shown that DopR expression in γ neurons is sufficient to fully support short-term and long-term memory (Qin et al. 2012). Our findings support a model in which DA-mediated CS-US association is formed in γ neurons, followed by communication between γ and α/β neurons, to drive consolidation (Fig. 3). Current efforts are focused on investigation of the role of γ–α/β neuron signaling in memory stabilization/consolidation.

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

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

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

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STEM CELLS, SIGNAL TRANSDUCTION, AND BRAIN FUNCTION

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Stem cells of the adult organism ensure that a tissue is properly maintained, damaged cells replaced, extrinsic signals correctly interpreted, and new challenges adequately met. Our research aims to identify stem cells and the changes they undergo when converting into fully differentiated cells. We generate animal models to study stem cells and their environment in adult tissue and the mechanisms that determine the division and differentiation choices of these cells. Our main focus is on neural stem cells of the adult brain. Several areas in the adult brain can continuously produce new neurons. These newly born neurons are important for learning and memory, neural tissue repair, and response to therapies. We are working to understand how stem cells of the adult brain are regulated during the life span of the organism, how they and their progeny affect cognitive function and mood, and whether they can be efficiently manipulated for therapeutic gains. We are also investigating stem cells in nonneuronal tissues, focusing on interactions between different types of stem cells that help coordinate tissue growth and repair. We continue to work on the biological roles of nitric oxide, uncovering new and unexpected modes of its action. We consider our research not only as a way to understand the basic mechanisms of tissue maintenance, but also as an entry point for designing new types of therapies to ameliorate the effects of aging and disease.

Neural Stem Cells and Brain Disorders

New neurons are continuously produced from neural stem cells in selected regions of the adult brain of animals and humans; one of these regions is the hippocampus, a region crucial for cognitive function. Adult hippocampal neurogenesis responds to a multitude of extrinsic stimuli. Emerging evidence indicates that it may be important for behavior, pathophysiology, brain repair, and response to drugs. Proneurogenic and antineurogenic stimuli may affect any step of the differentiation cascade or may converge on a few defined targets. Our goal is to unravel the basic rules and mechanisms controlling the quiescence, division, self-renewal, and differentiation of neural stem cells and their progeny. To identify and quantify the cellular targets of proneurogenic and antineurogenic stimuli, we developed a novel approach that is based on reporter transgenic mouse lines (which we often use in combination with other genetically modified lines) in which neural stem and progenitor cells or their progeny are marked by fluorescent proteins. These genetically encoded reporters enabled us to dissect the neurogenesis cascade in the adult hippocampal dentate gyrus (DG) into distinct steps and to assemble a detailed scheme of the neurogenic and astrogenic arms of the cascade. We are now using this comprehensive description of the hippocampal stem cell life cycle as a conceptual framework onto which to map the targets of various neurogenic stimuli, determine the commonalities in the modes of action of these stimuli, and compare different strategies by which stem cells may be harnessed for tissue regeneration or cognitive enhancement.

We have already used the basic blueprint of hippocampal neurogenesis to identify and compare the targets of antidepressant drugs, deep brain stimulation of selected brain regions, electroconvulsive shock, exercise, radiation, chemotherapeutic agents, trauma, ablation of dopaminergic neurons, and aging. We found that such diverse antidepressant therapies as fluoxetine, running, and deep brain stimulation converge on the same steps of the cascade and increase the number of divisions in the transit-amplifying progenitor population. Importantly, these treatments do not affect the pool of stem cells; in contrast, we found that electroconvulsive shock, a highly efficient antidepressant

therapy, and memantine, a widely used Alzheimer's disease drug, activate division of stem cells in the adult hippocampus. We have also found that trauma (e.g., moderate trauma of the cortex) and disease (ablation of dopaminergic neurons of the substantia nigra in a model of Parkinson's disease) can activate stem cells of the hippocampus, even when distant regions of the brain are damaged. This activation may reflect the induction of innate repair and plasticity mechanisms by the injured brain; however, it may also potentially lead to a premature exhaustion of the neural stem cell pool, particularly in the aging organism. With hippocampal neurogenesis being important for the cognitive function and with the continuing loss of stem cells during aging, we investigated whether the levels of neurogenesis can be modified in aging animals by treatments that have been shown to increase the life span. One such treatment is restriction of calorie intake, shown to increase the life span of a diverse range of species, including mammals. Life span can also be significantly extended by prolonged treatment with the mTOR (mammalian target of rapamycin) pathway inhibitor rapamycin, even when treatment is started late in life. We investigated whether these treatments affect neurogenesis in old animals. We determined specific subclasses of neural stem and progenitor cells and the steps of the neuronal differentiation cascade targeted by calorie restriction or rapamycin. Calorie restriction was found to increase the number of dividing stem and progenitor cells in the dentate gyrus of female mice. Our results suggest that restricted calorie intake may increase the number of divisions that neural stem cells undergo in the aging brain of females.

As the evidence increases that adult neurogenesis is important for cognitive function and mood, the prospect of being able to detect changes in the level of neurogenesis in the live brain also becomes important. During the last several years, we have been carrying out a broad collaboration with Dr. Helene Benveniste (Stony Brook University and Brookhaven National Laboratory), working to characterize changes in magnetic resonance spectra in the live human and animal brain indicative of changes in neurogenesis. By modifying known methods of analyzing in vivo spectra, we are able to reliably detect indicative signals in the live animal brain under normal conditions and after electroconvulsive shock. This approach is now being investigated in preclinical and clinical trials; if successful, these trials may present a new approach for following aging, neurological and psychiatric disorders, or cancer.

Stem Cells in Nonneural Tissues

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

This approach is, of course, not limited to reporter lines based on the regulatory elements of the *Nestin* gene, and we have been using reporter lines based on the promoters of the *Sox2, Gli1*, or *Lgr5* genes, alone or in combination (e.g., *Sox2*-GFP/Nestin-mCherry or *Lgr5*-mCherry/Nestin-GFP), to investigate stem cells in different adult tissues. Using this approach and working in collaboration with Dr. Alex Nikitin from Cornell University, we made an important discovery of a new population of stem cells in the ovary that normally act to repair the epithelial surface of the ovary, but can be easily transformed and become cells of origin of ovarian tumors. When an oocyte leaves the ovary during the ovulation, the ovarian surface epithelium is ruptured and must be repaired. We found a small population of cells in the hilum of the mouse ovary that has the main characteristics of stem cells: They cycle slowly and express typical markers of stem cells, and their progeny are seen to replenish the surface epithelium. Importantly, we found that these stem cells show increased propensity to be transformed after inactivation of the tumor suppressor genes *Trp53* and *Rb1*, whose pathways are altered frequently in the most aggressive and common type of human ovarian cancer,

high-grade serous adenocarcinoma. Our study provides experimental support for the idea that susceptibility of transitional zones between different types of epithelia (of which the epithelial cells of the hilum are an example) to malignant transformation may be explained by the presence of stem cell niches in those areas.

Our findings support the possibility that junction-associated stem cell niches are predisposed to cancer and suggest that similar junction/transitional areas in other organs, such as the uterine cervix, may also contain cancer-prone stem cell niches, thereby explaining susceptibility of such regions to malignant transformation. Our findings also put forward an attractive model for further studies of stem cell niches and of aberrations in the molecular and cellular mechanisms governing epithelium regeneration that may contribute to ovarian cancers.

Signaling in the Stem Cell Niche

Signaling pathways controlling adult neurogenesis are only beginning to be understood. Although the action of specific regulators of stem and progenitor cell division (e.g., Notch, Shh, or Wnt) are studied in detail, more general questions—such as the source of the signals, autocrine vs. paracrine vs. hormonal regulation, interaction between different cell types, or specific signaling messenger molecules—are often unknown even for the best-studied systems, including the brain. One such general mode of stem cell control is redox signaling. The changes in the redox status and production of reactive oxygen and nitrogen species (ROS/RNS) change proliferation and differentiation of various classes of stem and progenitor cells, including neural cells.

Although the general importance of redox signaling is acknowledged, the picture remains vague with respect to stem cells. There are gaps in understanding even the most basic features of redox signaling in stem cells (e.g., the source of the signal, distribution of activity within the cell, and changes during aging or upon stimulation). One approach to study signaling is to use genetically encoded sensors of redox signaling. We have demonstrated the utility of sensors for superoxide/hydrogen peroxide and for the cytoplasmic NAD/NADH in several settings, including neurons in vivo and in vitro, and have generated transgenic animals that express these genetically encoded sensors. Our goal is to combine two directions of research—genetically encoded sensors and genetically encoded reporters—to gain insights into redox signaling in adult neural stem cells and to observe dynamic changes in stem cells, their compartments, and their niche. We are now working to expand the panel of sensors and reporters to visualize multiple signaling events controlling stem cells.

Nitric Oxide Development, and Differentiation

During recent years, we discovered an essential role of nitric oxide (NO) in development and in stem cell regulation. We continue to discover the diversity of biological functions performed by NO. In our latest studies, we uncovered an important function of NO in multiciliated cells whose cilia beat in concert to generate directed flow of fluid across tissue. We found that this role of NO is conserved and its basic features are highly similar in such diverse settings as skin of the frog embryo and mammalian airways. We identified two distinct signaling pathways that control the polarity of cilium distribution and cilium length and determined the critical targets of NO signaling in both pathways. Our results suggest that some of the inborn and acquired human ciliopathies and related disorders may also be related to decreased availability of NO and may thus benefit from NO-based therapies.

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MOLECULAR ANALYSIS OF NEURONAL RECEPTORS AND ION CHANNELS

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N. Simorowski
N. Tajima

The research program in my lab is aimed at understanding the molecular basis for the functions of cell surface receptors or membrane proteins that initiate cellular signal transductions involved in neurotransmission in the mammalian brain. It is hoped that this work will help in the design of compounds with therapeutic potential in neurological diseases and disorders including schizophrenia, depression, stroke, and Alzheimer's disease. Toward this end, we are conducting structural and functional studies on neurotransmitter receptor ion channels and intramembrane enzymes, two classes of membrane proteins that regulate the processes of neurotransmission and dysfunction implicated in neurodegenerative diseases. To achieve our goals, we use X-ray crystallography to determine the three-dimensional atomic structures of target proteins and test structure-based mechanistic hypotheses by site-directed mutagenesis in combination with biochemical and biophysical techniques. Our main accomplishment in 2012 was obtaining high-resolution structures of the ligand-binding domain (LBD) of a ligand-gated ion channel, *N*-methyl-D-aspartate receptor (NMDAR), in complex with antagonists. The crystal structure shows overall conformational alteration in LBD upon binding to two types of antagonists compared to the agonist-bound form. We are currently validating functional hypotheses using a combination of techniques including electrophysiology, isothermal titration calorimetry, and analytical ultracentrifugation.

Structural Studies on NMDAR

NMDARs belong to the family of ionotropic glutamate receptors (iGluRs) that mediate the majority of excitatory synaptic transmissions in the mammalian brain. They are voltage-sensitive calcium ion channels composed of two copies each of the GluN1 and GluN2 subunits, which bind glycine and glutamate, respectively. Calcium signals facilitated by NMDARs have pivotal roles in cellular signal transduction, resulting in neuroplasticity essential for learning and memory formation. Dysfunctional NMDARs are implicated in various neurological disorders and diseases including depression, schizophrenia, Parkinson's disease, and Alzheimer's disease. The four distinct GluN2 subunits (A–D) control pharmacological properties of the NMDAR ion channels and show different spatial and temporal expression patterns; thus, there has been great interest in creating subtype-specific compounds that can target specific neuronal circuits. The NMDAR subunits are modular and are composed of distinct protein domains, including the amino-terminal domain (ATD), ligand-binding domain (LBD), transmembrane domain (TM), and carboxy-terminal domain (CTD) (Fig. 1).

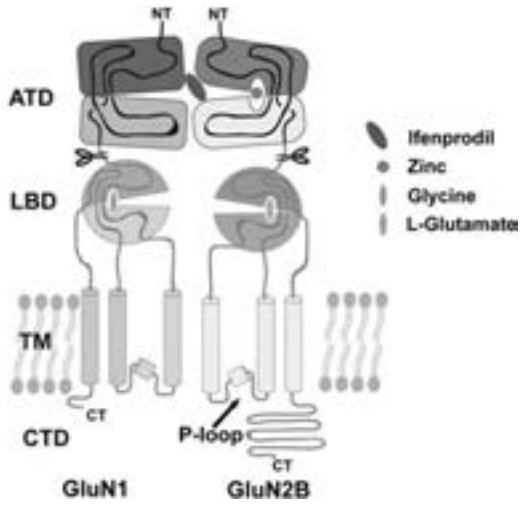

Figure 1. Domain organization of NMDAR subunits. NMDAR subunits are modular proteins composed of distinct domains including amino-terminal domain (ATD), ligand-binding domain (LBD), transmembrane domain (TM), and carboxy-terminal domain (CTD). Shown here are schematic presentations of the glycine-binding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

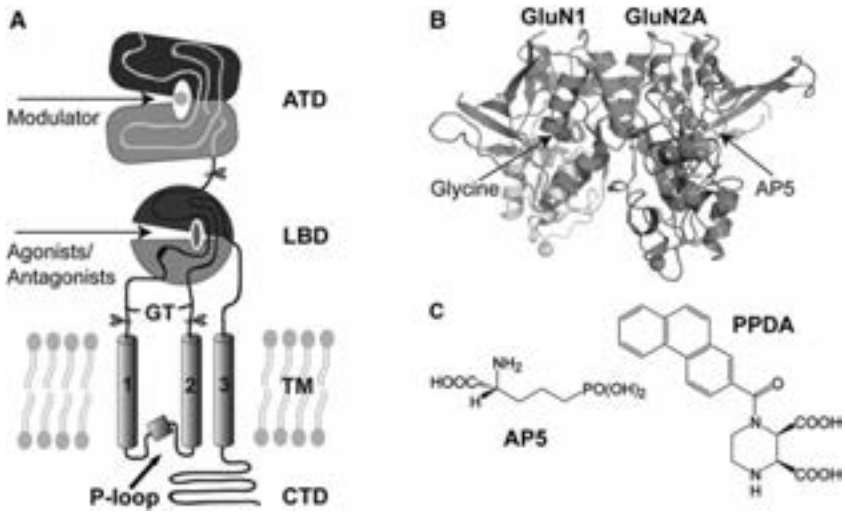

Figure 2. Crystal structure of GluN1/GluN2A LBD. (*A*) LBD was designed by excising (using scissors) the part of the extracellular domain. (*B*) Preliminary crystal structure of GluN1/GluN2A LBD in complex with glycine and AP5 at 1.9 Å. GluN1 and GluN2A LBDs have a bilobed "clam-shell-like" structure and the ligands bind at the cleft of the structures. (*C*) Chemical structures of the two classes of antagonists, AP5 and PPDA.

The focus of our current studies is the ATD and LBD domains, which mediate allosteric regulation and activation in the NMDAR ion channels upon binding to polyamine-based compounds and neurotransmitters, respectively. Despite intensive efforts in structural studies of NMDARs during the past decade, we still have no insight into how GluN2 targeting antagonists bind and mediate the inhibition of NMDAR ion channels. Furthermore, there was until now no understanding of how GluN2 antagonist compounds such as AP5 and PPDA, which have completely distinct chemical structures, could mediate similar antagonistic effects on NMDAR ion channels (Fig. 2C). To answer these questions, we have conducted X-ray crystallographic studies on the GluN1/GluN2A subtype in complex with the GluN2 antagonists AP5 and PPDA. Crystals have been created by mixing GluN1 and GluN2A LBD proteins in the presence of GluN2 antagonists such as PPDA and AP5 and in the presence of GluN1 agonist glycine. These crystals diffracted X rays to 1.9 Å (for AP5 crystal) and 2.1 Å (for PPDA crystal). The high-resolution structures that resulted from the high-resolution diffraction data unambiguously showed ligand-binding modes as well as overall protein conformational changes (Figs. 2B and 3).

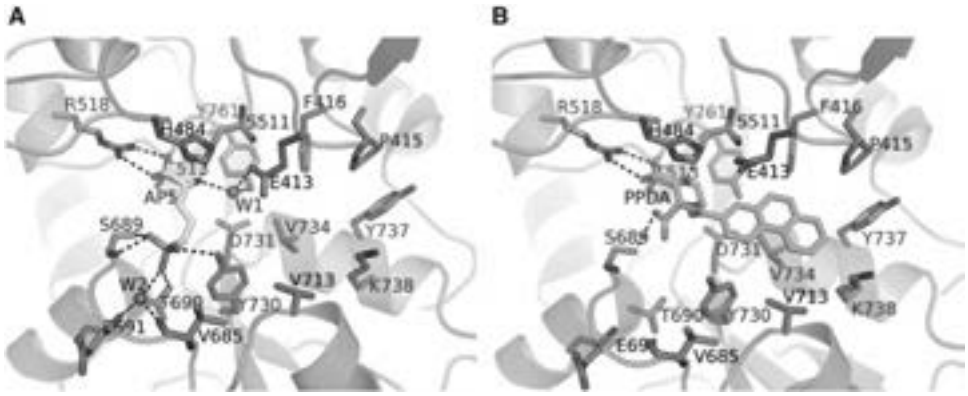

Figure 3. Antagonist binding site. Binding sites of AP5 (*A*) and PPDA (*B*) show two different modes of antagonist recognition. Dots represent polar interaction.

The crystal structures identified the antagonist binding site at the cleft of the clam-shell-like structure. As shown in Figure 3, the binding of AP5 (Fig. 3A) and PPDA (Fig. 3B) involves different residues. The binding of AP5 is mediated mainly by hydrophilic interactions between the α-carboxyl and phosphine groups. Binding of PPDA is mediated mainly by hydrophobic interaction between hydrophobic residues deep in the LBD cleft. However, in both cases, the clamshell-like architecture of LBDs is forced open similarly to the agonist (glutamate)-bound form, indicating that the antagonism of NMDAR involves conformational alteration. Based on the crystal structures, we will design hybrids of AP5 and PPDA to increase potency of the antagonists. Such reagents may be tested in animal models for neurological disorders and diseases to determine any potential therapeutic values.

Structure and Function of γ-Secretase

γ-Secretase is an intramembrane protease involved in the initiation of Notch signaling, which is critical for cell differentiation and stem cell maintenance as well as production of β-amyloid involved in the pathology of Alzheimer's disease. The formation of neurotoxic β-amyloid oligomers and deposition of β-amyloid fibrils are hallmarks of Alzheimer's disease. Furthermore, overactivation of Notch signaling is frequently observed in various forms of cancer. Thus, pharmacological regulation of γ-secretase activity may be an effective strategy in the treatment of those major diseases.

γ-Secretase is a multimeric protein complex of four transmembrane proteins including presenilin, nicastrin, APH-1, and PEN-2 (Fig. 4). Together, the protein complex mediates an unusual molecular event called regulated intramembrane proteolysis, in which the transmembrane substrate is proteolyzed within the biological membrane. This multimembrane protein complex cleaves a number of single transmembrane substrates, including Notch ligand and amyloid precursor protein. The mechanistic understanding of intramembrane proteolytic activities in γ-secretase is limited due to a complete absence of any atomic structure. My research program is aimed at obtaining an atomic map of γ-secretase to gain insight into intramembrane proteolysis, substrate specificity, inhibition, and functional modulation.

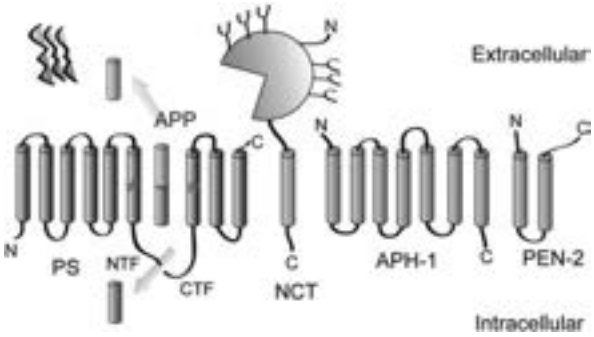

Figure 4. Components of γ-secretase. γ-Secretase is a protease complex composed of four integral membrane proteins: presenilin (PS), nicastrin (NCT), APH-1, and PEN-2. Wild-type PS contains active-site aspartyl residues within the transmembrane domains and becomes active only when it forms a complex with three other components. A single transmembrane substrate such as amyloid precursor protein (APP) is proteolytically processed within the membrane. The extracellular and cytosolic domains form neurotoxic β-amyloid oligomers or act as a transcriptional regulator, respectively. NCT and APH-1 are considered important for substrate recognition, whereas PEN-2 has a critical role in proteolyzing PS at the cytosolic loop, thereby converting PS into the active form.

In 2012, we continued to spend much effort toward understanding the mechanism of substrate recognition mediated by a γ-secretase component, nicastrin. Using the recombinant proteins that have been successfully expressed and purified by my group (as reported in 2011), we have conducted screening for crystallization conditions with minimal success. Several potential reasons for our unsuccessful crystallization attempts are that (1) nicastrin contains 20 glycosylation sites that could interfere with crystallization, (2) a substantial portion of nicastrin is intrinsically unstructured, and (3) the nicastrin protein is not stable for the long period of time (weeks), needed for crystallization. To circumvent these problems, we have found a way to prepare nicastrin proteins with minimal glycosylation (enzymatic removal of glycosylation, incorporation of mutations to putative glycosylation sites, and usage of a GlnT$^-$ cell line that homogeneously glycosylates proteins). To create crystal contacts, we also made monoclonal antibodies for nicastrin. These antibodies will be complexed with the purified nicastrin proteins, which will be used to facilitate crystallization. Thus far, we have a preliminary crystal for nicastrin complexed with antibodies. We will further optimize the crystallization conditions so that the sample could be

used for crystallographic studies. Overall, the structural and functional studies of γ-secretase have great potential for the development of compounds that can be used to lower the level of neurotoxic amyloid oligomers in Alzheimer's disease, as well as to alleviate overactive Notch signaling in cancer.

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ASSEMBLY AND FUNCTION OF GABA INHIBITORY CIRCUITS IN THE NEOCORTEX

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M. He K. Krishnan J. Lu H. Taniguchi P. Wu

Our research aims to understand the organization, development, and function of neural circuits in the cerebral cortex and their implications in neuropsychiatric disorders. We have pioneered the use of mouse engineering toward a genetic dissection of GABAergic inhibitory circuitry in the neocortex. We recently achieved the genetic targeting of major classes and lineages of cortical interneurons. Our strategy and tools are enabling a systematic and comprehensive analysis of GABAergic circuits throughout the mammalian brain. We have made discoveries in the cellular and molecular mechanisms underlying the specificity and activity-dependent development of GABAergic synapses, and in the role of GABAergic inhibition in regulating the critical period of neural plasticity. More recently, we have further developed strategies for cell-type-based genomic analysis in brain tissues. Genetic targeting of distinct neuron types establishes entry points for studying cortical circuits and builds a solid middle ground that coherently connects to system neuroscience, on the one hand, and molecular and development neuroscience, on the other hand. We aim to discover the genetic principles underlying the assembly and organization of cortical circuits and to provide insight into how altered development of these circuits contributes to mental disorders.

Origin and Organization of Chandelier Cells in the Mouse Neocortex

Diverse GABAergic interneurons regulate the functional organization of cortical circuits and derive from multiple embryonic sources. It remains unclear to what extent embryonic origin influences interneuron specification and cortical integration due to difficulties in tracking defined cell types. Using genetic fate mapping, we followed the developmental trajectory of chandelier cells (ChCs), the most distinct interneurons that innervate the axon initial segment of pyramidal neurons and control action potential initiation. A single ChC innervates hundreds of pyramidal neurons and might exert decisive influence in their firing, but the developmental origin and cortical organization of ChCs have been unclear. We discovered that ChCs mainly derive from the ventral germinal zone of the lateral ventricle during late gestation and require the homeodomain protein Nkx2.1 for their specification. They migrate with stereotyped routes and schedule and achieve specific laminar distribution in the cortex. The developmental specification of this bona fide interneuron type likely contributes to the assembly of a cortical circuit motif. Our findings demonstrate protracted neurogenesis and a novel progenitor source in the Nkx2.1 lineage that gives rise to a functionally powerful and evolutionarily expanding class of cortical interneuron. Genetic targeting of ChCs provides an entry point to studying cortical circuit assembly, organization, and dysfunction.

Impact of GABAergic Deficiency in Synaptic Transmission and Behavior

The rate-limiting enzyme for GABA synthesis, GAD67, is altered in several neuropsychiatric disorders, including autism, bipolar disorder, and schizophrenia (SZ). GAD67 expression is activity dependent, and its reduction in disease states is thought to result from decreased engagement of inhibitory circuitry. Although this common pathophysiological feature may be a major contributor to disease processes, whether and how GAD67 deficiency leads to impaired inhibitory synaptic transmission have not been examined. This is in part due to confounds from developmental compensation and the diversity of inhibitory interneurons. We have produced a cell-type-restricted molecular deficit, similar to what is observed in SZ, by specifically knocking out one allele of the gene for GAD67, *Gad1*, in PV interneurons in juvenile mice. This manipulation led to substantial deficits in synaptic transmission from parvalbumin

(PV) interneurons to pyramidal neurons assayed by paired recording in prefrontal cortex: IPSC (induced pluripotent stem cells) amplitude and decay time both decreased, producing a supralinear reduction in overall IPSC charge transfer. Single-cell recordings revealed a concurrent disinhibition of pyramidal neuron spiking and increased excitation/inhibition balance in PV cells. In adult mice, synaptic deficits recovered, possibly through a homeostatic response to increased feedback excitation of the PV cell network. These results suggest that GAD67 levels directly contribute to the strength of synaptic inhibition. In SZ specifically, GAD67 deficiency in PV cells likely contributes to cortical network dysfunction, but the reversibility observed in our model suggests nonpermanent damage to inhibitory circuitry.

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

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

Our approach establishes a cell-type-based experimental paradigm for epigenomic analysis in complex tissues and sets the stage for analysis of neuronal subtypes during both brain development and behavioral plasticity.

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

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

MeCP2 Regulates the Maturation of GABAergic Interneurons and Timing of Critical Period Plasticity in Primary Visual Cortex

Rett syndrome (RTT) in several ways epitomizes the challenge for understanding neuropsychiatric disorders. RTT is caused by mutations in the *MeCP2* gene encoding a chromatin and transcription regulatory protein. Although MeCP2 is broadly expressed in the developing and mature brain, RTT is characterized by a specific set of postnatal symptoms including language and cognitive deficits, stereotyped behaviors, and a myriad of sensory and neurological phenotypes. *MeCP2* mutations likely impact gene regulation in multiple neuronal and glial cell types and in multiple brain regions; the initial impact may further result in compensatory changes, including

maladaptive activity- and experience-dependent synaptic plasticity, leading to altered developmental trajectory of circuit formation and function. Although numerous mouse models of genetic etiology have been established, it has been difficult to identify primary molecular and cellular changes and trace their impact on circuit alterations that underlie behavioral deficits. Using *MeCP2-null* mice, we examined experience-dependent development of neural circuits in the primary visual cortex where GABAergic interneurons regulate a critical period of plasticity. The maturation of GABAergic neurons was accelerated upon vision onset, indicated by elevated GABA synthetic enzyme GAD67 and perineuronal nets, both associated with PA-positive interneurons. These changes correlated with a precocious critical period and deficient visual function—the orientation tuning of individual binocular neurons remained mismatched through the two eyes in mature animals. Our results trace molecular and cellular changes through aberrant circuit development to functional deficits in a model of neuropsychiatric disorder.

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NEUROBIOLOGY OF COGNITION AND DECISION MAKING

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The long-term goal of our research is to identify the computational and neurobiological principles underlying cognition and decision making. Using a reductionist approach, we translate psychological questions into the language of neuroscience by distilling them to quantitative behavioral tasks for rodents that enable the monitoring and manipulation of neural circuits mediating cognitive behaviors. We use state-of-the-art electrophysiological techniques to establish the neural correlates of behavior and then use molecular and optogenetic manipulations to systematically dissect their underlying neural circuits. Given the complexity of animal behavior and the dynamics of neural networks producing it, our studies require quantitative analysis and computational models to guide and sharpen the neurobiological questions. In addition, we started incorporating human psychophysics into our research to validate our behavioral observations in rodents by linking them with analogous behaviors in human subjects.

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

Neural Basis of Decision Confidence

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

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

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

From Metacognition to Statistics: Confidence Judgments in Humans

J. Sanders, B. Hangya

In our rodent studies of confidence, we use a computational framework to interpret behavioral and neural data. We are also interested in understanding the degree to which the theoretical concept of *decision confidence*

corresponds to the human notion of subjective confidence. In statistics, the confidence associated with a hypothesis test is a measure of the quality of evidence, and we believe that the subjective notion of confidence describes a similar process at work in the brain.

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

We have also developed other behavioral tasks testing confidence associated with general knowledge, only suitable for humans. We find that all these tasks share the basic patterns of confidence that are predicted by statistical confidence. On the basis of these results, we are now in a position to use these quantitative measures of decision confidence in humans, through collaborations, with neuroimaging and genetic approaches. Moreover, by showing that a single confidence measure is applicable to humans and rodents, our results strengthen the case for using the rat as a model system for studying decision confidence.

Integration of Decision Confidence and Reward Size in the Orbitofrontal Cortex

J. Hirokawa

Making optimal decisions requires integrating information from multiple sources. For instance, perceptual decisions involve an evaluation of the uncertainty about a stimulus, whereas free-choice paradigms require an estimation of the reward value of each option. Can rats combine experienced reward value and decision uncertainty to make optimal choices? And how does the brain dynamically compute and integrate these different estimates to make predictions?

We designed a reward-biased psychometric decision task to investigate interaction of reward estimates based on decision uncertainty and reward history. We found that the animals' choice was biased by the reward size and the extent was proportional to the amount of evidence of the correctness for the chosen option, suggesting that decision confidence and reward size were optimally integrated behaviorally. Multiunit recording from the OFC revealed that the largest population of neurons combined "decision uncertainty" with "small reward size" information to yield an integrated negative reward value representation. However, different portions of neurons still representing decision confidence were little affected by the reward size manipulation, and some other neurons represented reward size independent of decision confidence. These results show that rat OFC has the capacity to integrate different types of reward expectations for a chosen option that may contribute to maximizing future reward acquisition.

Microtraits in Rodent Models of Psychiatric Diseases

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

Basic research on psychiatric disorders requires the use of animal models. Usually, animal models are validated mainly on the basis of genetic insights and simple behavioral measures. However, in the case of cognitive diseases, this has become challenging because it is unclear how to map human behavioral deficits to animal models. To overcome these issues, we are taking a different approach to this challenge, based on the emerging field of computational psychiatry.

We focus on microtraits that can be easily quantified using psychometric tasks and can be directly related to humans, e.g., reward sensitivity, learning rate, and decision confidence.

For instance, our psychometric olfactory discrimination task enables us to measure several important

parameters of decision making: sensitivity to reward and punishment, accuracy of the discrimination, integration of the stimuli, flexibility, reward history, and decision confidence. We are currently using this task to study two rodent models for cognitive disorders: spontaneously hypertensive rats (SHR) for attention-deficit/hyperactivity disorder (ADHD) and congenital learned helplessness (cLH) rats for major depressive disorder (MDD). Our goal is to test both model rats in our task, determine behavioral microtraits, and eventually relate them to specific neural circuits such as OFC.

Division of Labor between Distinct Interneuron Subtypes during Behavior

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

The anterior cingulate cortex (ACC) in humans and rodents has been implicated in a variety of goal-directed behaviors including reward processing, inhibitory response control, conflict monitoring, and foraging decisions. Electrophysiological recordings from ACC show great diversity of neuronal responses to a specific behavioral variables. On the other hand, we know that the mammalian cortex is composed of a variety of cell types, among which GABAergic interneurons exhibit the largest diversity in connectivity, morphology, and intrinsic physiology. Does the anatomical and molecular variety of interneuron subtypes map onto the diversity of neuronal responses in behaving animals? To address this issue, we use optogenetics as a means to identify extracellularly recorded neurons in freely moving mice, focusing on inhibitory interneurons, which exhibit the largest diversity of cell types in the cortex. We tested the overarching hypothesis that neurons belonging to the same subtype share fundamental commonalities in response properties during behavior, whereas distinct subtypes specialize in distinct functional roles.

We showed that Pv and a subtype of Som neurons form functionally homogeneous populations showing a double dissociation between both their inhibitory impact and behavioral correlates. Of a number of events pertaining to behavior, a subtype of Som neurons selectively responded at reward approach, whereas Pv neurons responded at reward, leaving encoding preceding stay duration. These behavioral correlates of Pv and Som neurons defined a behavioral epoch and a decision variable important for foraging (whether to stay or to leave), a crucial function attributed to ACC. Furthermore, Pv neurons could fire in millisecond synchrony, exerting fast and powerful inhibition on principal cell firing, whereas the inhibitory impact of Som neurons on firing output was weak and more variable, consistent with the idea that they respectively control the outputs of and inputs to principal neurons. These results suggest a connection between the circuit-level function of different interneuron types in regulating the flow of information and the behavioral functions served by the cortical circuits. Moreover, these observations bolster the hope that functional response diversity during behavior can in part be explained by cell-type diversity.

Function of VIP Interneurons in Cortical Microcircuits

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

We are interested in understanding how the diversity of cortical inhibitory interneurons underlies distinct neural circuit dynamics that direct perception and behavior. As part of this broader project, we have begun to study vasoactive intestinal peptide (VIP)-expressing interneurons. Although VIP neurons constitute less than 2% of total cortical neuronal population, previous studies indicate that VIP might have a distinct function in the local cortical column. VIP neurons are mostly bipolar, and their processes are vertically oriented. Interestingly, these neurons specifically target other inhibitory neurons and avoid pyramidal cells. Therefore, we hypothesized that VIP neurons regulate a disinhibitory circuit and tested it in two functionally different cortical regions: auditory and medial prefrontal cortices.

Using a combination of in vivo and in vitro physiology, we showed that VIP interneurons mediate disinhibitory control in multiple areas of the neocortex. By combining optogenetic activation with single-cell recordings, we examined the functional role of VIP interneurons in awake mice and investigated the underlying circuit mechanisms in vitro in auditory and medial prefrontal cortices. We identified a basic disinhibitory circuit module in which activation of VIP interneurons transiently suppresses most somatostatin-expressing and a fraction of parvalbumin-expressing inhibitory interneurons, specialized to control the input and output of principal cells, respectively. In the

auditory cortex, we found that reinforcement signals (punishment and reward) strongly activated VIP neurons, and in turn, VIP recruitment modulated the tone-responsiveness of a functional subpopulation of principal neurons. We propose that this disynaptic disinhibitory mechanism represents a cortical circuit motif that enables long-range inputs to modulate the gain of local circuits.

Neural Representation of Social Decisions and Rewards

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

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

We started our studies by recording from part of the amygdala that processes information from the vomeronasal system and detects behaviorally significant chemical cues, such as pheromones. We have designed a behavioral task that presents different urinary proteins to our mice while we are performing electrophysiology in the medial amygdala. Moreover, we characterized molecular markers that enable us to further dissect the social amygdala circuit. Currently, we are carrying out optogenetic experiments as well as electrophysiological recordings to map behavioral relevance onto specific medial amygdala neurons.

We are also interested in understanding the basic rules that mice use to choose the appropriate mating partner for themselves. For this purpose, we are developing psychophysical social behavior tasks to combine with our electrophysiology studies. This system improves stimulus delivery precision and repertoire. To deliver social stimuli and determine their "value" for a mouse, we developed a new behavioral setup, the "mouse carousel." In the future, we plan to characterize social preferences and neural circuits using this behavioral paradigm.

Optogenetic Dissection of Nucleus Basalis during Sustained Attention

B. Hangya, S. Ranade, M. Lorenc

The nucleus basalis (NB) is a vitally important yet poorly understood neuromodulatory system that is thought to have significant roles in cognitive functions, including learning, memory, and attention. Cognitive deficits in Alzheimer's disease, Parkinson's dementia, age-related dementias, and normal aging are correlated with the extent of deterioration of NB cholinergic neurons. Yet, despite the association of NB with higher cognitive functions and a host of disease states in humans, it is surprising how little we understand about its function during behavior. Previous research, mostly using lesions, pharmacology, and microdialysis, revealed that NB can have strong and confusingly diverse effects on downstream targets and behavior. However, there are no functional studies that reveal what NB neurons actually do during behavior. To overcome these challenges, we combine optogenetic and electrophysiological approaches to record from identified cholinergic projection neurons in NB during behavior. We have successfully developed visual and auditory versions of sustained attention tasks for mice. In these tasks, mice report target stimuli occurring at uncertain times. Performance and reaction time are used as indicators of attention. We manipulate temporal expectancy by using specific distributions for stimulus timing, thus modulating attention in a temporally precise, graded manner, also reflected in the reaction times of the animals. At present, our efforts are focused on recording from identified cholinergic neurons to assess their role in sustained auditory attention and optogenetic manipulations of cholinergic neurons to influence behavioral variables.

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Adam Kepecs, Ebru Demir

THEORETICAL AND COMPUTATIONAL NEUROSCIENCE

A. Koulakov B. Kolterman Y. Wei

Our laboratory develops theories of neural computation. We work in parallel on three topics. First, we formulate mathematical models for combining genetic information and experience (nature and nurture) during formation of connections between neurons. Our models describe how genes can help build neural networks and how neural activity adds a layer of plasticity to the network topology that reflects learning and experience. These models have been tested on circuits that are formed in the visual system and can be rewired through genetic, surgical, and pharmacological manipulations. This year, we have applied our theory to the visual maps of ocular dominance observed in visual cortices of many higher vertebrates, such as humans. We were able to relate the orientation of ocular dominance patterns to the distribution of molecular labels that control establishment of connectivity between the cortex and thalamus. Second, we have been developing the neural network theory for olfactory processing. We previously argued that information about smells can be represented in the olfactory system in the form of temporal sequences. This year, we proposed a network model for the processing of temporal sequences by the olfactory cortex. Our model is consistent with the known features of anatomy and physiology of the piriform cortex. Finally, we have attempted to apply neural network theory to the networks regulating gene expression in cells. We considered the impact of fusion of two cells that reside in different conflicting states. We argued that this event may make cells into spurious attractors—meaning they occupy abnormal states inaccessible during normal cell differentiation. Our mathematical formalism allowed a precise definition of the Waddington epigenetic landscape through the Lyapunov function of the underlying network. Finally, we argued that the phenomenon of collision of two or more networks and the resulting dynamics may be more general and apply outside of biology to processes in economics, politics, and society.

Odors in the Olfactory Bulb Are Defined by a Short Discrete Temporal Sequence: Recognition by Brute Force Conversion to Spatial Patterns

A. Koulakov, B. Kolterman [in collaboration with H. Sanders and J. Lisman, Brandeis University, Waltham, Massachusetts; D. Rinberg, HHMI Janelia Farm, Ashburn, Virginia and New York University]

Recordings in awake behaving rodents have recently revealed new properties of olfactory coding. Mitral cells, the principal neurons in the olfactory bulb, respond to odorants by firing bursts of action potentials called sharp events. The time of the onset of the sharp event for a particular mitral cell occurs at a precise moment with respect to the sniff cycle for a given odor, but sharp events occur at different times for different cells and for different odors. In this project, we studied the onset of sharp events and found that they occur phase-locked to the ongoing gamma frequency oscillation. Because there are not many gamma cycles during a single sniff, the signature of an odor is a discrete sequence that is relatively short. These findings suggest a new class of solutions to the problem of odor recognition. Specifically, the cortex might contain a small number of modules, each forming a persistent snapshot of what occurs in a certain gamma cycle. The collection of these snapshots forms a spatial pattern that represents the entire sequence and that could be recognized by standard attractor-based mechanisms. We show that such a "brute force" solution can occur in a neurally plausible way.

An Exactly Solvable Model of Random Site-Specific Recombinations

Y. Wei, A. Koulakov

The Cre-Lox system is used as a genetic tool to control site-specific recombination events in genomic DNA. If multiple recombination sites are organized

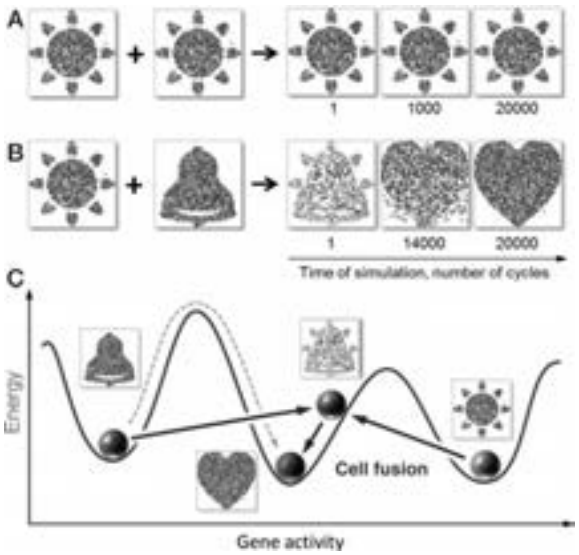

Figure 1. Simulated cell fusion can change cell types. The state of this network is represented by an array in which activated genes are indicated in light gray, inhibited genes in dark gray, and genes with basal activity in white. The entire network contains 2304 (482) genes, and thus its state is visualized by a 48 × 48 array. (*A*) Fusion of two cells of the same type leads to a hybrid of the same type. (*B*) Fusion of two cell types (sun and bell) results in a hybrid that belongs to another cell type (heart). (*C*) Interpretation of middle cells. Cell fusion can transport a cell from one attractor (bell) to the basin of another (heart) by creating an intermediate product (bell + sun) with another cell (sun). This mechanism bypasses the need to overcome the hills of the epigenetic space.

in a compact cluster within the same genome, a series of stochastic recombination events may generate substantial cell-specific genomic diversity. This diversity is used, for example, to distinguish neurons in the brain of the same multicellular mosaic organism, within the brainbow approach to neuronal connectome. Thus, every cell in an organism could be identified on the basis of a relatively short unique DNA barcode. Here we study an exactly solvable statistical model for site-specific recombination operating on a cluster of recombination sites. We consider two types of recombination events: inversions and excisions. Both of these events are available in the Cre-Lox system. We derive three properties of the sequences generated by multiple recombination events. First, we describe the set of sequences that can in principle be generated by multiple inversions operating on the given initial sequence. Second, we demonstrate that after a large number of stochastic inversions, every sequence that can be generated is generated with equal probability. Finally, we derive equations for the probability of finding a sequence as a function of time in the limit when excisions are much less frequent than inversions, such as in shufflon sequences.

The Problem of Colliding Networks and Its Relation to Cell Fusion and Cancer

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

Cell fusion, a process that merges two or more cells into one, is required for normal development and has been explored as a tool for stem cell therapy. It has also been proposed that cell fusion causes cancer and contributes to its progression. These functions rely on a poorly understood ability of cell fusion to create new cell types. We suggest that this ability can be understood by considering cells as attractor networks whose basic property is to adopt a set of distinct, stable, self-maintaining states called attractors. According to this view, fusion of two cell types is a collision of two networks that have adopted distinct attractors. To learn how these networks reach a consensus, we model cell fusion computationally (Fig. 1). To do so, we simulate patterns of gene activities using a formalism developed to simulate patterns of memory in neural networks. We find that the hybrid networks can assume attractors that are unrelated to parental attractors, implying that cell fusion can create new cell types by nearly instantaneously moving cells between attractors. We also show that hybrid networks are prone to assume spurious attractors, which are emergent and sporadic network states. This finding means that cell fusion can produce abnormal cell types, including cancerous types, by placing cells into normally inaccessible spurious states. Finally, we suggest that the problem of colliding networks has general significance in many processes represented by attractor networks, including biological, social, and political phenomena.

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

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

Experience-Dependent Synaptic Modification in the Central Amygdala Fear Circuit

The amygdala is essential for fear learning and expression. The central amygdala (CeA), once viewed as a passive relay between the amygdala complex and downstream fear effectors, has emerged as an active participant in fear conditioning. However, how CeA contributes to the learning and expression of fear is unclear. We have found in mice that *fear conditioning induces robust plasticity of excitatory synapses onto inhibitory neurons in the lateral subdivision of CeA (CeL).* This experience-dependent plasticity is cell-specific, bidirectional, and expressed presynaptically by inputs from the lateral amygdala. In particular, preventing synaptic potentiation onto somatostatin-positive (SOM^+) neurons impairs fear memory formation. Furthermore, activation of these neurons is necessary for fear memory recall and sufficient to drive fear responses. These findings support a model in which the fear-conditioning-induced synaptic modifications in CeL favor the activation of somatostatin-positive neurons, which inhibit CeL output, thereby disinhibiting the medial subdivision of CeA and releasing fear expression (Fig. 1). Our ongoing study on this project is revealing the mechanisms by which the inhibitory circuits of CeL respond to fear conditioning and contribute to both the learning and expression of fear. Although fear conditioning modifies multiple synapses, the fear-conditioning-induced potentiation of excitatory synaptic transmission onto SOM^+ CeL neurons appears to be crucial, as suppression of this potentiation severely impaired fear memory. Our results also support a model in which CeA stores fear memory in series with lateral amygdala. Such serial organization of fear memory allows the regulation of fear conditioning at multiple levels. Moreover, as transmission is potentiated following fear conditioning, both at the auditory thalamus–lateral amygdala synapses and at the lateral amygdala–CeL synapses, the signal carrying conditioned stimulus (CS) information can be reliably transmitted from the auditory thalamus to CeA via lateral amygdala while maintaining specificity.

These findings delineate cellular and circuit mechanisms that may explain previously reported observations: (1) pharmacological inactivation of CeL during conditioning impairs fear learning; (2) fear conditioning is followed by the appearance of two functionally distinct cell populations in CeL, the CeL_{on} and CeL_{off} neurons, which show opposite responses to CS; and (3) the appearance of CeL_{on} neurons is associated with (medial division of CeA) activation, rather than inhibition. Further studies will be required to elucidate the detailed cellular and molecular changes in distinct CeL inhibitory circuits during fear conditioning and to determine how they are related to fear memory acquisition, consolidation, and expression.

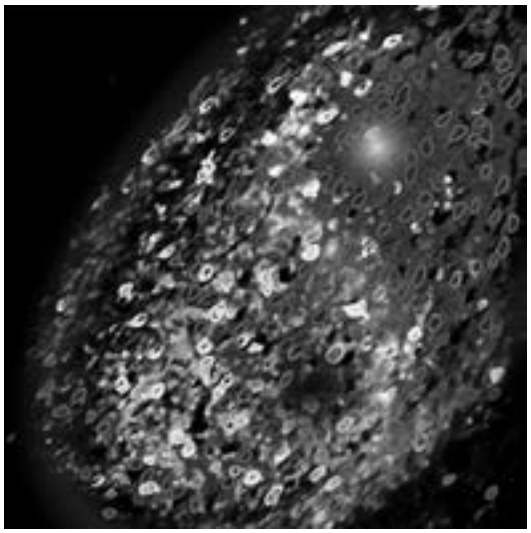

Figure 1. The yin and yang of the central amygdala. Image shows neurons in the lateral subdivision of the central amygdala (CeL). Darkly labeled cells are somatostatin-positive (SOM^+) neurons, which promote fear; brightly labeled cells are another set of neurons known as PKC-delta$^+$ cells, which inhibit fear.

The Habenula Circuitry in the Learned Helplessness Model of Depression

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

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

The Thalamic Reticular Nucleus Circuit that Controls Attention

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

Rule selection and input selection represent distinct attentional processes that likely require the coordinated action of cortical and subcortical structures. We found that suppression of SOM^+ TRN neurons (by the chemical-genetic method) impaired performance in both the input-selection and rule-selection tasks, indicating that these neurons are indispensable. On the other hand, increasing cortical

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

These results uncover a critical role of a cortico–TRN synaptic circuitry in goal-directed attention, and provide insights on cellular and circuit mechanisms by which TRN dysfunction may occur and contribute to a consistently observed cognitive impairment in major psychiatric disorders. Our results suggest that either aberrantly increased or decreased TRN neuronal activity can result in impairment in rule selection, a key aspect of executive control. The rule-selection task used in our study mimics, to some extent, the Stroop task, a test commonly used to assess cognitive deficit in schizophrenia. The finding that ErbB4 deficiency selectively impairs rule selection but not input selection, parallels a clinical observation, that schizophrenia specifically affects performance in the Stroop task and does not impair attentional functions in general. Deletion of a single copy of *ErbB4* is sufficient to produce a robust effect, suggesting that ErbB4 signaling is exquisitely regulated in SOM+ TRN neurons in order to properly maintain TRN function. Thus, our findings reveal circuitry and cellular mechanisms that may underlie a form of cognitive deficit observed in mental disorders.

In Press

Haohong Li, Penzo M, Taniguchi H, Kopec CD, Josh Huang Z, Li B. 2013. Experience-dependent modification of a central amygdala fear circuit. *Nat Neurosci* **16:** 332–339.

Kristen Delevich

INTEGRATIVE SYSTEMS NEUROBIOLOGY

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

We study complex neurobiological systems using a combination of experimental and computational approaches. The primary area of experimental work in our laboratory is the Mouse Brain Architecture (MBA) project, with a goal to generate the first brain-wide mesoscale connectivity map in the mouse. In collaborative studies, we also apply the methods developed for the MBA project to multiple other species, including zebra finch, marmoset, macaque monkey, and human. Our neuroinformatics research involves the development and application of analytical tools to large volumes of neurobiological data. A specific area where we have concerted our efforts is the development of informatics infrastructures for data and knowledge integration for the various brain connectivity projects. We have several major ongoing collaborations with research groups, both inside and outside CSHL. At CSHL, we joined with Josh Huang on a project on GABAergic interneuron expression in mouse models of autism. Outside CSHL, we participate in the Brain Architecture Project, with collaborators at multiple institutions, notably Harvey Karten (University of California, San Diego), Kathy Rockland (Massachusetts Institute of Technology), Michael Hawrylycz (Allen Institute of Brain Research), and Jin Hyung Lee (University of California, Los Angeles). Laboratory personnel continuing in the Mitra laboratory in 2013 are Pascal Grange (Postdoctoral Fellow), Jamie Jones (Laboratory Technician), Sandra Michelsen (Administrator), Vadim Pinskiy (Postdoctoral Fellow), Alex Tolpygo (Laboratory Manager), Gregor Havkin (Computational Science Manager), Caizhi Wu (Research Associate), Kevin Weber (Laboratory Technician), Neil Franciotti (Laboratory Technician), and Caitlin Powell (Laboratory Technician). Christin Bergano (Laboratory Technician), Amit Mukherjee (Postdoctoral Fellow), Yingbin Zhang (Computational Science Manager), and Ferenc Mechler (Computational Science Analyst) joined us during the last year.

Mouse Brain Architecture Project

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

The function of the brain is served and constrained by its wiring. Understanding this fundamental dependence is hindered by our crucially incomplete knowledge of mammalian brain connectivity. We have set out to address this problem in the MBA project by a comprehensive mapping of the patterns of neural connectivity between brain regions. The MBA project is in its third year, currently funded by National Institutes of Health (NIH) grants (a running Transformative-R01 and an RC1 Challenge Grant completed last year) and support from CSHL.

Using a pair of anterograde tracing methods and a pair of retrograde tracing methods, we map brain connectivity in the C57BL/6J mouse line in four different ways and align these results to the Allen Brain Atlas. For tracers, we use classical histochemical labels as well as genetically engineered viruses that cannot jump synapses and express fluorescent protein in the cytoplasm of the invaded neuron. For anterograde tracing, we use biotinylated dextran amine (BDA) and adeno-associated viruses (AAVs); for retrograde tracing, we use cholera toxin subunit B (CTB) and a modified rabies virus (RRV). Computer-guided precision injections are targeting one of 262 predetermined sites systematically mapped on a three-dimensional grid that covers the entire brain volume. The project aims to cover all combinations of injection sites and tracer types with repeated injections (at least two animals), which requires processing ~2100 mouse brains in the MBA pipeline. We now have ~50% completion of this plan (Fig. 1), with a significant subset of processed brains already released for public access on the MBA web portal (http://mouse.brainarchitecture.org).

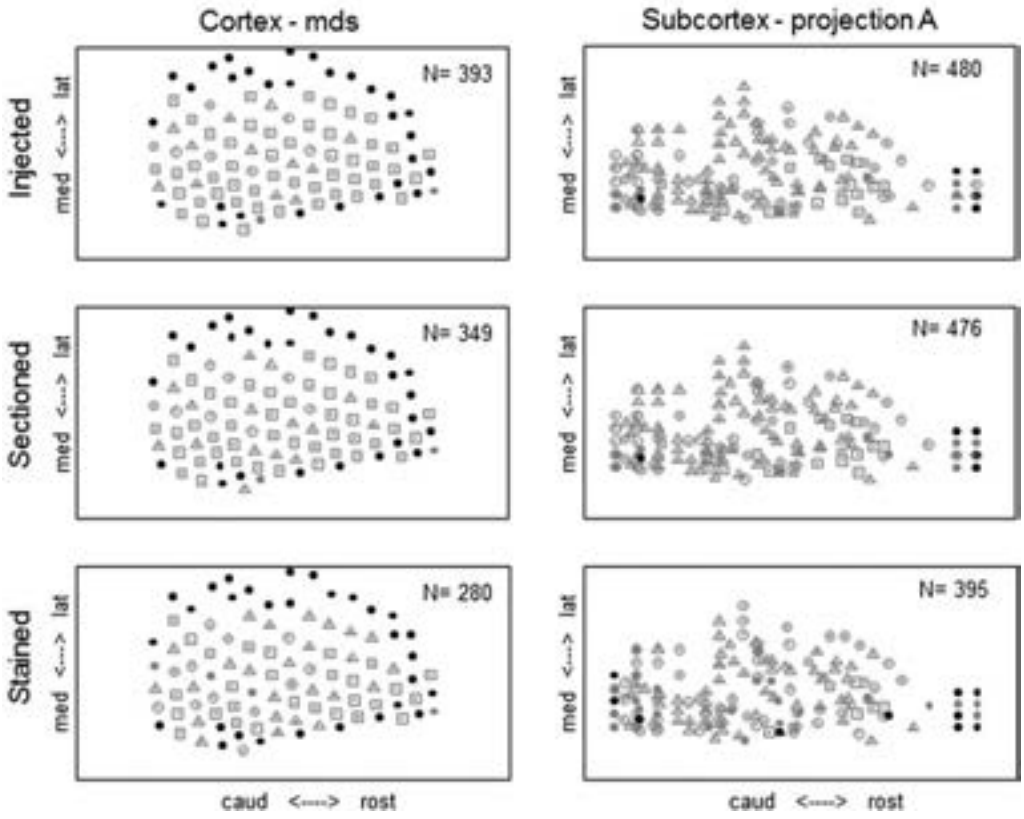

Figure 1. Pipeline statistics. Summary of the brains injected in cortical (*left*) and subcortical (*right*) sites for three successive states of production in the MBA project (brains injected, sectioned, and stained). Injection sites (*left*) are rendered in two dimensions by multidimensional scaling of the cortex and stereotaxic projection of subcortical sites. Planned sites (black circles) are overlaid by one of four colored symbols that indicate grades of completion (1, 2, 3, or 4 tracers by dot; gray circle, triangle, and square, respectively).

We also made progress in the continued development of the automated data processing pipeline. We developed algorithms and software for two-dimensional image registration (Fig. 2), three-dimensional volume reconstruction (Fig. 3), and three-dimensional cell detection and density estimation (not shown).

In addition to the MBA core data sets, we are continuing to process and post auxiliary data sets. One consists of four complete Nissl-stained brains imaged at high-depth resolution using dense optical sectioning to be used for our own reference brain. Other notable examples include a web-posted tutorial on the myelin-stained mouse brain by Kathleen Rockland, and a set of web-based instruction modules meant for clinical neurologists developed in collaboration with Dr. Joseph Safdieh at the Weill Medical College of

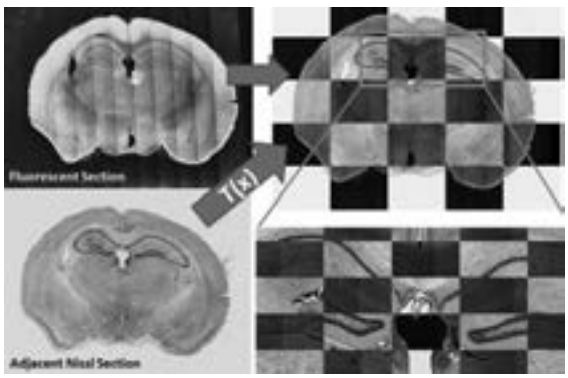

Figure 2. Two-dimensional registration. Section images are aligned by rigid transformation. This operation can be applied to sections of different image modalities (dark field for sections with fluorescent tracer; bright field for Nissl-stained alternate sections)

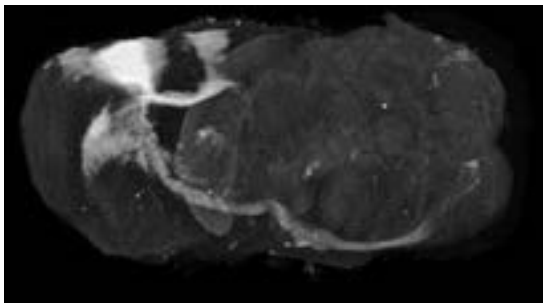

Figure 3. Three-dimensional reconstruction of whole brain. Left lateral view of a volume rendered mouse brain that was reconstructed from fluorescent section images. The brain was double injected in the left primary motor cortex (MOp) with fluorescent anterograde adeno-associated virus (supragranular site with GFP-AAV and the infragranular site with RFP-AAV) to highlight efferent projections from MOp.

Cornell University. We expect that the MBA project, with its publicly accessible data and the open source pipeline technologies, has the potential to impact the entire neuroscience community and directly address several items on the agendas of at least eight of the 20 NIH institutes. One indirect but quantifiable indicator of the scientific impact of the project and the interest it continues to generate is the number of unique visitors to the MBA portal (22,781 during June 1–Dec 12). Of these, 22% are returning visitors who maintain continuing engagement with the data on the portal. For more on the Brain Architecture Project, see our website: http://brainarchitecture.org.

Alterations in Brain-Wide GABAergic Neuroanatomy in Autism Mouse Models

V. Pinskiy, A. Tolpygo [in collaboration with K. Krishnan (Huang Lab), Z. Collins (intern), S. Mukhopadhyay (Wigler Lab), Cold Spring Harbor Laboratory]

With support from the Simons Foundation Autism Research Initiative (SFARI), we joined with Josh Huang in a collaboration to address the developmental neuroanatomy of dysfunctional inhibition implicated in autism spectrum disorders (ASDs). In a systematic brain-wide approach, we generated whole-brain maps of genetically targeted key subpopulations of inhibitory neurons. The project focuses on four GABAergic neuron subtypes: neurons expressing parvalbumin (PV), somatostatin (SOM), corticotrophin-releasing hormone (CRH), and vasointestinal peptide (VIP).

We aim to quantify the distribution and long-range projection patterns of the inhibitory neurons in two genetic mouse models of ASD (*16p11.2(df)* and *CNTNAP2 –/–* mutants) and assess how they differ from the C57/B6 wild-type controls, both in adulthood and during postnatal development. The project was started in the summer of 2011. In the first year, the MBA pipeline processed brains of wild-type animals that the Huang lab had genetically engineered to express green fluorescent protein (GFP) in the nuclei in each of the five selectively targeted GABAergic neurons. This soma-label data set is well suited for cell density estimation, but not for tractography. In the past year, the Huang lab succeeded in engineering animals that simultaneously express nuclear GFP and a diffuse cytoplasmic red fluorescent protein (tdTomato) that helps us to visualize axon projections. With these animals, we are now able to simultaneously quantify both neuron density and the axonal projection pattern of selectively targeted GABAergic neurons in the same animal. Figure 4 shows the sparse distribution of the somatostatin-positive (SOM+) GABAergic bitufted interneurons in dual-labeled wild-type mouse.

In the next phase of this project, we will focus on breeding and processing dual-labeled ASD animals. Eventually, we would like to quantify how the ASD genotypes differ from the wild type in the brain-wide distribution and projection pattern of select subtypes of GABAergic neurons and to gain insight into the postnatal development of pathological inhibition in ASD.

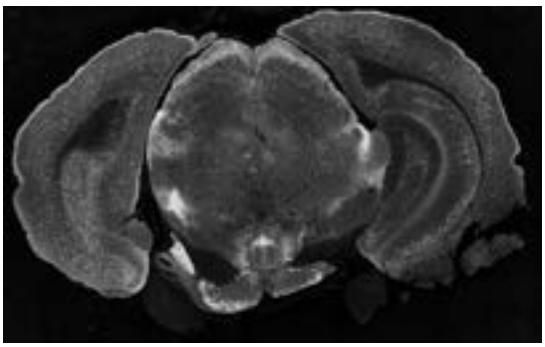

Figure 4. Sparse brain-wide distribution of (SOM+) GABAergic bitufted interneurons in an adult (p56) dual-labeled C57/B6 animal. (SOM-Ires-Cre:HG:Ai14 C57/B6 knockin). Bright specks indicate labeled neurons in the low-magnification fluorescent image of a representative coronal section (~Bregma –3.60).

Mapping Long-Range Neuronal Circuits in the Postmortem Human Brain

V. Pinskiy, C. Powell

This is a method development project, supported by the G. Harold and Leila Y. Mathers Charitable Foundation, with the aim of developing a practical method of studying long-range connectivity in postmortem human tissue. Our approach has three steps: (1) Use electrophoretic DiI tracing of fiber tracts in thick brain sections, (2) resection the stained specimen into thin sections, and (3) digitally scan high-resolution images of the thin, large-format, whole sections. Each processing step is trivial in small specimens, but scaling them up to cope with the size of the human brain poses formidable technical challenges. Our past and current efforts, outlined below, were and are focused on finding innovative ways of overcoming these challenges.

- *Tractophoresis*. We have improved the tractophoretic apparatus to cope with long-term current application and prevent dehydration and shrinkage by adding a dual-phase gel heat trap and silicone oil insulation around the tissue and the polyacrylamide conductive gel phase.
- *Embedding and Sectioning*. We implemented an old method of embedding the brain in oxidized agarose base that forms covalent cross-links with the tissue, providing adequate mechanical support of the specimen. We recently acquired a refurbished whole-body cryomicrotome that, unlike vibrotomes, can section specimens of human brains; it is waiting to be adjusted and tested.
- *Imaging*. To image the large slides required by human brain specimens, we have acquired a large-format confocal slide scanner (TISSUEscope by Huron Technologies). TISSUEscope maintains high spatial resolution while accommodating a broad range of slide sizes (25×75 mm to 150×200 mm). It is being adjusted and tested for large-format high-throughput use. We are currently running initial trials with whole sheep brains. Our plan is to continue making improvements on the performance of the tractophoretic and imaging apparatus and then begin working with Dr. Hof of Columbia University on high-throughput digitization of human samples from his extensive collection.

Computational Analysis of Brain-Wide Gene Expression

P. Grange

A Matlab toolbox for computational neuroanatomy. The main objective of this collaboration with the Allen Institute (AI) is to use mapped gene expression energies to study coexpression patterns of genes related to addiction and to identify the footprint of those patterns in mouse neuroanatomy. The mapping is to the Allen Reference Atlas (ARA), a volumetric map of the adult mouse brain with 200-μm voxel resolution. We developed computational techniques to identify sets of genes that mark brain regions as annotated in the ARA. In particular, we imposed positivity constraints on sets of genes and optimized the fitting scores, which measure how well an expression profile follows the shape of a given brain region. The solutions tend be sparse (they involve few genes). We allow for a parameter to increase the sparsity (using an L1-L2 optimization scheme). Of the 12 main regions of the left hemisphere in the ARA, the midbrain is the one for which optimal sets of genes bring the largest improvement with respect to single genes (Grange and Mitra 2012). We incorporated these new developments in a Matlab toolbox, BrainGeneExpressionAnalysis (BGEA), available from our web portal: http://brain-architecture.org/allen-atlas-brain-toolbox. BGEA also includes work on coexpression networks in the brain, marker genes, and the estimation of the brain-wide density of certain cell types. We plan to adapt these methods to the ARA of the human brain (mindful of the limitations inherent in the nonvoxelized nature of this atlas) and to the atlas of the zebra finch brain (currently under development at the Mello lab, Oregon Health and Science University).

Applications to databases of condition-related genes. The methods of the BGEA toolbox were applied to databases of genes related to nicotine addiction (NicSNP), and to autism (AutRefDB, maintained by collaboration at MindSpec). The 288 genes we analyzed from NicSNP are not more coexpressed than expected by chance (Grange et al. 2013). However, we have identified a collective behavior in sets of autism-related genes: These autism gene sets are significantly coexpressed, and significantly better

fitted to the cerebellar cortex than sets of genes of the same size, with a particular enrichment in the granular layer.

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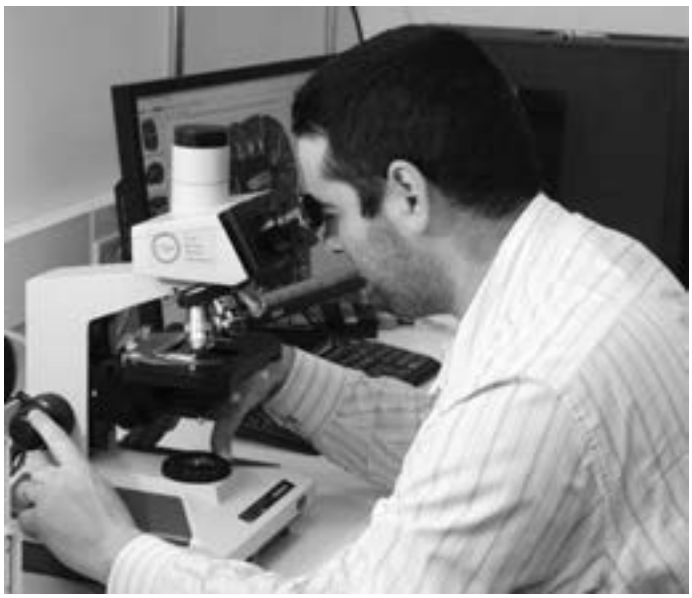

Vadim Pinskiy

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

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

Gaining an understanding of the brain regions and circuits that govern behavior is a central goal of systems neuroscience. During the last 4 years, research in our laboratory has been focused on the development of a novel method for mapping behavior-evoked neuronal activation in the mouse brain at cellular resolution. Our approach is based on the visualization of the immediate-early gene c-*fos*, a molecular marker of neuronal activation, by serial two-photon (STP) tomography in transgenic c-*fos*-GFP mice. Whole-brain STP tomography data sets are analyzed by a pipeline of computational methods that detect the activated green fluorescent protein (GFP)-labeled neurons, warp their distribution to a reference brain volume registered to the Allen Mouse Brain Atlas, identify activated brain regions by statistical tests, and plot anatomical connectivity between the activated regions using the Allen Mouse Connectivity database (Figs. 1–3). This method opens the door to systematic screening of mouse brain circuits under normal conditions and in genetic and other mouse models of human brain disorders.

Mapping Social Behavior in the Mouse Brain by STP Tomography

To demonstrate the power of our methods in studying brain functions underlying complex behaviors, we decided to map whole-brain activation evoked during social behavior—the so-called "social brain circuit"—in normal (wild-type) mice (Fig. 3). These experiments were done by comparison between three groups of mice: (1) A "home-cage group" of nonhandled mice, which served as a baseline control; (2) an "object group" that was allowed to explore a novel object added to the home cage environment for 90 sec, and (3) a "social group" that was allowed to interact with a conspecific ovariectomized female in the home cage for 90 sec. The comparison between the social and object group revealed all regions previously determined by other methods to be involved in rodent social behavior and a number of additional brain regions (Fig. 3; note the high level of statistical significance for many core circuit regions, with FDR-corrected values $<10^{-5}$). Because our c-*fos* data are registered in the Allen Brain Atlas format, we used the anatomical anterograde projection data from the Mouse Connectome project (http://connectivity.brain-map.org) to draw a connectivity matrix between all significantly activated brain areas (Fig. 3). These data represent the first comprehensive map of social-behavior-evoked neuronal activation in the mouse brain. We conclude that STP-tomography-based visualization of c-*fos*-GFP induction represents a highly sensitive method for mapping behavior-evoked brain activation in the mouse brain. This work was presented at last year's SFN meeting in San Diego and the manuscript is in preparation.

The Study of Brain Circuits in Genetic Mouse Models of Autism

The goal of this work is to identify brain-circuit-based endophenotypes related to autism in genetic mouse models using our c-*fos*-GFP-based brain screening described above. Recently, we have discovered that mice carrying a deletion of the 16p11.2 locus, which is associated with autism, schizophrenia, developmental delay, seizures, and obesity, have increased seizure propensity and abnormal neuronal circuit activation in two distinct brain circuits. One circuit centers on the subiculum, which is the main output of the hippocampus, and several downstream connected regions,

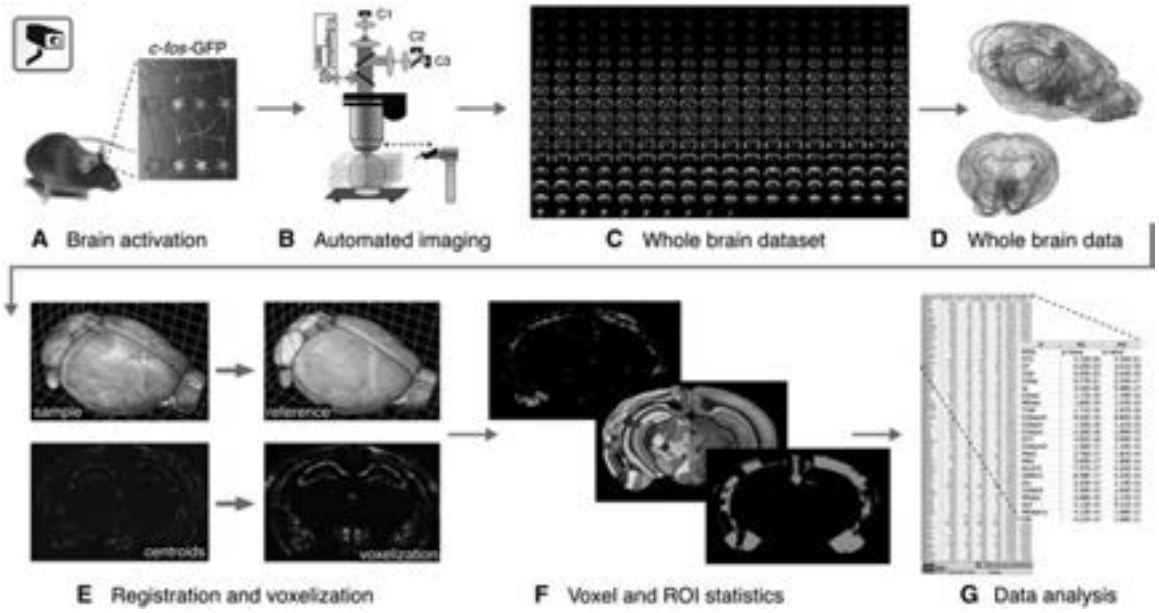

Figure 1. Mapping brain activation. (*A*) Brain activation causes the induction of c-*fos*-GFP. (*B,C*) The brain is imaged by STP tomography. (*D*) c-*fos*-GFP cells are computationally detected. (*E*) The data sets are warped to a "reference" brain. (*F*) The distribution of c-*fos*-GFP cells is compared by statistical analyses. (*G*) Results are tabulated in a spreadsheet of brain regions, with FDR-corrected *q* values for each region.

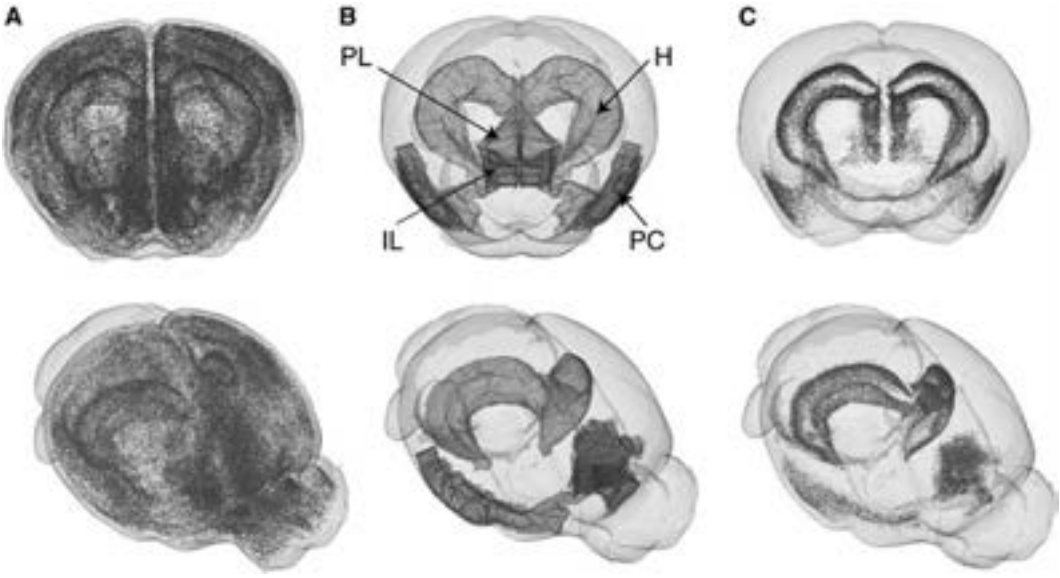

Figure 2. Brain Atlas–based analysis of c-*fos*-GFP data. (*A*) 367,378 c-*fos*-GFP cells detected in one mouse brain data set. (*B*) Anatomical segmentation showing the hippocampus (H), prelimbic (PL), infralimbic (IL), and piriform (PC) cortex. (*C*) Visualization of c-*fos*-GFP cell counts in the hippocampus (38,170), prelimbic (3305), infralimbic (3827), and piriform (10,910) cortex.

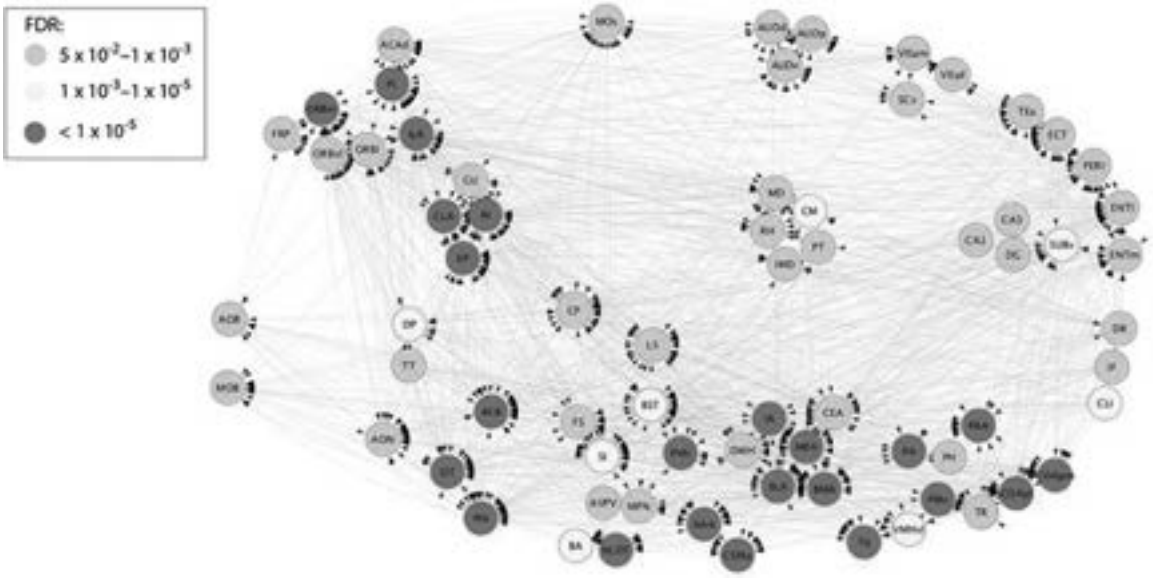

Figure 3. Social brain circuit in the mouse. Brain regions with significantly higher c-*fos* induction in the social group versus the object group are shown in an approximate layout of a sagittal view of the mouse brain. Connectivity is plotted based on data from the Mouse Connectome project (www.connectivity.brain-map.org). Statistical significance is gray-scale coded as shown in the upper left inset. Note the high significance for well-established areas in social behavior, such as amygdala (MEA, BMA, BLA), cortical amygdala (COA), or paraventricular hypothalamus (PVH). The abbreviations used are based on the Allen Brain Atlas (www.mouse.brain-map.org).

including the nucleus accumbens, amygdala, entorhinal cortex, and thalamus (mammillary body, dorsal premammillary nucleus, and tuberomammillary nucleus). These structures are involved in memory, as part of the Papez circuit, goal-oriented behaviors, and defensive behaviors, as well as control of arousal, sleep, and circadian rhythm. A second subcircuit is formed by areas of the prefrontal cortex (PFC), centered on the orbital area and including infralimbic, prelimbic, and cingulate cortices, which control emotional processing, decision making, empathy, and autonomic functions.

Our ongoing work on this project is focusing on the characterization of the circuits in the PFC, which we consider to be the primary candidate area for neuronal deficits related to at least some clinical manifestations of autism. We have also initiated collaborations with researchers at Seaside Therapeutics (Cambridge, Massachusetts) and Roche (Basel, Switzerland) to begin testing novel therapeutic strategies. We focus on two candidate drugs: CTEP, a selective mGluR5 inhibitor, and R-baclofen, a GABA-B receptor agonist. Both drugs are currently being tested in clinical trials in children with fragile-X syndrome (FXS), the most common cause of inherited intellectual disability and autism. We will test the effects of these drugs using our c-*fos*-GFP-based brain screening in the Fmr1 KO mouse model of FXS, as well as in the 16p11.2 df/+ mouse model. These experiments will allow us to test the translational strength of our methods, because we will be able to compare the outcomes of our assays in the Fmr1 KO mice to the outcomes of the ongoing clinical trials in children with FXS. In addition, the studies in the 16p11.2 df/+ mice will test whether FXS-targeted treatments may have broader indications in other genetic forms of autism.

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NEURAL CIRCUITRY FOR SOCIAL COMMUNICATION

S. Shea B. Cazakoff G. Ewall
K. Crump B. Lau
H. Demmer K. Tranchina
D. Eckmeier

The overarching research goal in our laboratory is to understand how processing in specific brain circuits works to support natural communication behaviors. We aim to reveal neural mechanisms that allow organisms to detect and recognize familiar individuals, to gather information about their identity and social status, and to select appropriate behaviors.

Mice are capable of acquiring detailed profiles on one another from the smells and sounds experienced during their social encounters. These dossiers may include information on a mouse's sex, genetic identity, reproductive state, levels of distress, or sexual interest, or even recently consumed foods—details that are indispensable for survival and mating success. Initially, we are working to understand the neuronal activities and mechanisms in primary sensory brain areas that support these forms of communication. In the future, we anticipate moving deeper into the brain to ascertain where the sensory data from those regions are collected and integrated into hormonal and electrical signals that promote appropriate behavioral choices.

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

There are two broad areas of research in the lab. The primary focus is on neural circuits that facilitate detection, discrimination, and memories for olfactory cues. In particular, we are interested in how these circuits help an animal flexibly select appropriate behavioral decisions toward potential mates, rivals, offspring, predators, and food sources. Our approaches range from detailed in vivo synaptic physiology in anesthetized animals, to functional imaging and physiology in head-fixed mice, to recording and manipulating neural activity in awake, freely behaving animals. We ultimately hope to uncover the neural encoding of social cues during a live encounter with another mouse.

The secondary focus of our research program is aimed at understanding the plasticity of cortical circuits that enable vocal communication between mice. Specifically, we have been working on how this circuitry and the attendant behavior are altered in mice that have impaired function of the gene *MeCP2*, which is mutated in humans with Rett syndrome.

Noradrenaline Stores Olfactory Memories through Dynamic Regulation of Inhibition

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

How do we remember individuals whom we have previously encountered? Substantial evidence indicates that many animals remember each other based on olfactory cues. Memories are especially strong for individuals encountered during key life events such as mating with a new partner or the birth of a litter of young. These important events typically evoke massive release of the neurochemical noradrenaline (NA), initiating a heightened state of emotion and arousal.

This surge appears to cause long-lasting modifications to the responses to odorants in the olfactory bulb, which is the first processing station for scent in the mammalian brain. Indeed, it was hypothesized that the coincidence of an odor stimulus with a surge of NA is minimally sufficient to store a memory.

We exploited the intimate relationship between NA, olfactory bulb activity, and behavior to create and study olfactory memories in the anesthetized mouse. We discovered that indeed, when NA release is evoked by stimulating locus coeruleus (the source of most NA) while the sleeping mouse sniffed an odorant, neural responses to that odor underwent specific long-term alterations. Remarkably, once awake, the mouse's subsequent behavior toward the odorant was also changed. In other words, the mouse seemed to remember the odor and treat it as though it were familiar. Memories are widely believed to be stored as changes to the synaptic connections among the neurons in our brains. How are olfactory memories for individuals stored mechanistically among the specific synaptic connections of the various neuronal types in the olfactory bulb? To answer this question, Dr. Heike Demmer developed techniques for making technically challenging targeted recordings from a specific type of inhibitory neuron (granule cells) that seems to regulate odor perception and behavior by inhibiting the mitral cells that form the output of the olfactory bulb. She then made these recordings during the induction of NA-dependent plasticity to examine how they contribute to memories. The data provide the first evidence that naturalistic stimulation of NA release in vivo suppresses granule cells, thus transiently increasing the excitability of mitral cells by relieving them from inhibition. In our model, this temporary disinhibition of mitral cells is a key trigger for the synaptic plasticity that likely underlies memory. Indeed, this controlled disinhibition leads to a selective long-term *increase* in inhibition onto cells that respond to the learned odor, and we propose that this is a critical substrate for odor learning (Fig. 1). To test this proposal, we are planning to use disinhibition of mitral cells by optogenetics techniques to recreate the neural dynamics that we now know are set up by NA and assess its sufficiency for plastic changes.

Dr. Dennis Eckmeier has been taking a different approach to observe population mechanisms of NA-dependent memory formation with functional neural imaging. He uses a technique in which the sensory neurons that provide input to the olfactory bulb are labeled with a fluorescent activity sensor that allows him to monitor the strength of activation in foci called "glomeruli." By plotting the response strength of each of these glomeruli before and after NA release, Dr. Eckmeier has shown that NA weakens the response to paired odors (Fig. 2). This effect is only observed in cases where NA is released during odor perception by stimulation of a brainstem structure called locus coeruleus (LC). These data surprisingly establish the synaptic input to the main olfactory bulb (MOB) as a target of noradrenergic modulation and present an opportunity to observe how memories shape sensory representations of large ensembles of neurons, as opposed to single cells.

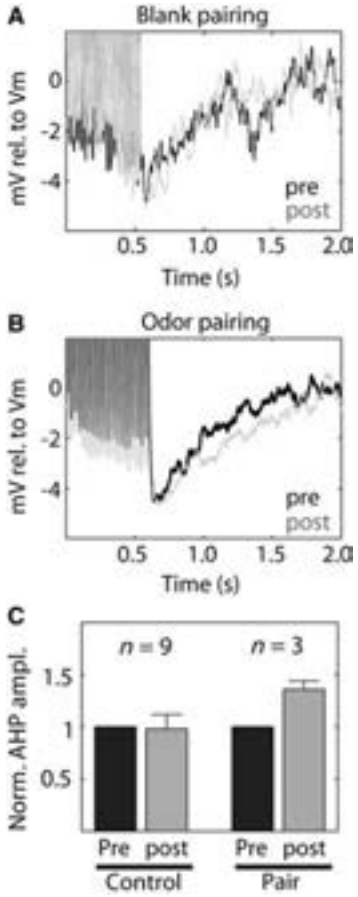

Figure 1. LC stimulation strengthens GC inhibition onto MC. (*A,B*) Pairing an odor with LC stimulation potentiates recurrent inhibition. Note the prolonged AHP postpairing. Potentiation was not seen after pairing LC stim with blank controls. (*C*) These effects were consistent for a population of cells.

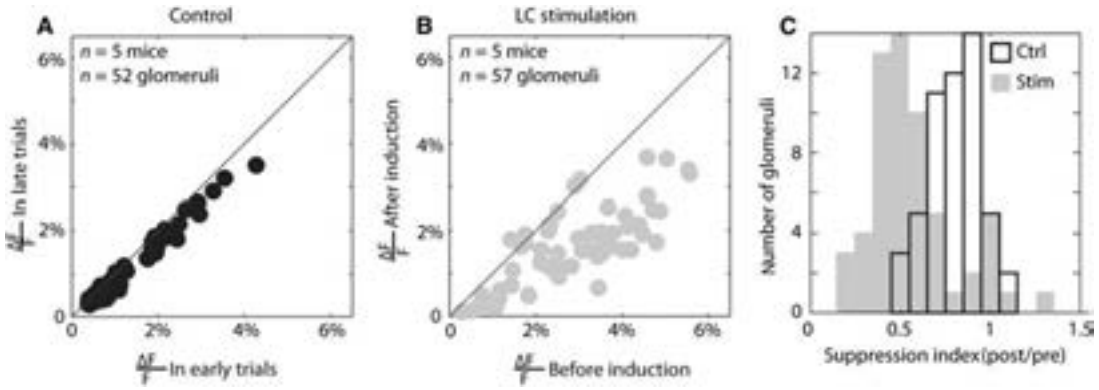

Figure 2. LC stimulation suppresses population responses to paired odors. (*A*) Scatterplot of the responses of glomeruli from five mice, plotting late trials over early trials from after and before control sham LC stimulation. (*B*) Scatterplot of the responses of glomeruli from five mice, plotting post-LC stimulation response strength over pre-LC stimulation response strength. (*C*) Distribution of suppression ratios for all glomeruli in control animals (open bars) and LC-stimulated animals (gray bars).

Granule Cells of the Olfactory Bulb Exhibit Dramatically State-Dependent Activity

B. Cazakoff, B. Lau, K. Crump [in collaboration with A. Koulakov, Cold Spring Harbor Laboratory]

The granule cells in the olfactory bulb are widely considered to be critical for olfactory discrimination and learning; however, their activity patterns in vivo are poorly understood. Moreover, their electrophysiological properties in awake animals are completely unknown. This is due to the fact that the granule cells do not yield to conventional recording techniques. Therefore, we applied the techniques developed by Heike Demmer to mice that are awake with their heads fixed but running freely on a foam ball and receiving water rewards from a lick tube. CSHL Undergraduate Research Program student Kerensa Crump initially set up this approach during her 10-week research internship this year, and the work has subsequently been expanded by Watson School of Biological Sciences student Brittany Cazakoff and postdoctoral fellow Dr. Billy Lau. Collectively, they have found that the activity patterns of granule cells in awake mice are dramatically different from those observed under anesthesia, which was the condition for all previous studies of granule cells (Fig. 3). Ms. Cazakoff's thesis will be focused on expanding this paradigm to include discrimination of odors and associative learning to study how the firing patterns of granule cells reflect experience with and behavioral significance of an odor. In this study, and the study of NA-dependent memories, we are collaborating with CSHL professor Dr. Alexei Koulakov to test some surprising predictions that emerge from his model of population changes in granule cell activity during odor learning. So far, these unusual predictions have been consistent with our data.

Vocal Communication Is Impaired in a Mouse Model of Rett Syndrome

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

Far outside the range of our hearing, in the ultrasound range, mice are constantly holding conversations with one another in a language that is poorly understood at best. Many types of vocalizations are emitted by males and females, juveniles, and adults in a variety of behavioral contexts. We would like to better understand the perceptual significance of these calls to the mice and how they are used to guide behavioral choices. One form of vocalization that is reasonably well understood is the ultrasonic distress vocalization (USV). Young mice before having vision and full mobility will occasionally become separated from the nest. This is stressful for them and they will therefore call out to their mother with a very high frequency peep. New mothers develop sensitivity to these cries and respond by moving toward their source (phonotaxis) to retrieve the pup. Moreover, females who have never given birth do not innately show approach responses to pups or

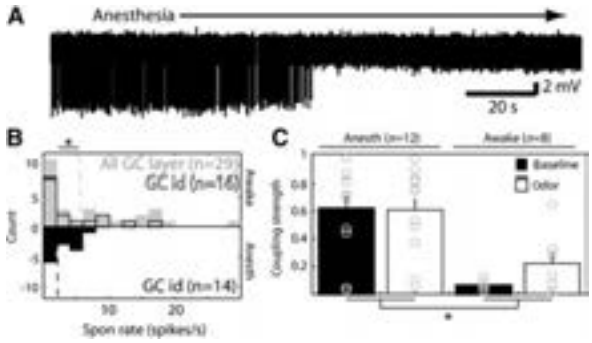

Figure 3. GC spontaneous and sensory-driven activities are highly state dependent. (*A*) Histogram comparing mean spontaneous firing rates between anatomically identified GCs recorded under anesthesia (isoflurane [10/140 or ketamine/xylazine {4/14}], black bars) and in awake animals within the GC layer neurons (open) and anatomically identified as GCs (gray). (*B*) Comparison of mean spontaneous and odor-evoked respiration phase coupling strength between GCs recorded under anesthesia and in wakefulness. (*C*) Raw trace from an anatomically identified GC as the mouse transitions to anesthesia. *$p < 0.05$, ANOVA.

their calls; however, they can learn to perform the behavior with experience.

We are collaborating with CSHL professor Dr. Josh Huang and his postdoctoral fellow Dr. Keerthi Krishnan to examine how this behavior is affected in mice that are missing one copy of a gene called *MeCP2*. Impairments in the function of this gene are understood to cause the autism spectrum disorder Rett syndrome, and the Huang lab's findings regarding mutations in *MeCP2* suggest that they may affect inhibitory networks in the auditory cortex that may be important for development of pup call sensitivity. Indeed, we find that females who possess only a single copy of *MeCP2* are not able to develop proficiency at gathering pups (Fig. 4). Future studies will assess whether the impairment of the gene only in the auditory cortex is enough to produce the learning deficit. In accordance with this possibility, we are also measuring responses to auditory stimuli in the cortex of mutant and wild-type animals to determine the circuit basis of the aberrant behavior.

Neural Activity during Social Encounters

D. Eckmeier

We have begun experiments that are ultimately aimed at recording individual neurons during social encounters and other behavioral assays involving the perception of social and nonsocial information. There are two broad related goals to this approach. The first goal is to examine the encoding of social information such as body odors and vocalizations in primary sensory structures of awake animals. We hypothesize that activity in response to these signals may be labile to associative learning, attention, and arousal which we may be able to manipulate in the context of social encounters. The second goal is to record from neurons in deep brain neuromodulatory centers during these encounters as well. Neurons that release noradrenaline and dopamine are likely responsive to social signals and may modulate encoding of sensory data and associative plasticity. Understanding the context-dependent activity patterns of these neurotransmitters is therefore critical to developing models of how they affect behavior. Critical to this approach is the development of techniques for observing neural activity with implantable probes that are sufficiently lightweight and low profile to not interfere with normal social behaviors, yet are also

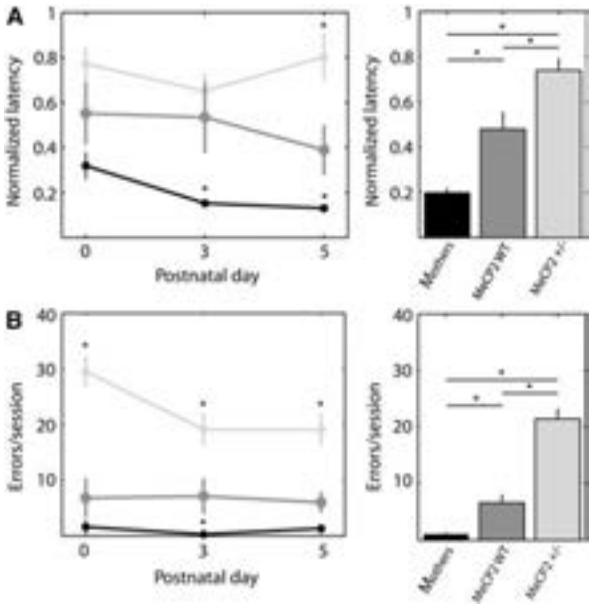

Figure 4. MeCP2 +/– mutants exhibit impaired maternal behavior. MeCP2 mutants and wild-type littermates were housed in a cage with a pregnant mother to assess their ability to learn maternal gathering behavior. Mutants took longer to gather (*A*) and made more errors (*B*) than their wild-type counterparts. *$p < 0.05$, ANOVA.

stable enough to allow steady, low-noise recordings during highly interactive behaviors such as mating. Our initial experiments make use of fixed silicon probes etched with a high-density array of electrical contacts. The data show that these probes can be used to record multiple isolated neurons in the socially significant modulatory nucleus locus coeruleus. We are now using these probes in awake animals to assess the structure of LC output during social learning with high temporal resolution.

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

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

The brain has a tremendous capacity to form different memories. These memories are, under normal circumstances, highly accurate for particular stimuli and situations. This is precisely the facility that is lost in diseases such as Alzheimer's and other dementias. Our overall goal is to understand how the brain forms such precise memories: How does the specificity arise? How does the brain derive such tremendous capacity for forming different memories? We address these questions by studying olfactory memory formation in *Drosophila*. Just like Pavlov's dogs, *Drosophila* learn to form associations between smells and reward or punishment. A specific area of the fly brain, known as the mushroom body (MB), is essential for the flies to form olfactory memories. We are investigating how the neural activity patterns in this brain area are used to form specific olfactory memories. To achieve this, we monitor activity using both electrophysiological and functional imaging techniques. We have found that MB neurons exhibit highly odor-specific responses, and activity patterns are relatively sparse across the population of MB neurons. This specificity is thought to underlie the accuracy of memory; modifying the synapses of highly odor-specific neurons would lead to relatively precise memories. Sparse representations by highly stimulus-specific neurons are a general feature of brain areas involved in learning and memory, including hippocampus and cerebellum in humans. Using the simplicity and genetic manipulability of *Drosophila*, our goal is to understand several fundamental properties of neural responses in learning- and memory-related brain areas: What mechanisms give rise to the stimulus specificity? What exactly is meaningful about the activity patterns? Is it simply which cells respond or does the precise timing of activity matter? How are these response patterns modified by learning? Ultimately, what are the roles of the many genes implicated in learning on network-level activity in the brain?

Robust Sparse Coding in the Mushroom Body

K. Honegger, R. Campbell

Theoretical studies indicate that sparse representations are useful for learning and memory. However, it is crucial to distinguish between truly sparse representations and weak responses observed simply because the stimuli tested are suboptimal (i.e., not those the system evolved to detect) or because strongly responsive cells are spatially segregated. In this study, we used imaging to establish that odor representations are genuinely sparse in the MB. We showed that natural stimuli which have intrinsic meaning to the fly do not elicit qualitatively distinct responses from artificial, monomolecular odors. Additionally, there is no evident spatial segregation of responses which would suggest that olfactory information is channeled into distinct anatomical pathways. Rather, odors evoke responses in a small fraction of the MB population, with cells randomly distributed throughout the MB. This format is likely useful for memory storage and recall. It is a simple way to minimize the overlap between representations of different stimuli: If each odor evokes a response in 10% of the MB neurons, and for each odor the 10% are randomly distributed across the population, the representation of any two odors will overlap by only 1%. Consequently, any changes in the properties of neurons that respond to one of the odors should only have a tiny effect on the representations of other odors. Overall, this format optimizes the circuit to form accurate memories with specific odors. We examined this explicitly in the next project.

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

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

Do the neuronal response patterns in the MB convey the specificity of memory formation? If so, what features of neural activity underlie that specificity? To address these fundamental questions, we tested whether we could use the activity patterns we measure in the MB to predict the odor specificity of flies' memories. We examined this in the following two contexts: when flies learn (1) fine *discriminations* between highly similar odors and (2) an association with one odor and then *generalize* that association to a different but related odor. To assess the odor specificity of the memories, we used the standard Pavlovian conditioning protocol, training flies to form an association with one odor and testing the specificity of those memories by giving flies a choice between that odor and a second odor. We compared this behavioral measure with the specificity of the response pattern of ~150 MB neurons to these odors. We found that we could use the MB activity patterns to accurately predict the specificity of memory formation subsequently measured in behavioral experiments. To evaluate the features of neural activity that underlie accurate memory formation, we developed techniques to track activity of ~80% of the 2000 neurons in the MB. The unprecedented scope of these experiments enabled us to find the small differences between different odor representations. We found that, even when flies are forced to choose between two odors that are near the limit of their discrimination ability, there are neurons that respond to only one odor of the pair, so that binary differences are present between the two odor representations. These differences are sufficient to train a biologically realistic model network to respond specifically to different odors. We have also used the activity patterns we measure in the MB to predict generalization of odor memories. There are two important conclusions from this study: (1) Analog differences between odor representations in the olfactory receptor neurons are converted to binary differences in the MB and (2) a simple learning scheme of changing synaptic strength of all neurons that respond to an odor is sufficient to accurately determine odor identity. These results show how a simple coding scheme can enable accurate but generalizable memory formation.

Dendritic Claws of Mushroom Body Neurons Integrate the Olfactory Code

E. Gruntman

How is it possible to recognize an odor as a unified smell (e.g., coffee) when it has a large number of different components, which in turn have many different chemical features? One idea is that olfactory receptor neurons (ORNs) in the nose (fly's antennae) each recognize a particular feature of an odor, and the overall combination of activated ORNs conveys the identity of the odor. But are there neurons at deeper layers of the brain that read this combinatorial code and respond to specific combinations of co-active inputs? In this work, we show that the integration of these different inputs occurs on the dendritic trees of individual MB neurons. We addressed this issue by examining odor responses of individual dendritic input sites, making this one of a handful of studies to examine sensory responses of dendrites in vivo. To achieve this, we expressed a calcium-sensitive fluorescent protein in single MB neurons, and then used two-photon imaging to construct an odor tuning curve for each dendritic input site. By comparing tuning curves for different dendritic sites, we directly showed that some MB neurons integrate combinations of different inputs. To evaluate how these inputs are integrated, we used optogenetic techniques to control activity of the input neurons. Our results provide an elegant explanation for why MB neurons are so odor selective: They not only *receive* different types of inputs, but *require* those different inputs to be co-active in order for the MB neuron to spike. Examining the time course of synaptic summation showed a rapid initial depolarization that quickly plateaued at a level well below spike threshold. Summation of inputs from different dendritic sites is also strongly sublinear. Nevertheless, if sufficient numbers of different input sites are activated, membrane potential can reach spike threshold. Contrary to prevailing models for generating stimulus-specific responses, which rely mainly on supralinear summation of coincident inputs, our results show a surprising variant: a demand for strong multiple concurrent inputs due to sublinear summation.

Decoding Sparse Representations

T. Hige

Sparse representations are useful for learning and memory, but how do downstream neurons integrate this information? Ultimately, the information in layers with sparse representations must be converted into a behavioral response. How does this process occur, and how do neurons downstream from a sparsely responding brain area integrate that information? In *Drosophila*, this process appears to occur immediately after the MB, as the 2000 MB neurons converge onto an estimated 35 output neurons. Individual members of this output population can be labeled with green fluorescent protein (GFP) using the genetic tools available in *Drosophila*, making this an excellent system to determine the basic principles of information processing downstream from sparse representations. Using GFP-targeted intracellular recordings, we have characterized the odor responses of one of these MB output neurons. In contrast to the highly odor-specific tuning of MB neurons, this output cell responds to a broad array of different odors. These functional properties are reflected in the cell's anatomy: It has very extensive dendritic processes within the output lobes of the MB, suggesting that it receives highly convergent input from many MB neurons. Although the neuron responds to almost all tested odors, it nevertheless responds distinctly to different odors, suggesting that this neuron is capable of carrying odor-specific information. However, the odor-tuning properties of this neuron vary among individual flies; the same neuron has very different tuning curve shapes in different animals. One explanation for this variability is that it reflects synaptic plasticity that operates on a developmental timescale. Our working hypothesis is that the tuning curves of the MB output neurons are a product of the prior olfactory experience of the individual fly. We are currently testing this by examining the role of olfactory experience in shaping the response properties of these neurons.

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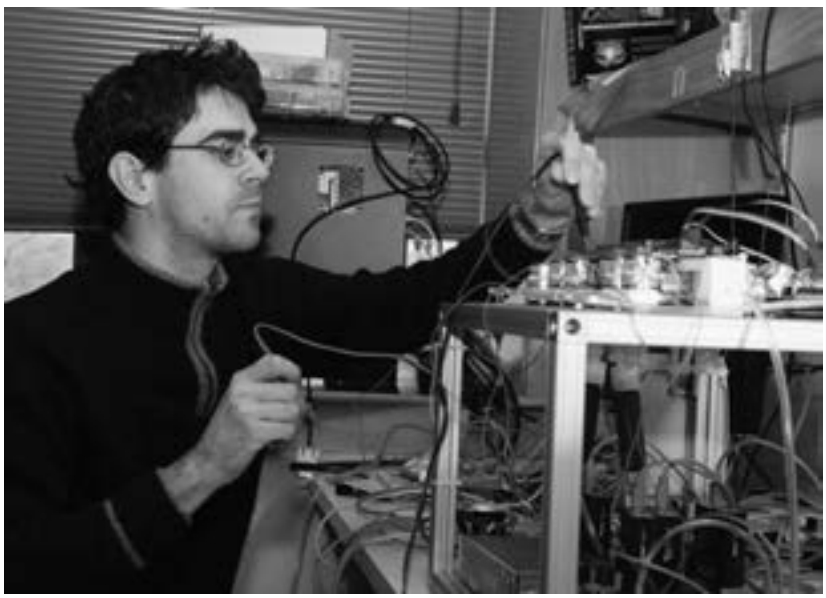

Alexander Campbell

NEURAL CIRCUITRY OF UNDERLYING NORMAL AND ABNORMAL PROCESSING IN THE CORTEX

A. Zador K. Borges, B. Burbach, D. Gizatullina, S. Jaramillo, J. Kebschull, S. Koh, F. Marbach, H. Oviedo, H. Oyibo, I. Peikon, A. Reid, Q. Xiong, H. Zhan, P. Znamenskiy

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

Sequencing the Connectome

D. Gizatullina, J. Kebschull, H. Oyibo, I. Peikon, H. Zhan [in collaboration with J. Dubnau, A. Koulakov, G. Hannon, Cold Spring Harbor Laboratory and L. Enquist, Princeton University]

The brain is a complex network, consisting of billions of neurons connected by trillions of synapses. The details of these connections—which neurons form synaptic connections with which other neurons—are crucial in determining brain function. Malformation of these connections during prenatal and early postnatal development can lead to mental retardation, autism, or schizophrenia; loss of specific connections later in life is associated with neurodegenerative diseases such as Alzheimer's. An efficient method for determining the brain's wiring diagram would transform neuroscience research. Inspired in part by the success of the Allen Brain Atlas, the Human Genome Project and other major efforts, there is growing excitement in neuroscience to determine the complete connectivity diagram—the "connectome"—of the brain. So far, the complete connectome has been established for only one organism, the tiny worm *Caenorhabditis elegans,* with 302 neurons connected by ~7000 synapses. However, determining the connectome of even this simple nervous system was a heroic task, requiring more than 50 person-years of labor to collect and analyze the electron micrographs. All current approaches to determining the connectome are based on microscopy. Unfortunately, microscopy is poorly suited to the study of neural connectivity because brains are macroscopic structures, whereas the synaptic connections between neurons require electron microscopy. Reconstructing the complete wiring diagram of such a brain is akin to piecing together the complete roadmap of the United States from a collection of postage-stamp-sized photographs. To circumvent the considerable challenges associated with determining the connectome based on microscopy, we are developing an entirely novel approach based on high-throughput DNA sequencing technology. Sequencing technology has not previously been applied in the context of neural connectivity. The appeal of using sequencing is that it is already fast—sequencing hundreds of millions of individual pieces of DNA in a single day is now routine—and, like microprocessor technology, getting faster exponentially. Moreover, the cost of sequencing is plummeting: Predictions are that it will be possible to sequence an entire human genome (~3B nucleotides) for $1000 within a few years. Thus, by converting brain connectivity from a problem of microscopy to a problem of sequencing, we render it tractable using current techniques. An inexpensive and rapid method for deciphering the wiring diagram of a neural circuit of an entire brain would have a profound impact on neuroscience research. Knowing the neuronal wiring diagram would provide a foundation for understanding neuronal function and development, in the same way that knowing the complete genomic sequence provides the starting point for much of modern biological research in the postgenomic era. Moreover, many neuropsychiatric diseases such as autism and schizophrenia are thought to result from disrupted neuronal connectivity, but identifying the disruptions even in mouse models is a major challenge given current technology. Our approach may help to usher in an era when understanding the connectivity of a new mouse model of neuropsychiatric disease is the routine first step to analyzing its deficits.

Role of Inhibitory Interneurons in Auditory Cortex Function

A. Reid, T. Hromadka

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

Processing of Spatial Information in the Auditory Cortex

S. Koh

In vision and somatosensation, spatial information is already present at the receptor level. In contrast, spatial information is not available at the cochlear level and must be computed using information from both hemispheres. A subpopulation of neurons in the primary auditory cortex conveys sound location and sound motion information, but little is known at the neural circuit level due to technical difficulties of identifying neurons during in vivo recordings. We have previously developed a technique called PINP (photostimulation-assisted identification of neuronal populations) that allows us to "tag" subpopulations of neurons based on their axonal projections. The tag is a light-gated ion channel channelrhodopsin-2 (ChR2), which can be triggered by a brief flash of blue laser with millisecond precision. We inject herpes simplex virus (HSV), which can be taken up by axons and travels in a retrograde fashion in order to deliver ChR2 specifically to the neurons projecting to the infected area. ChR2-tagged neurons can be identified during in vivo recording by responsiveness to a flash of blue light. We are using PINP to test the hypothesis that layer-3 ACx neurons projecting to the contralateral ACx are more sensitive to auditory spatial information than other subpopulations in ACx. Layer-3 neurons seem to be ideal candidates, deduced from the following facts: (1) Many layer-3 ACx cells project to contralateral ACx, unlike visual and somatosensory cortex, where layer-3 cells predominantly project to ipsilateral cortical areas; and (2) processing of spatial information requires inputs from both hemispheres.

Circuits Underlying Auditory Representations and Decisions

S. Jaramillo, P. Znamenskiy, K. Borges

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

Local and Long-Range Circuitry in the Auditory Cortex

H. Oviedo

We are studying the underlying mechanisms of the prefrontal cortex's (PFC) ability to select relevant signals and suppress irrelevant ones. Diminished inhibitory influence from PFC to the auditory cortex impairs our ability to ignore irrelevant sounds. To inhibit the auditory cortex, the PFC must send excitatory output via pyramidal neurons that target local inhibitory neurons in the auditory cortex. Little is known about the anatomical and physiological properties of this projection. Because of the variety of inhibitory

neurons and differences in their computational roles, we are examining the role of the most prevalent types we can target with transgenic mice: parvalbumin, somatostatin, and vasoactive intestinal polypeptide. Our approach is to use channelrhodopsin-2-assisted circuit mapping to investigate the top-down inhibitory control of auditory cortical activity.

Role of Corticostriatal Plasticity in Auditory Decisions

Q. Xiong, P. Znamenskiy

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

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Sang-Geol Koh

NEURAL BASIS OF LEARNING AND MEMORY IN *DROSOPHILA*

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

Food Odor Value Coding in the Fly Brain

J. Beshel

Stimulus valuation is a critical step in determining how we relate to the world. Yet the way the brain computes and represents value remains a matter of much debate. The assessment of potential food sources provides an expedient framework to address value representation in the brain given its ubiquity in nature. Under normal conditions, most animals, including *Drosophila*, are extremely discerning about what food sources to approach, even when given the choice between multiple viable options; odors are one of the most important sensory cues all animals use to track, evaluate, and select among available foods. *Drosophila* are an unparalleled model organism with which to study such questions given their complex behavior and relatively tractable nervous system, as well as the wealth of genetic tools available to both observe and manipulate targeted neural populations. We first established *Drosophila's* partiality for differing food odors behaviorally. We then examined with single-cell resolution, using in vivo two-photon calcium imaging, the relationship between observed food-odor values and activity in targeted subsets of olfactory and neuromodulatory neural populations. We were specifically interested in the role of *Drosophila* neuropeptide F (dNPF) neurons, the functional homolog of mammalian orexigenic neuropeptide Y, which is a prominent regulator of food-related appetitive behaviors. We show that dNPF activity precisely defines a hierarchy of odor value with a perfect correlation between neural activity and behavior: The greater the odor-evoked dNPF response, the greater the attraction to that odor. The neuron's activity also explicitly accounts for behavioral changes observed due to alterations in motivational state, in this case hunger, by increasing its activity accordingly. We confirmed that dNPF activity is necessary for the attraction we observe to food odors as genetically disrupting the dNPF pathway abolishes odor attraction. Consistent with these results, stimulating dNPF activity was sufficient to drive asymptotic levels of the food-odor approach regardless of the satiety state. Taken together, these experiments describe how the value of a specific class of stimuli, food odor, is flexibly represented in the brain.

Rac-Dependent Forgetting in Fruit Fly

Y. Shuai, A. Sodsiri, Y. Ai

The biological process underlying forgetting is not well known. We intend to study the molecular and cellular mechanisms of forgetting by using *Drosophila* olfactory associative memory as a model. The paired odor-shock conditioning paradigm is well characterized in the fruit fly. Labile memory forms generated after single-session conditioning are vulnerable to forgetting. They will decay within a few hours and are prone to disruption by interference from new learning and reversal learning. We have shown in our recent study that both decay and interference-induced forgetting are regulated by Rac small-G protein activity in the mushroom body (MB). We hypothesize that Rac-dependent forgetting is an adaptive strategy that can actively modulate the transience of memory. We seek to further determine (1) how Rac activity is regulated during forgetting and (2) how Rac forgetting affects established memory traces. For the first question, we take advantage of a fluorescence resonance energy transfer (FRET)-based Rac activity probe. We combine in vivo imaging of Rac activity with transgenic RNA interference (RNAi) to screen for Rac regulators in the adult MB. The initial screen focused on the guanine nucleotide exchange factor (GEF) and GTPase-activating proteins (GAPs), two families of proteins known to regulate RhoGTPase activity. Screening through ~40 GEF and GAP candidates has uncovered one GEF and one GAP that can up- and down-regulate Rac activity in the MB, respectively. We are now in the process of evaluating

their significance during forgetting. For the second question, we combine live animal calcium imaging by GCaMP3 with acute genetic manipulation of Rac activity to probe the cellular effect of Rac forgetting. We are testing the hypothesis that collapse of a memory stabilization network involving the α′/β′ subtype of MB neurons subserves forgetting.

NF1 and Memory in *Drosophila*

C. Xu

Neurofibromatosis 1 (NF1) is a genetic disorder affecting one in 3500 people in the population. Affected individuals manifest overgrowth of various types of tissue and ~40% of the patients show cognitive defects. The mutated gene responsible for the NF1 disorder is called neurofibromin, and it is conserved among species. When the neurofibromin gene is deleted, the *Drosophila* animal model has both learning and long-term memory phenotypes with aversive associative conditioning. In contrast to the aversive conditioning, appetitive conditioning is more effective in forming long-term memory by single training trails, whereas aversive conditioning needs many trails. It is not known whether appetitive memory also requires neurofibromin. We are currently investigating this question with neurofibromin mutant flies.

Decision Making in *Drosophila* Larvae

S. Khurana

Decision making, and its underlying mechanisms, whether in the context of learning or sensory response, is one of the key areas of inquiry in contemporary neuroscience. Understanding of the behavior involved in state transition from one repetitive behavioral pattern to another has been relatively unexplored. Using automated tracking of larvae of *Drosophila melanogaster,* we are studying the behavioral patterns of locomotion, sensory responses, and conditioning to explore various steps involved in the orientation strategies. Although automated tracking has been increasingly used in neuroscience, including the larvae of fruit fly, the focus of the field has been to explore only one or a few simple parameters of behavior. We are looking at changes of behavior in the form of the most measurable behavioral parameters from one step to another, i.e., state transitions, something that has not been explored. In larvae, there are two broad phases: repetitive locomotion patterns, called fast phases, where the curvature of tracks is relatively small under most circumstances, and slow phases, where animals slow down and explore their environment. Exploration of slow phases, its fine structure, and relation of one slow phase as a function of previous slow and fast phases, conditions that can elicit changes in fast phase and its state dependence, has revealed a pattern of transitions where a previous history of the animal influences its next step orienting to or away from a stimulus. We are currently completing the streamlining of automation for this analysis, which generates a large amount of data, similar to genomic analysis from relatively few experiments, and gearing toward two kinds of manipulations to explore the meaning of such patterns. The first set of experiments involves the manipulation of stimulus parameters, the behavioral arena, and the state of animal to reveal changes in this orientation strategy. The second set involves a conditional knock-out and knock-in of various neurotransmitters, neuromodulator systems, and brain regions, using the inducible UAS-GAL4 system to explore the underlying mechanisms regulating the different phases and transitions. We hope that in the long run, application of this detailed behavioral approach to various disease model systems, including mammalian systems, will be useful in the exploration of various facets of neuropsychiatric disorders and the application of combinatorial drug therapy needed for many such disorders.

PLANT BIOLOGY

David Jackson and colleagues study genes and signals that regulate plant growth and architecture. They are investigating a unique way in which plant cells communicate, by transporting regulatory proteins via small channels called plasmodesmata. These channels, which direct the flow of nutrients and signals through growing tissues, are regulated during development. The team discovered a gene, *CCT8*, that controls the transport of a transcription factor, SHOOTMERISTEMLESS (STM), between cells in the plant stem cell niche, or meristem. STM is critical for stem cell maintenance, and studies of the *CCT8* gene indicate that movement of STM between cells is required for this function. The lab also continues to identify other genes that control plant architecture through effects on stem cell maintenance and identity. Recent examples include discovery of a subunit of a heterotrimeric G protein that is conserved throughout animals and plants, and their studies indicate that this gene controls stem cell proliferation. In animal systems, the G protein interacts with a class of receptors that are called GPCRs (G-protein-coupled receptors), but they have found that in plants, the same protein interacts with a completely different class of receptors. Their discovery helps explain how signaling from diverse receptors is achieved in plants. This past year, they also demonstrated that weak mutations in one of the receptor proteins can enhance seed production in maize, which could lead to yield increases. Separately, the lab has characterized system-wide networks of gene expression in inflorescence development, using "next-gen" profiling methods, and is developing a collection of maize lines that can drive expression of any reporter or experimental gene in any tissue type—tools of great interest to maize researchers that are being made available to the broader scientific community, enabling experiments never before possible in crop plants.

Zachary Lippman's research focuses on identifying genes that cause tomato plants to produce their flowers in an elegant repetitive zigzag arrangement on a branching structure called an "inflorescence." Proceeding from a base of knowledge they have built regarding a universal growth habit represented by tomato, called sympodial growth, Lippman's lab is addressing the question of zigzag flower patterning by focusing on the fundamental question of how plants are able to stop making leaves and start making flowers. Of particular interest is how these "reproductive phase transitions" have contributed to the evolution of diverse inflorescence branching patterns in tomato's larger *Solanaceae* family, which includes plants that make just one flower in each inflorescence, to plants whose inflorescences produce dozens of branches and hundreds of flowers. Using classical and modern genetic techniques, Lippman is dissecting the gene networks that are responsible for the variation in inflorescence branching found in nature. He hopes to leverage these discoveries to improve crop yields in several *Solanaceous* plants such as pepper, eggplant, and potato. Already, a collaboration between Lippman's group and scientists at Hebrew University has identified a gene called florigen, which is responsible for making flowers in plants, and can be altered using simple genetic tricks to push hybrid tomato plants to increase their yield by as much as 60%. Using a systems-biology approach and next-generation sequencing technology to capture the transcriptome of stem cells at five different stages of maturation enabled the lab to identify nearly 4000 genes that represent the "maturation clock" in tomato plants. Most recently, the team built on this with the discovery of a gene, Terminating Flower or TMF, that acts as a key regulator on this clock, slowing flowering down so it does not happen too precociously. This year, the Lippman lab was also involved in sequencing the "Heinz" tomato and its wild ancestor in order to better understand the evolution of the domesticated variety.

Epigenetic mechanisms of gene regulation—chemical and conformational changes to DNA and the chromatin that bundles it—have had an important impact on genome organization and

inheritance and on cell fate. These mechanisms are conserved in eukaryotes and provide an additional layer of information superimposed on the genetic code. **Robert Martienssen**, a pioneer in the study of epigenetics, investigates mechanisms involved in gene regulation and stem cell fate in yeast and model plants including *Arabidopsis* and maize. He and colleagues have shed light on a phenomenon called position-effect variegation, caused by inactivation of a gene positioned near densely packed chromosomal material called heterochromatin. They have discovered that small RNA molecules arising from repeating genetic sequences program that heterochromatin. Martienssen and colleagues have described a remarkable process by which "companion cells" to sperm in plant pollen grains provide them with instructions that protect sperm DNA from transposon damage. This year, they published research showing that some of these instructions, or epigenetic marks, could be inherited in the next generation. With collaborators in Mexico, Martienssen has also coaxed *Arabidopsis*, a flowering plant, to produce egg cells without meiosis, an important step toward a long-time goal of plant breeding: generating clonal offspring to perpetuate hybrid vigor. The lab has also showed that when RNA polymerase II has transcribed a stretch of DNA, the RNA interference mechanism causes the enzyme to release its hold on the DNA and fall away. This allows the replication fork to progress smoothly and the DNA strands to be copied; histone-modifying proteins, which follow right along, establish heterochromatin. Martienssen's group also continues to work on problems related to the creation of plant-based biofuels.

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

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

D. Jackson R. Balkunde, P. Bommert, C. Brooks, A. Eveland, A. Farahani, S. Hiraga, B. Il Je, M. Pautler, L. Sahl, C. Schwoyer, S. Vi, Y.G. Wolfenson, Q. Wu, F. Yang, T. Zadrozny

Our research aims to identify genes, signals, and pathways that regulate plant growth and development. All organisms develop by carefully controlling the flow of information ("signals") that passes between cells and tissues. We are particularly interested in discovering these signals and finding out how they are transmitted, and how they function. In one project, we are investigating a unique way in which plant cells communicate, by transporting regulatory proteins via small channels called plasmodesmata. These channels, which direct the flow of nutrients and signals through growing tissues, are regulated during development. We discovered a chaperone protein-encoding gene *CCT8* that controls the transport of the transcription factor SHOOTMERISTEMLESS (STM) between cells in the plant stem-cell niche, or meristem. STM is critical for stem-cell maintenance, and studies of the *CCT8* gene indicate that movement of STM between cells is required for this function. We also continue to identify other genes that control plant architecture through effects on stem-cell maintenance and identity.

Recent examples include discovery of a subunit of a heterotrimeric G protein that is conserved throughout animals and plants, and our studies indicate that this gene controls stem-cell proliferation. In animal systems, the G protein interacts with a class of receptors that are called GPCRs (G-protein-coupled receptors), but we have found that in plants, the same protein interacts with a completely different class of receptors. Our discovery helps explain how signaling from diverse receptors is achieved in plants. This past year, we also demonstrated that weak mutations in one of the receptor proteins can enhance seed production in maize, which could lead to yield increases. Separately, our lab has characterized system-wide networks of gene expression in inflorescence development, using "next-gen" profiling methods, and we are developing a collection of maize lines that can drive expression of any reporter or experimental gene in any tissue type. These tools are of great interest to maize researchers and are being made available to the broader scientific community, enabling experiments never before possible in crop plants.

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

R. Balkunde

Cell-to-cell communication functions in specifying cell fate and coordinating development in all multicellular organisms. A special paradigm for such communication in plants is the selective trafficking of signaling macromolecules, e.g., transcription factors and small RNAs, through plasmodesmata (PDs), channels that traverse the cell wall and connect all plant cells. In addition to cell-fate specification, PDs are involved in viral movement, transport of metabolites, and cell-to-cell spread of RNA interference (RNAi), which points to their fundamental importance in coordinating plant defense, metabolism, and development. Despite the discovery of PDs more than 100 years ago, and our increasing recognition of their functional significance, the underlying components and mechanisms of PD trafficking remain poorly understood. Hence, we are taking an unbiased genetic strategy to dissect these molecular components and mechanisms, using a transgenic reporter system.

Ethylmethanesulfonate (EMS) mutagenesis screening using a "trichome rescue" system followed by Illumina high-throughput sequencing identified a mutation in a gene encoding CCT8, a chaperonin subunit. This led to an exciting discovery that chaperonin facilitates KN1 cell-to-cell trafficking and stem-cell maintenance in *Arabidopsis*, which clearly supports the functional relevance of chaperonin-mediated trafficking through PDs. These results suggest that chaperonin dependency is a general mechanism for protein

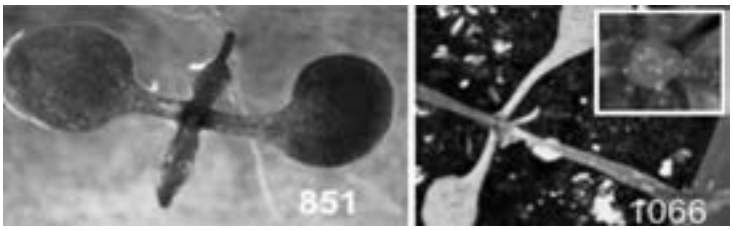

Figure 1. Trafficking mutants with interesting developmental phenotypes. (*Inset*) Close-up of the short apex, showing the enlarged meristem.

trafficking and highlight the importance of conformational changes for PD trafficking. In addition to their trafficking and stem-cell defects, *cct8* mutants exhibit another striking phenotype in leaf polarity where the leaves are curled upward. An exciting possibility is that this could result from altered trafficking of a leaf polarity signal. Alternatively, it could result from altered protein folding or misexpression of one or more polarity regulators.

Current efforts are focused on understanding leaf polarity defects of *cct8* mutants and on identifying new trafficking regulators. Our efforts to detect additional KN1 trafficking regulators have resulted in identifying potential mutants with interesting developmental defects (Fig. 1). We will clone these additional mutants using next-generation sequencing. We have also initiated a new screening approach for trafficking mutant identification. Here, we will be using the activation tagging approach to identify additional genes that regulate PD trafficking.

Illuminating Maize Biology: Using Fluorescent Proteins for High-Throughput Analysis of Protein Localization and Function in Maize

Q. Wu, S. DeBlasio, A. Goldshmidt, T. Zadrozny
[in collaboration with A. Chan, J. Craig Venter Institute; A. Sylvester, University of Wyoming]

Recent advances in sequencing technology have generated genome sequences from nearly all groups of organisms. These massive data sets have revolutionized the types of biological questions that can now be addressed. Despite the ability to predict the function of some proteins by comparative bioinformatics, experimental validation remains necessary to assign function definitively. One effective approach to validate gene function is to develop visualization and biochemical tools associated with fluorescent protein (FP) technology.

FP technology is a powerful tool that can be used to noninvasively mark protein expression and localization to study various subcellular processes in vivo. We have successfully optimized this technology for maize and have created a public resource of cellular biomarkers that we and other members of the plant community use for both small-scale analysis of individual genes and global expression and functional profiling. To date, we have generated more than 100 stable, natively expressed, maize FP fusion lines that highlight most subcellular compartments, and these lines have allowed us to study various cellular pathways including hormone signaling, cytoskeletal behavior, vesicle trafficking, and stem-cell maintenance and differentiation. We are also using these lines to immunoprecipitate tagged proteins to identify previously uncharacterized components of these important pathways. In the case of FP-tagged transcriptional regulators, chromatin immunoprecipitation (ChIP) is being used to identify putative DNA-binding sites and/or to confirm gene targets that have been detected by transcriptional profiling of the respective mutant.

To study maize development on a broader scale, we are optimizing the LhG4 two-component *trans*-activation expression system for use in maize. This system relies on the transcription factor LhG4 being expressed by a cell- or tissue-specific promoter to drive the expression of a reporter in *trans*, via its pOp-binding sites. The reporter can be any gene, such as GUS, FPs, an FP fusion protein, a gene of interest for tissue-specific expression, a silencing construct for transcriptional knockdowns, or a gene encoding a toxin for tissue ablation experiments. We have already generated several constitutive, meristem- and leaf-specific promoter LhG4 drivers,

including p*UBIQUITIN*, p*RAMOSA3*, p*WUSCHEL*, and p*YABBY14*, as well as a pOp responder line, pOp-FT, as a means to test the specificity of the system with a readout in flowering time. We are now working to create additional vascular-, root-, and embryo-specific LhG4 driver lines and other responder lines. Our goal is to produce an array of cell- and tissue-specific LhG4 lines, which we will use to (1) isolate specific cell types by FACS (fluorescence-activated cell sorting) for transcriptional profiling and (2) drive the misexpression of developmentally regulated genes in order to better understand the changes that occur within stem-cell niches during differentiation and development. Data on the characterization of our FP and LhG4 transgenic lines, including confocal micrographs, movies, and recent publications, can be found on our website: http://maize.jcvi.org/cellgenomics/index.php.

Control of Shoot Meristem Size and Phyllotaxy by the Maize Glutaredoxin *Abphyl2*

F. Yang, M. Pautler [in collaboration with V. Llaca, DuPont Crop Genetics, Wilmington, Delaware; R. Johnston, Cornell University, Ithaca, New York; B. Ha Lee, Sogang University, Korea; H. Sakai, DuPont Crop Genetics, Wilmington, Delaware]

Phyllotaxy is a major taxonomic indicator and affects plant architecture and light capture efficiency. Auxin and its polar transport through PINFORMED1 (PIN1) proteins are crucial for controlling phyllotactic patterns. Recently, our studies on *ABPH1* in maize have shown that cytokinin hormone signaling, as well as its cross-talk with auxin, has an important role in this process. *abphyl1* (*abph1)* mutants change maize phyllotaxy from alternate to decussate and develop an enlarged shoot apical meristem (SAM). Here, a similar phyllotaxy mutant, *Abph2*, is described. *Abph2* is caused by a dominant mutation and also has an enlarged SAM and a decussate leaf pattern that becomes visible at leaf ~4–5 stage. Map-based cloning brought *Abph2* into a region of ~20 kb on chromosome 7, containing five predicted genes in the reference B73 genome. However, direct sequencing of the open reading frames of these five genes, as well as their transcript level analysis by reverse transcriptase–polymerase chain reaction (RT-PCR), did not give any obvious clues as to the identity of the *Abph2* gene. Therefore, a bacterial artificial chromosome (BAC) library generated from the *Abph2* mutant was screened using probes located within the 20-kb mapping interval. BAC sequencing revealed a 4.5-kb fragment inserted into the 20-kb mapping interval. This inserted fragment contained a predicted glutaredoxin gene identical to a gene (named *Msca1*) located ~800 kb upstream. A transgenic line containing this 4.5-kb fragment fused with yellow fluorescent protein (YFP) tag phenocopied the dominant *Abph2* phyllotaxy defect, demonstrating that the inserted glutaredoxin gene (named *Abph2* thereafter) is the cause of the decussate leaf phenotype. Meanwhile, putative knockout lines of the dominant *Abph2* mutation were screened by EMS mutagenesis. Sequencing the *Abph2* gene in these knockout lines found two point mutations, leading to conserved amino acid changes in each line, further supporting that the inserted glutaredoxin gene is *Abph2*.

RNA in situ hybridization indicated that the *Abph2* transcripts accumulate in P_0 leaf primordia and vasculature. However, this expression pattern was unaltered in *Abph2* mutants. More careful investigation showed that *Abph2* transcripts start to accumulate in embryos from 12 days after pollination (DAP), but we detected a misexpression of *Abph2* at 18 DAP when approximately four leaves have been initiated. In *Abph2* mutant embryos, the *Abph2* transcripts were detected in the entire meristem, compared to a P_0-localized expression pattern seen in wild type. This altered expression pattern likely explains the altered phenotype in the mutant.

The *Abph2* gene is identical to *Msca1*. Loss-of-function mutation in *Msca1* leads to male sterility but a normal phyllotaxy. A detailed meristem size comparison revealed a significant decrease in *msca1* mutants. This finding demonstrates that *Msca1* promotes meristem growth. *Abph2*/*Msca1* encodes a CC-type glutaredoxin protein, similar to the *Arabidopsis ROXY1* and *2* genes. Recently, *ROXY1* and *2* were found to interact with basic leucine zipper (bZIP) transcription factors, including PERIANTHIA (PAN), which is related to FASCIATED EAR4 (FEA4) recently cloned in our lab. Loss of *FEA4* function results in a larger meristem, as well as altered phyllotaxy, similar to *Abph2*. Using yeast two-hybrid and bimolecular fluorescence complementation (BiFC) assays, we confirmed that ABPH2 and FEA4 interact, and *msca1 fea4* double-mutant analysis showed a synergistic interaction. These findings

suggest that these genes may define a new pathway in meristem proliferation control.

The Regulation of Meristem Size in Maize

B. Il Je, M. Pautler, P. Bommert, Q. Wu, A. Eveland
[in collaboration with M. Komatsu and H. Sakai, Dupont Crop Genetics]

All plant organs derive from populations of stem cells called meristems. These stem cells have two purposes: to divide to replace themselves and to give rise to daughter cells, which will differentiate into lateral organs. Consequently, plant meristems must precisely control the size of the stem-cell niche via a network of positive and negative feedback signals. A loss of function in a negative regulator of stem-cell fate can result in an enlarged or fasciated meristem phenotype and a dramatic alteration in the morphology of the maize ear and tassel.

Maize is an excellent genetic model system because of a large collection of developmental mutants and a sequenced reference genome. Our lab has undertaken a forward genetic approach to identify key regulators of stem-cell homeostasis and meristem size. Two previously cloned mutants, *fasciated ear2* and *thick-tassel dwarf1*, encode orthologs of the *Arabidopsis thaliana* genes *CLAVATA1* and *CLAVATA2*, indicating the well-known *CLAVATA-WUSCHEL* regulatory feedback loop is conserved from dicots to monocots. Work published this year has shown that natural variation in FEA2 can enhance maize seed yield traits. However, little else is known about the control of this important developmental process in maize. Here, we describe our progress in identifying additional genes contributing to stem-cell niche homeostasis.

Plant growth and development depend on the balancing of meristem proliferation that is controlled by a negative feedback loop between the CLAVATA pathway and the *WUSCHEL* homeobox gene. CLAVATA signaling involves a secreted peptide, CLAVATA3 (CLV3), and its perception by cell surface leucine-rich repeat (LRR) receptors, including the CLV1 receptor kinase and an LRR receptor-like protein, CLV2. However, the signaling mechanisms operating downstream from these receptors are not fully understood, especially for the LRR receptor-like proteins, which lack an intracellular signaling domain. Here, we describe our progress in understanding the downstream signal of the CLAVATA pathway by characterizing *COMPACT PLANT2 (CT2).*

ct2 is a classical mutant of maize that exhibits a fasciated inflorescence phenotype as well as semidwarfism. *ct2* mutants have a range of phenotypes, including a semidwarf stature and shorter and wider leaves. The mutants also show striking inflorescence defects, including strongly fasciated ears. Via a map-based cloning approach, we discovered *ct2* to encode the α subunit of a heterotrimeric GTPase, a membrane-associated protein involved in the transduction of extracellular signals to induce specific cellular responses by activating downstream effectors. We made a CT2 fusion with YFP driven by its endogenous promoter, and see localization to plasma membrane. *ct2* is epistatic to *fea2* with respect to spikelet density, which correlates with meristem size, suggesting they act in a common pathway. In coimmunoprecipitation experiments (Co-IPs), we found that FEA2 and CT2 interact, implicating heterotrimeric G-protein signaling in the CLAVATA signaling pathway. To gain a better understanding of the cross-talk between G-protein signaling and the CLAVATA pathway, we are using fluorescence resonance energy transfer (FRET) and other biophysical methods to investigate the factors that affect signal transmission between FEA2 and CT2. The preliminary FRET data suggest that FEA2 and CT2 interact with each other directly. This research introduces a new paradigm in G-protein signaling, because G proteins interact exclusively with seven-pass transmembrane receptors in mammals and fungi.

Another fasciated ear mutant that we recently cloned is *fasciated ear 3* (*fea3*). *fea3* shows an overproliferation of the inflorescence meristem. We cloned this gene using map-based cloning, and the mutant results from the insertion of a partial retrotransposon into an exon of the *FEA3* locus. We confirmed this identity by isolation of new alleles of *fea3* from an EMS-targeted mutagenesis. *fea3* encodes a predicted LRR receptor-like protein related to *fea2*. In situ hybridization and red fluorescent protein (RFP)-tagged transgenic plants show that FEA3 is expressed in the organizing center of the shoot meristem, and it is also expressed in the root apical meristem. FEA3 protein is localized in the plasma membrane. To ask if FEA3 responds to a CLV3-related (CLE) peptide, we tested its sensitivity to different peptides. *fea3* mutants showed reduced peptide sensitivity, but interestingly, they

responded to a different CLE peptide compared to FEA2. Double mutants of *fea2*/*fea3* and *td1*/*fea3* have additive and synergistic fasciated phenotypes (Fig. 2), indicating that they act in independent pathways that converge on the same downstream target to control meristem size. These results indicate that the function of FEA3 as a receptor protein is in a new pathway distinct from that of TD1 and FEA2.

Another mutant, *fasciated ear4*, is a semidwarfed maize mutant with fasciated ears and tassel (Fig. 3). These phenotypes are caused by enlarged vegetative and inflorescence meristems, which have lost control of the pathways normally regulating meristem size. We had previously mapped *fea4* to a small region of chromosome 6 containing 30 genes. Sequencing of candidate genes in the reference allele revealed a bZIP transcription factor with an EMS-induced base transition causing a premature stop codon. Two additional alleles also contained mutations in the gene consistent with EMS mutagenesis. We subsequently obtained a series of transposon-induced mutant alleles through a collaboration with DuPont-Pioneer, confirming that we have identified a novel gene required for the control of meristem size in maize.

We have subsequently investigated the function of *fea4* through several parallel approaches. We used in situ hybridization to determine the expression pattern of *fea4* during different stages of development. During vegetative development, *fea4* is expressed in the peripheral zone of the SAM and is dramatically excluded from the stem-cell niche and the site of leaf initiation. This unique expression pattern suggests that *fea4* does not act by directly influencing the stem cells in the central zone of the SAM, but rather by influencing the process of differentiation in the peripheral zone. We have generated transgenic plants expressing a translational fusion of YFP with FEA4 under the control of the native promoter. These transgenic plants express the fusion protein in the nucleus of cells in the expected domain, closely matching the mRNA expression pattern (Fig. 3). These stable transgenic lines will be useful in the future for functional genomics and proteomics experiments, such as ChIP-Seq and immunoprecipitation (IP)-mass spectrometry (MS). We have also obtained a global picture of transcriptional changes in the mutant by Illumina mRNA-sequencing and are beginning to follow up on downstream genes that may mediate *fea4* action. Additional approaches include making double-mutant combinations between *fea4* and other meristem regulation mutants. So far, these analyses have suggested that *fea4* acts outside of the canonical meristem size regulation pathways, consistent with a role in buffering the balance between division and differentiation.

In addition, we are in the process of mapping additional fasciated ear mutants from nontargeted EMS mutagenesis screens. Our current approach involves creating F_2 mapping populations and rough mapping by bulked segregant analysis. We take advantage of the precise quantitative genotyping capability of the Sequenom MassArray system to look for areas of the genome that are linked to the phenotype. We have determined rough positions for several of these mutants and are proceeding with fine mapping and molecular cloning.

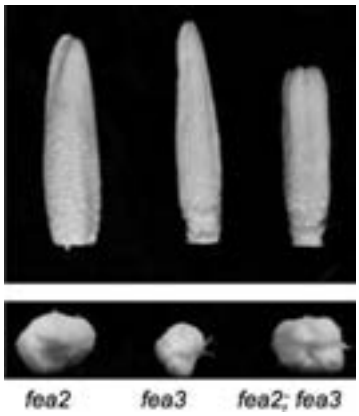

Figure 2. *fea* (mutant ears); *fea2*; *fea3* double mutants are more severe than single mutants.

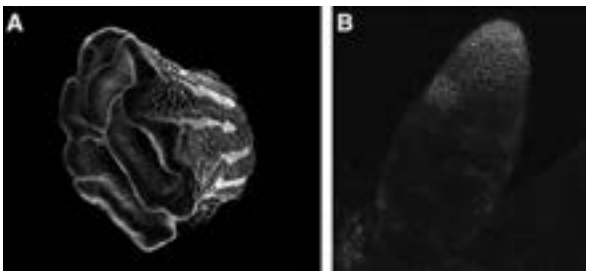

Figure 3. (*A*) Scanning electron micrograph of a *fasciated ear4* ear primordium showing a massively fasciated inflorescence meristem. (*B*) Confocal microscope image showing expression of YFP-FEA4 in the nucleus of cells in the inflorescence meristem.

Defining the Regulatory Networks Controlling Inflorescence Architecture in Maize

A.L. Eveland, A. Goldshmidt, M. Pautler, S. Hiraga
[in collaboration with D. Ware, Cold Spring Harbor Laboratory; E. Vollbrecht, Iowa State University; K. Morohashi and E. Grotewold, The Ohio State University]

Inflorescences bear the fruits and grains that we eat, and thus understanding the genetic and regulatory basis for how these structures are formed has clear agronomic importance in determining yield. Inflorescence architecture among cereal crops is diverse, yet characterized by a unique morphology, where flowers are borne on short, determinate branches called spikelets. In maize, these spikelets are paired, an exclusive feature of the tribe *Andropogoneae*, which includes other important cereal and bioenergy crops. Variations in inflorescence branching patterns arise from position and developmental fate of differentiating stem-cell populations called meristems. The coordinated actions of key developmental regulators, as well as genetic interactions between them, modulate meristem initiation and determinacy during maize inflorescence development. Our goal is to understand the mechanisms by which these regulators act to control inflorescence architecture, what other factors and/or biological processes are involved in this regulation, and how these regulatory networks coordinate development in space and time. Inflorescence architecture in maize is largely determined by grass-specific meristem types and, therefore, findings from this work can be translated to other important cereal crops with comparable inflorescence architectures. The primary objective of this work is to improve our understanding of how inflorescence architecture is modulated at the molecular level so that we can identify control points for targeted improvement of important agronomic traits such as yield. We are using systems-level analyses to elucidate the gene networks that control identity and determinacy of axillary meristems, which contribute to specific branching patterns found among grass inflorescences. Our approach integrates large-scale genomics datasets, such as gene expression profiles achieved by high-throughput RNA sequencing, with discrete morphological features in development. Here, we characterized the precise timing of developmental transitions at the molecular level in maize male and female inflorescences, tassel and ear, respectively, by associating spatiotemporal dynamics in gene expression with morphological changes resulting from genetic perturbations in a pathway controlling branching. The latter includes loss-of-function mutants in three important regulators of the *ramosa* (*ra*) pathway, which controls stem-cell fate and the decision to branch. Using a k-means clustering approach, we classified genes with similar expression profiles during development into coexpression signatures and identified predominant signatures that coincided with key stages of inflorescence development (Fig. 4a). One

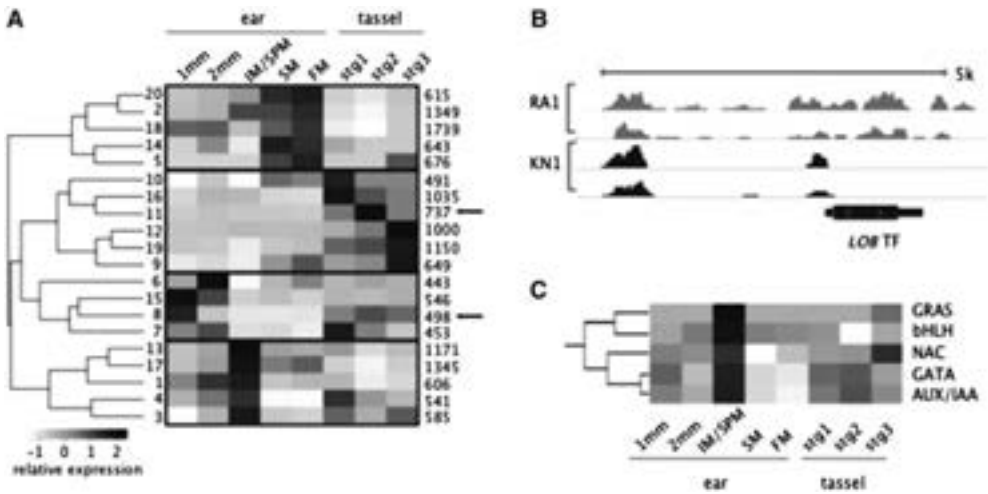

Figure 4. Spatiotemporal expression signatures during maize inflorescence development; arrows denote clusters enriched for *ra1* modulated genes (*a*). RA1 and KN1 cotarget TFs (*b*), which show specific coexpression signatures during development (*c*).

particular signature was strongly enriched for genes that were altered in *ra* mutants and included many known determinacy factors previously shown to work together in other developmental contexts. Here, these determinacy factors were further coexpressed across *ra* mutant backgrounds in a developmentally dependent manner, revealing a grass-specific module for spikelet pair meristem determinacy that also included genes of unknown function, many of which were grass specific. The latter represent candidate genes for novel regulators of meristem determinacy. Functional characterization of these candidates is currently under way, including in situ hybridizations and analysis of loss-of-function mutant alleles and crossing these to *ra* mutants to reveal potential genetic interactions. To expand our coexpression network and gain insight into the mechanisms of gene regulation in the *ramosa* pathway, we identified in vivo gene targets of the RA1 transcription factor (TF) on a genome-wide scale using chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-Seq). We used transgenic *ra1* mutant maize lines expressing RA1 tagged with either HA or YFP tags in two parallel experiments to identify ~1000 high-confidence target genes that were bound in both. Of these, about one-fourth showed altered expression in the *ra1* background, suggesting that they are modulated targets of RA1. We also showed that RA1 acts as both an activator and a repressor of gene expression, which is interesting given that it possesses two EAR repressor domains shown to confer repressive function. Strikingly, activated and repressed targets are exclusively coexpressed, suggesting that the mechanism of RA1 action is dependent on spatiotemporal context. We further integrated ChIP-Seq data available for the KNOTTED1 (KN1) TF to determine whether RA1, which imposes determinacy on spikelet pair meristems, and KN1, which maintains indeterminacy in all shoot meristems, share common targets. This revealed the potential for combinatorial binding for these two TFs at multiple loci in the maize genome, including 176 bound and modulated targets. Targets shared between RA1 and KN1 networks were enriched for TFs and suggest points of regulation that interface meristem maintenance and determinacy (Fig. 4b,c). We are further investigating potential regulatory motifs that are enriched within the binding sites of RA1 to resolve consensus TF binding sites for RA1 and/or cofactors that may be involved in regulation of its downstream targets. Because *ra1* has been implicated as an important locus in the domestication of modern maize and is found only in Panicoid grasses, these data are being used in comparative analyses with other grasses to further understand RA1 function and the evolution of grass inflorescence architecture.

Control of Branching and Determinacy in Plant Shoots

Y.G. Wolfenson, S. Goldshmidt, L. Sahl, S. Vi, S. Hiraga

RAMOSA (*RA*) genes in maize function to impose determinacy on axillary meristem growth; consequently, *ra* loss-of-function mutants (*ra1, ra2,* and *ra3*) have more highly branched inflorescences. *RA1* encodes a C2H2 putative transcription factor that appears to have had a key role in maize domestication and grass evolution. *RA2* also encodes a putative transcription factor, a LOB domain protein, whereas *RA3* encodes a predicted metabolic enzyme, a trehalose phosphate phosphatase. The disaccharide trehalose is not abundant in plant tissues and may have a regulatory role because it has been implicated in stress protection, control of sugar signaling, and regulation of photosynthetic rates. This is the first indication that trehalose has a specific developmental function. *RA3* is expressed in a localized domain at the base of axillary inflorescence meristems, and it localizes to nuclear and cytoplasmic compartments, suggesting that its effect on development is not simply metabolic. Interestingly, genetic and molecular studies suggest that both *RA2* and *RA3* are required for proper *RA1* expression and act upstream of *RA1*. All three genes are expressed in overlapping domains; *RA2* is expressed in the axillary inflorescence meristems themselves, whereas *RA1* and *RA3* expression is only at the base of the axillary inflorescence meristems, suggesting that they may control a mobile signal that regulates meristem determinacy. The *RAMOSA* (*RA*) genes therefore define a boundary domain that surrounds the developing branch meristem, rather than being deployed in the meristem itself. These data support the hypothesis that *RA* genes may serve as mediators of signals, maybe a sugar signal, originated at the boundary domain and regulating determinacy. *RA3* itself may have a transcriptional regulatory function, because it affects the expression of specific genes.

To find specific genes that are differentially expressed in the *RA*-specific domain, we are using FACS

followed by mRNA-Seq. p*WUSCHEL*::RFP:NLS and pRA3::RFP:NLS reporter lines crossed to *ra* mutants are used for this purpose. The p*WUSCHEL* reporter is a marker for cells of the organizing center of the meristem. We expect that cell-specific profiling will enable identification of genes that act directly in the RAMOSA signaling pathway.

In another approach, we will use yeast one-hybrid analysis to identify transcription factors that regulate *RA3* gene transcription. The *RA3* promoter was cloned into a reporter vector and integrated into the yeast genome. This strain provides a host for library screening. A cDNA library of DNA-binding proteins, which are expressed as fusions to the yeast GAL4 transcription activation domain (GAL4 AD prey proteins), will then be screened, in collaboration with Lifang Zhang (Ware lab). After screening, further analysis of identified transcription factors will be done in order to learn more about their role in determinacy. Furthermore, testing whether *RA3* gene transcription is affected by other factors, such as sugars, could be done in the yeast reporter system. These studies will therefore contribute additional information about *RAMOSA*-mediated determinacy pathways.

The *Arabidopsis thaliana* genome contains a family of 10 genes related to *RA3*. Null mutants of all *AtTPP*s were tested, and the *AtTPPI* mutant was found to have an interesting phenotype that is connected to development and determinacy. Its late-flowering phenotype is accompanied by reduced size and shorter roots, as well as fusion of the cauline leaves to the main stem. In addition, double mutants with *superman*, an *ra1* homolog, show increased indeterminacy, suggesting conservation of this developmental module. A close paralog of *AtTPPI*, At*TPPJ*, has a similar expression pattern. We identified T-DNA insertions, and *tppi;tppj* double mutants show enhanced phenotypes, as well as defects in floral development, including additional and abnormal sepals and petals (Fig. 5).

Additionally, we opted for genetic approaches to identify factors that act in the same pathway with RA3 to control spikelet pair meristem determinacy, by screening for enhancer/suppressor modifiers of the *ra3* phenotype. Typically, *ra3* mutants in a B73 background have three to eight branches only at the base of the ear. We mutagenized *ra3* mutants and looked for plants that have more branches and/or have branches at the upper part of the ear. So far, we have identified several enhancers (Fig. 6) and are in the process of making mapping populations and mapping by bulk segregant analysis.

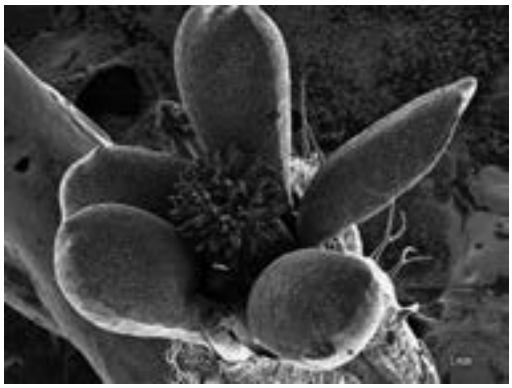

Figure 5. Floral defects in *tpp* mutants.

Natural Variation and Inflorescence Architectures

S. Vi, S. Goldshmidt, P. Bommert

Maize inflorescence architectures have been a target for extensive selection by breeders since maize domestication, hence different maize inbreds vary greatly in these particular traits. The genetic basis underlying this diversity is largely unknown and is of great interest for both fundamental and applied science. Now with the wealth of genomic data, we are equipped to study them. To identify natural variation relevant to inflorescence traits, we looked for inbred backgrounds

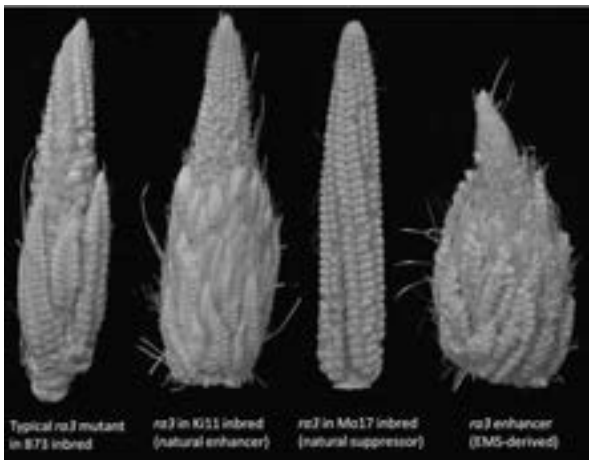

Figure 6. Modifiers of *ramosa3* branching phenotype. Note the differences in the number of branches on ears of the modifiers.

that can enhance/suppress the phenotypes of the mutants on which we work. We focused on the 25 NAM (nested association mapping) founder inbreds because they were selected to capture the diversity of maize germplasm and because of the genetic tools available for these inbreds. We have crossed these 25 inbreds to our collection of mutants (often in B73 background) and screened the F2 for plants with suppressed/enhanced phenotype compared to the mutant in the original inbred background.

So far, we have identified a suppressor of *ramosa3* (*ra3*) coming from the Mo17 inbred and an enhancer coming from the Ki11 inbred (Fig. 6), as well as an enhancer of *fea2* coming from the NC350 inbred. Segregation ratios suggest one semidominant locus for the Mo17-derived suppressor, two or more loci for the Ki11-derived enhancer, and one recessive locus for the NC350-derived enhancer. We are now in the process of rough mapping by bulked segregant analysis in F2 populations. Additionally, because the natural modifiers are often the result of quantitative trait loci (QTL) rather than single-gene effects, we also plan to cross the mutants to the corresponding NAM-founder/B73 RILs, in order to identify and map potential modifying QTL.

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PLANT DEVELOPMENTAL BIOLOGY, STEM CELLS, AND FLORAL BRANCHING SYSTEMS

Z. Lippman K. Jiang C. MacAlister S. Thomain
K. Liberatore S. Park

In plants, self-maintained populations of undifferentiated cells called shoot apical meristems (SAMs) produce all above-ground organs and guide overall morphology. The basic structure of a flowering plant can be reduced to two phases of meristem growth: the vegetative phase and the reproductive phase. Initial SAM growth following germination produces a shoot with leaves and lateral (axillary) meristems, which form in the axils of leaves. Axillary meristems can themselves give rise to shoots, leaves, and additional leaf-producing axillary meristems. The SAM then gradually enters the reproductive phase by transitioning to an inflorescence meristem (IM), which initiates flowers and axillary meristems that can either produce additional flower-producing axillary meristems or immediately become flowers.

Although useful for understanding basic principles of meristem activity and potential, this simplified framework fails to explain the vast architectural diversity in the plant kingdom, especially the remarkable variation in the number and arrangement of branches. This is because branching variation traces back differences in meristem production, growth, and size. In other words, when and where meristems form, whether they begin growing immediately or experience dormancy, how long they grow, how large they become, and the number of additional meristems they generate all depend on plant-specific sensitivities to the environment and differential regulation of physiological and genetic programs.

Our research aims to expose and understand the genetic and molecular mechanisms guiding branching, especially within inflorescences, which are responsible for plant reproductive success. For reasons outlined below, we use tomato as a model system to address the hypothesis that the rate at which meristems transition to a reproductive state (meristem maturation), along with meristem size, is responsible for evolutionary differences in inflorescence architecture and flower production and provides a foundation for improving crop yields.

Origins of Tomato Plant Architecture

Although a generic protein hormone called florigen is responsible for flowering induction in all angiosperms, the reproductive transition causes the SAM to gradually change fate over time, and the impact of this "meristem maturation" on shoot branching differs dramatically between different plants. One of the most poorly understood differences in meristem behavior following the reproductive transition involves "monopodial" versus "sympodial" growth. In monopodial plants such as *Arabidopsis* and maize, the inflorescence meristem continues to grow apically following the reproductive transition, and most axillary meristems soon become flowers. In contrast, in sympodial plants such as tomato and related nightshades (Solanaceae), meristems end growth by forming a terminal flower, but not before new axillary meristems are initiated that themselves undergo reproductive transitions and floral termination.

This meristem initiation and termination of sympodial plants results in compound shoots derived from distinct types of meristems (Fig. 1). In tomato, the primary shoot meristem (PSM) from the embryo produces 7–12 leaves before switching to reproductive growth. The PSM then gradually enlarges until it ends growth by differentiating into the first flower of the primary inflorescence. At the base of this flower, an axillary meristem initiates and quickly transitions into a flower itself, but not before giving rise to a new axillary meristem at its base. These "sympodial inflorescence meristems" (SIMs) undergo rapid reiteration and termination to form a zigzag inflorescence of several flowers. Vegetative growth then continues from the axillary meristem in the axil of the last PSM leaf. This "sympodial vegetative meristem" (SYM) generates three leaves before transitioning to the first flower of the next inflorescence. A new SYM then forms in the axil of the last leaf produced by the previous SYM, and this process reiterates to produce a

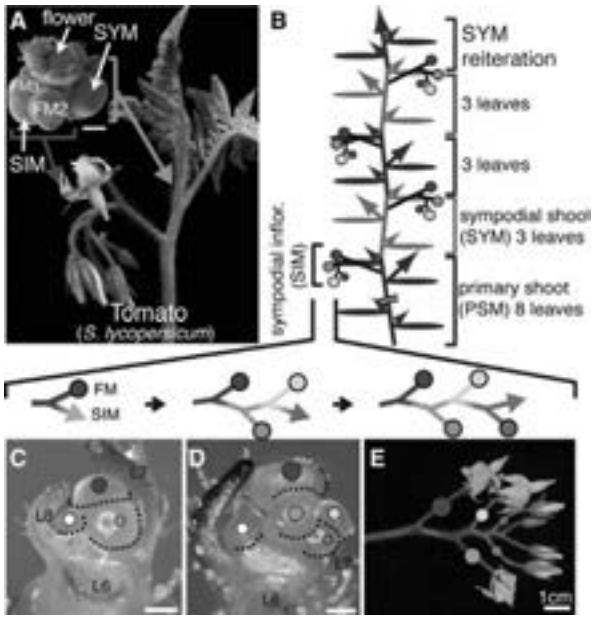

Figure 1. Tomato plant architecture. (*A*) Tomato plant and meristems. (*B*) Diagram of tomato shoot growth. Meristems terminate in flowers, and growth continues from specialized axillary (sympodial) meristems. (Ovals) Leaves; (arrows) side shoots. (*C–E*) Sequential meristem images (*C,D*) and inflorescence (*E*) showing zigzag reiteration of SIMs. White dot indicates SYM. Bars, 200 µm.

compound vegetative shoot. Axillary meristems hosted by lower leaves produce primary, sympodial, and axillary meristems in turn. Thus, tomato undergoes multiple flowering transitions throughout life to produce many multiflowered inflorescences. However, inflorescence structure varies considerably in related Solanaceae. For example, pepper and petunia produce only a single IM per reproductive transition, resulting in single-flowered inflorescences; however, in many Solanaceous trees, IMs proliferate to generate dozens of branches and hundreds of flowers.

Meristem Maturation Drives Inflorescence Diversity in Tomato and Related Nightshades

C. MacAlister, S.J. Park, K. Jiang

The basis for the remarkable range of inflorescence complexity in the Solanaceae family remains poorly understood; however, we previously showed that mutations in the homeobox transcription factor gene *COMPOUND INFLORESCENCE* (*S*) and the floral specification complex encoded by the F-box gene *ANANTHA* (*AN*) and its transcription factor partner *FALSIFLORA* (*FA*) cause highly branched inflorescences by delaying (*s* mutants) or blocking (*an* and *fa* mutants) flower formation. We have now explored the basis for simple inflorescences like those of pepper and petunia by studying a unique and previously uncharacterized tomato mutant called *terminating flower* (*tmf*), whose primary inflorescence is composed of a single flower.

Mutants of tomato have been described that cause inflorescences to become highly branched (e.g., *s*), but *tmf* is the only known mutant that produces a single-flower inflorescence. We identified the *TMF* gene by positional cloning using tools from our sequencing of the wild tomato species, *S. pimpinellifolium* (Sato et al. 2012), and found that it encodes a small transcriptional cofactor that functions to prevent the SAM from precociously differentiating into a flower during the vegetative phase of growth. *TMF* is expressed most when the meristem is in the vegetative phase and is dramatically down-regulated in reproductive meristems to promote flower formation. We find that *TMF* functions independently of the florigen pathway and maintains vegetative identity by repressing precocious activation of the highly conserved AN-FA flower specification complex. Thus, *TMF* defines a previously unknown step in the flowering process that allows precise synchronization of a gradual florigen-guided flowering transition with flower formation. Indeed, by driving *AN* expression precociously, we can transform all multiflowered inflorescences of tomato into solitary flowers, recreating the shoot organization and inflorescence architectures of tomato relatives such as pepper, petunia, and tobacco. Thus, by timing *AN* activation, *TMF* synchronizes flower formation with the gradual reproductive transition, which, in turn, has a key role in determining simple versus complex inflorescences (MacAlister et al. 2012).

Inflorescence Architecture and the Control of Meristem Size

K. Liberatore, C. MacAlister

Even within domesticated tomato, inflorescence architecture varies dramatically from a single zigzag branch of flowers to dozens of branches with hundreds of flowers. Most branching traces back to mutations

in the *S* gene, but there are also varieties with weaker branching that trace back to an increase in meristem size, referred to as "fasciation." Meristem size is tightly controlled through a process of "meristem maintenance," which continuously replenishes stem cells lost to lateral organ formation, and thus, the control of meristem size is a major developmental program regulating inflorescence branching.

A deep understanding of meristem maintenance in plants has developed from analysis of *Arabidopsis* fasciated mutants that exhibit thickened and fused stems, increased flower and floral organ number, and larger seed pods. Most knowledge comes from detailed analyses of three *clavata* (*clv*) mutants and their underlying genes: *CLV1, 2,* and *3.* Our lab recently identified the genes responsible for two fasciated mutants, *fasciated and branched (fab)* and *fasciated inflorescence (fin),* which produce mildly branched inflorescences and fasciated flowers and fruits (Fig. 2). *fab* was found to be defective in the ortholog of the classical membrane receptor kinase *CLAVATA1* (*CLV1*), providing the first functional link to the *CLV* pathway in tomato. Interestingly, however, *fin* was found to be defective in a gene of unknown function encoding a putative transmembrane protein, suggesting that it may act as a receptor or as a cofactor in a receptor complex. Tellingly, *fab;fin* double mutants show remarkable synergism, developing extremely enlarged meristems that rarely produce inflorescences, and the few flowers that do form give rise to extremely large fruits lacking seeds. This synergism suggests that the *FIN* gene acts in a separate pathway parallel to the CLV pathway. The discovery of *FIN* has raised many unanswered questions regarding the control of meristem size in sympodial plants. For example, to what extent are meristem maintenance mechanisms shared or different between monopodial and sympodial plants? Do the relevant genes and pathways vary for different types of meristems? Can changes in meristem size affect inflorescence branching and flower production? On the basis of our early findings, it is our expectation that the *CLV* pathway is not the main mechanism controlling meristem size in tomato and related sympodial plants, and we are currently performing deeper genetic and molecular analyses to better understand the functions of *FIN* relative to *FAB*.

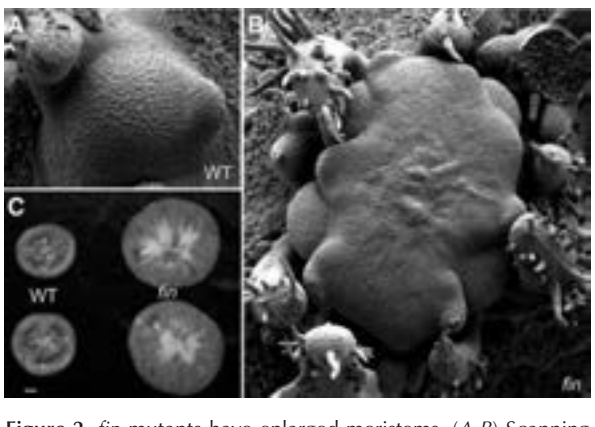

Figure 2. *fin* mutants have enlarged meristems. (*A,B*) Scanning electron micrographs of wild-type (WT) (*A*) and *fin* (*B*) meristems of the same magnification. (*C*) Enhanced fruit size in *fin*.

A Role for *FIN* Genes in Pollen Tube Development

C. MacAlister

FIN encodes an unknown protein of 373 amino acids with a predicted amino-terminal signal peptide and a transmembrane domain. *FIN* is a member of a deeply conserved plant-specific gene family (one to six members in all species), including four members in *Arabidopsis*. Given the vast genetic resources in *Arabidopsis*, we asked whether an analysis of the four *FIN*-like (*FINL*) genes might facilitate and expedite our studies in tomato. By analyzing insertion lines disrupting the expression of the three *FIN-like* family members in *Arabidopsis*, we have found that *finl1;finl2* double mutants are pollen-sterile. Although double-mutant pollen is produced and is capable of germination and tube elongation on the stigma and style, growth quickly becomes disorganized, tube growth arrests upon entering the ovary, and ovules are not targeted for fertilization (Fig. 3). Two proteomics screens in *Arabidopsis* have identified FINL2 as a component of the plasma membrane, and our fluorescent reporters confirm an association with membrane compartments in the cell. Curiously, a large-scale interaction screen has revealed a physical interaction between FINL2 and two plant glutamate-like receptors (GLRs). Plant GLRs are homologs of mammalian ionotropic glutamate receptors (iGluRs) that function in calcium flux and neuronal signaling. Excitingly, recent work has identified *Arabidopsis* GLRs as important regulators of the calcium flux necessary for pollen tube growth, providing a link to our *finl* pollen defects. We are now working

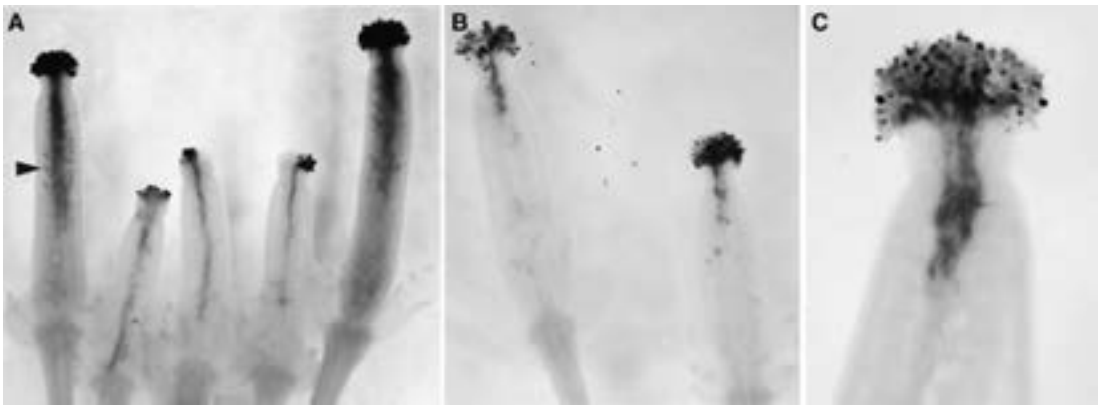

Figure 3. *finl1;2* double-mutant pollen fails to target ovules. (*A*) *finl1* single-mutant pollen tubes stained dark 7 h after pollination (7hap). At this advanced time point, wild-type tubes have grown all the way down the fruit. Arrowhead marks a pollen tube leaving the transmitting tract to target an ovule. (*B,C*) *finl1;2* double-mutant pollen 7hap. Growth ends in a disorganized mass of tubes that do not progress down the transmitting tract of the style and fail to target ovules.

to characterize the role of *FINLs* and their interaction with *GLRs* in pollen tube growth and guidance. Because a relationship between meristem maintenance and pollen tube development has never been reported, our studies of the *FIN* gene family across systems should provide new knowledge that unites these seemingly disparate sporophytic and gametophytic developmental pathways under a common novel mechanism with dual impacts on reproductive growth.

Single-Gene Heterosis, Flowering, and Tomato Yield

K. Jiang, S.J. Park

By demands of modern agriculture, "determinate" tomato varieties have been developed with increased inflorescence, flower, and fruit production from a more compact, bushy plant. This determinacy is based on a mutation in a major flowering gene known as *SELF PRUNING* (*SP*), which normally functions to repress flowering in opposition to the florigen hormone. Interestingly, we previously exposed a remarkable example of single-gene heterosis in tomato that increased yield up to 60% from having only one functional copy of the florigen gene, *SINGLE FLOWER TRUSS* (*SFT*), in the *sp* mutant background (Park et al. 2012). The resulting semideterminate plants generate more inflorescences, flowers, and fruits, and we hypothesized that these yield improvements stemmed from a dose-dependent suppression of *sp*-imposed determinacy.

To test this hypothesis, we profiled gene expression dynamics by RNA-Seq from developing meristems at precise stages of meristem maturation from wild-type, *sft* mutants, and *sft*/+ heterozygous plants. Using our previously established meristem maturation clock, we found that *sft*/+ heterozygous plants exhibit a clear semidominant molecular dosage effect in the SAM just after germination, resulting in an intermediate rate of meristem maturation between wild-type and *sft* homozygous mutants. Likewise, sympodial meristems of *sft*/+ heterozygotes experience a dose-dependent delay in maturation, indicating that an *sft*-induced dosage effect is sensed in all sympodial shoots, enabling a few more sympodial shoots and inflorescences to form prior to *sp*-imposed determinacy. These findings suggest that having only one functional copy of florigen weakly represses meristem termination in a semidominant manner in the primary and subsequent sympodial and axillary shoots, translating to cumulative yield overdominance for flower and fruit production. The critical role of sympodial growth in florigen-mediated single-gene heterosis was illustrated by a parallel experiment in *Arabidopsis*, a monopodial plant having only a single flowering transition. In *Arabidopsis ft/+;tfl1* plants (the equivalent of tomato *sft*/+;*sp*), we observed semidominant maturation of shoot growth similar to that of tomato; however, this dosage effect failed to translate into cumulative heterosis, because

there is only one flowering event that can sense the change in florigen dosage compared to the multiple sympodial flowering transitions in tomato. Our cross-species findings provide a framework for further exploration of single-gene overdominance in which localized dosage effects of highly pleiotropic growth factors such as florigen gradually progress into global transgressive effects.

On the basis of the finding that suppressing *sp* with flowering mutations such as *sft* can improve yield through mutant heterozygosity, we have characterized new flowering mutants that suppress *sp*. Importantly, these mutants also produce a semideterminate growth habit with more inflorescences and flowers like *sft*/+ heterozygosity. Two of these *suppressor of sp* (*ssp*) mutants have been found to result from defects in a transcription factor that physically interacts with florigen in the SAM to induce flowering. A third mutant was found to be a new weak allele of *SFT*, which leads to a plant that is only mildly late flowering, causes reversion to a typical three-leaf sympodial growth structure, and increases flower and fruit production in each inflorescence. On the basis of the principle that heterosis can be caused by heterozygous mutations in flowering genes, our *ssp* mutants provide a new set of tools to create semideterminate tomato varieties that can boost yield both in the field and under greenhouse conditions. In addition to mixing and matching our mutants in different homozygous and heterozygous combinations to generate novel shoot architectures not found in existing breeding germplasm, we are also exploring the molecular basis for *SSP*-induced semideterminacy by profiling gene expression dynamics of each new mutant by RNA-Seq during meristem maturation. Early results suggest that differences in the local balance of *SP* activity relative to both *SFT* and *SSP* are crucial for modulating quantitative differences in determinacy among different types of tomato meristems. Future work will focus on investigating the potential breeding value of all tomato flowering mutants in diverse commercial tomato types.

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EPIGENETIC INHERITANCE AND REPROGRAMMING IN PLANTS AND FISSION YEAST

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Plants and fission yeast provide excellent model organisms to address the question of how epigenetic information is propagated to daughter cells, including transposon regulation, heterochromatic silencing, and gene imprinting, important both for plant breeding and for human health. We are investigating the role of RNA interference (RNAi) in the inheritance of heterochromatic silencing in the fission yeast *Schizosaccharomyces pombe* and in the plant *Arabidopsis thaliana*, as well as continuing our work on *Zea mays*. In fission yeast, we have found additional evidence that DNA replication promotes spreading of heterochromatin and that RNAi promotes replication by releasing RNA polymerase II (RNA pol II). In plants, we have found that the genome undergoes reprogramming of DNA methylation in pollen guided by DNA glycosylases and small RNA. Our results suggest a model for imprinting, transposon control, and the origin of epialleles. In plant genomics, we have completed the methylome of maize, and demonstrated its impact on epigenetic inheritance and gene expression. We have also identified the gene rearrangement underlying the classical mutant of maize, *Tunicate 1*, which has been the subject of controversy in connection with the origin of corn. We continue to develop duckweeds as a source of biofuel and have characterized the genome of *Lemna gibba* as well as genes involved in lipid accumulation. This year, we said good-bye to Chunlao Tang, Patrick Finigan, and Antoine Hocher. We welcomed visiting fellow Atsushi Shimada, postdocs Hyun-Soo Kim, Filipe Borges, and Seung-Cho Lee, and master's student Alex Canto Pastor.

RNAi Promotes Heterochromatic Silencing and Spreading through Transcription, Replication, and Repair

J. Ren, S. Castel, H.-S. Kim, A.-Y. Chang, B. Roche [in collaboration with B. Arcangioli, Institut Pasteur, Paris, France; Z. Cande, University of California, Berkeley]

Heterochromatin comprises tightly compacted repetitive regions of eukaryotic chromosomes and has widespread roles in chromosome integrity, stability, and silencing. The inheritance of heterochromatin requires RNAi, which guides histone modification during the DNA replication phase of the cell cycle. However, the underlying mechanism is poorly understood. In *S. pombe*, the alternating arrangement of origins of replication and noncoding RNAs transcribed during S phase in the heterochromatic pericentromeric region provokes the collision of RNA polymerase with replication machinery. We propose that it is resolved by cotranscriptional RNAi, allowing replication to complete and couple the spreading of heterochromatin with fork progression. In the absence of RNAi, stalled forks are repaired by homologous recombination without histone modification. The molecular basis of this model and its genome-wide impact will be further investigated and may explain the participation of RNAi and DNA

replication in heterochromatin inheritance in other systems.

Programmed Replication Fork Pauses Control Imprinting and Spreading in Fission Yeast

S. Castel [in collaboration with M. Zaratiegui, Rutgers University; B. Arcangioli, Institut Pasteur, Paris; S. Lambert, Institut Curie-CNRS, Orsay, France; Y. Shi, Harvard Medical School]

In the fission yeast *S. pombe*, a chromosomal imprinting event controls the asymmetric pattern of mating-type switching. The orientation of DNA replication at the mating-type locus is instrumental in this process. However, the factors leading to imprinting are not fully identified and the mechanism is poorly understood. We have found that the replication fork pause at the *mat1* locus (MPS1), essential for imprint formation, depends on the lysine-specific demethylase Lsd1. We have demonstrated that either Lsd1 or Lsd2 amine oxidase activity is required for these processes, working upstream of the imprinting factors Swi1 and Swi3 (homologs of mammalian Timeless and Tipin, respectively). We have also shown that the Lsd1/2 complex controls the replication fork terminators found within the rDNA repeats. These findings reveal a role for the Lsd1/2 demethylases in controlling polar replication fork progression, imprint formation, and subsequent asymmetric cell divisions. Previously, we demonstrated that Lsd1 is localized at heterochromatin boundaries and is required to prevent spreading of pericentromeric heterochromatin into the surrounding euchromatin. A role in replication fork pausing may account for this activity.

Reprogramming of DNA Methylation in Pollen Guides Epigenetic Inheritance via Small RNA

J.P. Calarco, F. Borges, M.T.A. Donoghue, F. Van Ex [in collaboration with J. Becker, J. Feijo Instituto Gulbenkian de Ciencia, Portugal; F. Berger, Temasek Life Sciences Laboratory, Singapore]

Epigenetic inheritance is more widespread in plants than in mammals, in part because mammals erase epigenetic information by germline reprogramming. We sequenced the methylome of three haploid cell types from developing pollen—the sperm cell, the vegetative cell, and their precursor, the postmeiotic microspore—and found that unlike in mammals, the plant germline retains CG and CHG DNA methylation. However, CHH methylation is lost from retrotransposons in microspores and sperm cells and restored by de novo DNA methyltransferase guided by 24-nucleotide small interfering RNA (siRNA), both in the vegetative nucleus and in the embryo after fertilization. In the vegetative nucleus, CG methylation is lost from targets of DEMETER (DME), REPRESSOR OF SILENCING 1 (ROS1), and their homologs. These targets include imprinted loci and recurrent epialleles that accumulate corresponding small RNA and are premethylated in sperm. Thus, genome reprogramming in pollen contributes to epigenetic inheritance, transposon silencing, and imprinting, guided by small RNA.

Widespread Transposon Control by miRNAs and Epigenetically Activated Secondary siRNAs in the Absence of DNA Methylation

K.M. Creasey, J. Simorowski, U. Ramu, M. Regulski [in collaboration with J. Kendall, Cold Spring Harbor Laboratory; B. Meyers, University of Delaware; V. Colot, IBENS, Paris, France]

Transposons in *Arabidopsis* give rise to abundant 21-nucleotide "epigenetically activated" small interfering RNAs (easiRNAs) in *DECREASE IN DNA METHYLATION1* (*ddm1*) and *DNA METHYLTRANSFERASE1 (met1*) mutants, as well as in pollen from wild-type plants, in which heterochromatin is lost during reprogramming. easiRNA biogenesis is dependent on *ARGONAUTE1* (*AGO1)*, *DICER-LIKE4 (DLC4),* and *RNA-DEPENDENT RNA POLYMERASE6 (RDR6*), resembling 21-nucleotide *trans*-acting siRNAs (tasiRNAs) and other secondary siRNAs in this respect. However, specificity of targeting has remained a mystery. We have found that specificity is provided by targeted cleavage of thousands of transposon transcripts by at least 50 microRNAs (miRNAs), some of which are themselves encoded by transposons, but most of which are highly conserved with well-known roles in plant development. Interestingly, the loss of easiRNAs in *ddm1 rdr6* is compensated by the gain of 24-nucleotide heterochromatic siRNAs (hetsiRNAs) and subsequent restoration of DNA methylation. This suggests that *RDR6*-directed easiRNA production acts antagonistically to *RDR2*-directed hetsiRNA production,

thereby inhibiting RNA-directed DNA methylation (RdDM) and transcriptional gene silencing. Widespread targeting of transposons may reflect the evolutionary origin of miRNA in genome surveillance.

Epigenetic Transposon Regulation in Response to Drought Stress

F. Van Ex, F. Borges, J. Calarco, A. Hocher, M. Donoghue

Both DNA methylation and histone modifications have been linked to drought stress response in different plant species. Recent work has also shown that heat stress can lead to the reactivation of previously silent retrotransposons. However, little is known about the regulation of transposons after a prolonged exposure to drought stress. The drought-related miRNA (miR845b) is conserved in monocots and dicots. Although miR845b is absent in normal leaf tissue in *Arabidopsis*, we have found that it is very abundant in sperm cells of pollen. Interestingly, target prediction of this miRNA reveals exclusively retrotransposons as putative targets. To assess whether miR845b actually regulates these transposons, we developed sensor lines consisting of a green fluorescent protein (GFP) gene transcriptionally fused to the sequence of the predicted transposon target. Consistent with the absence of miR845b, we were able to detect activity of the sensor line in leaf tissue. In pollen, GFP expression was limited to the VN and pollen cytoplasm. No activity of the sensor was detected in sperm cells, where levels of miR845b are abundant. Our preliminary findings through RNA-Seq analysis reveal a significant up-regulation of a specific copia retrotransposon in drought-stressed plants.

Epigenome Dynamics during the Cell Cycle in *Arabidopsis* and Maize

C. LeBlanc, C. Underwood, Y. Jacob [in collaboration with B. Thompson, G. Allen, and L. Hanley-Bowdoin, North Carolina State University]

Our aim is to understand how epigenetic marks are transmitted through DNA replication and cell division in plants by analyzing the *Arabidopsis* DNA methylome during the cell cycle (G_1, early S, mid S, late S and G_2). To do this, genomic DNA extracted from nuclei by fluorescence-activated cell sorting (FACS) was subjected to bisulfite treatment and conversion and sequenced on the HiSeq platform. We are also studying transcriptional activity during the cell cycle via RNA polII binding. We have performed RNA polII chromatin immunoprecipitation sequencing (ChIP-Seq) with DNA extracted from maize root tips at different stages of the cell cycle. We are also generating RNA-Seq libraries to analyze the maize transcriptome from different stages of the cell cycle in root tips. Finally, we are making histone H3K56ac ChIP-Seq libraries to determine whether this acetylation is enriched during S phase in maize.

The Sperm Cell-Specific Histone H3 AtMGH3 Reprograms the Euchromatic Epigenome in the Male Germline of *A. thaliana*

Y. Jacob, C. LeBlanc

Large-scale reprogramming events in the germline of eukaryotes reset the epigenome before fertilization and formation of the zygote. In the model plant *A. thaliana*, resetting of the epigenome in the male and female reproductive tissues has been shown to involve both changes in DNA methylation and histone exchange. We have analyzed the subgenomic deposition of the male-specific histone H3 AtMGH3/HTR10 (Fig. 1). AtMGH3 is expressed specifically in sperm cells during pollen development and is removed from the early embryo and the endosperm after fertilization. Our results suggest that deposition of AtMGH3 in the male germline is confined to the euchromatin. Disruption of heterochromatin in the *ddm1* mutant leads to mislocalization of MGH3 and/or structural defects in the nucleus of sperm cells (Fig. 1). Taken together, our results suggest that AtMGH3 reprograms euchromatin in the male germline and that DDM1 spatially controls the deposition of AtMGH3.

Pod Corn Is Caused by Rearrangement at the *Tunicate1* Locus

J.-J. Han [in collaboration with D. Jackson, Cold Spring Harbor Laboratory]

Pod corn (*Zea mays* var tunicata) was once regarded as ancestral to cultivated maize, and it was prized by

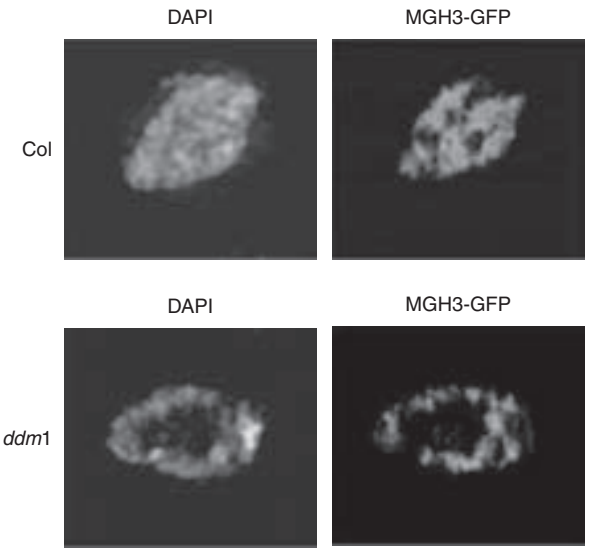

Figure 1. A mutation in *ddm1* disrupts the nuclear structure of sperm cells in *Arabidopsis*. We previously demonstrated that the chromatin remodeler DDM1 is strongly expressed in sperm cells of *Arabidopsis* pollen. Sperm cells from Col (wild-type) plants and *ddm1* mutant pollen expressing fusions of the male germ cell histone H3 (MGH3) with green fluorescent protein (GFP) were visualized using high-resolution OMX microscopy and stained for DNA using DAPI (4′-6-diamidino-2-phenylindole). The GFP signal is associated with euchromatin in wild type, but it is redistributed in *ddm1* mutants, along with DNA, to the periphery of the nucleus.

pre-Columbian cultures for its magical properties. *Tunicate1* (*Tu1*) is a dominant pod corn mutation in which kernels are completely enclosed in leaflike glumes. We have found that *Tu1* encodes a MADS box transcription factor normally expressed in leaves whose 5′ regulatory region is fused by a 1.8-Mb chromosomal inversion to the 3′ region of a gene expressed in the inflorescence. Both genes are further duplicated, accounting for classical derivative alleles isolated by recombination, and *Tu1* transgenes interact with these derivative alleles in a dose-dependent manner. In young ear primordia, TU1 proteins are nuclearly localized in specific cells at the base of spikelet pair meristems. *Tu1* branch determination defects resemble those in *ramosa* mutants, which encode regulatory proteins expressed in these same cells, accounting for synergism in double mutants discovered almost 100 years ago. The *Tu1* rearrangement is not found in ancestral teosinte and arose after domestication of maize.

The Maize Methylome Influences mRNA Splice Sites and Reveals Widespread Paramutation-Like Switches Guided by Small RNA

M. Regulski [in collaboration with J. Kendall, D. Ware, and J. Hicks, Cold Spring Harbor Laboratory; A. Rafalski and S. Tingey, Dupont Pioneer, Wilmington, Delaware]

The maize genome, with its large complement of transposons and repeats, is a paradigm for the study of epigenetic mechanisms such as paramutation and imprinting. We have determined the genome-wide map of cytosine methylation for two maize inbred lines, B73 and Mo17. CG (65%) and CHG (50%) methylation (where H = A, C, or T) is highest in transposons, whereas CHH (5%) methylation is guided by the 24-nucleotide siRNA, but not the 21-nucleotide siRNA. CG methylation in exons (8%) deters Mutator transposon insertion, whereas CHG methylation at splice acceptor sites appears to inhibit RNA splicing. Using the methylation map as a guide, we used low-coverage sequencing to show that parental methylation differences are inherited by recombinant inbred lines and influence gene expression. However, frequent methylation switches, guided by siRNA, persist for up to eight generations, suggesting that paramutation is much more common than previously supposed. The methylation map will provide an invaluable resource for epigenetic studies in maize.

Developing *Lemnaceae* (Duckweeds) as a Source of Hydrocarbon Biofuels

E. Ernst, S.-C. Lee, A. Molla-Morales, A. Pastor [in collaboration with D. Pappin, Cold Spring Harbor Laboratory; J. Shanklin and J. Schwender, Brookhaven National Laboratories; V. Citovsky, SUNY Stony Brook]

The aquatic monocotyledonous *Lemnaceae* are the fastest growing flowering plants, capable of producing 64 grams of biomass per gram starting weight in a week. They offer an attractive alternative to corn and algae as biofuel feedstocks because of their low lignin content, robust growth in open ponds, and excellent metabolic characteristics. *Lemnaceae* are cultivated aquatically, typically on animal wastewater, and thus they do not contribute to resource competition for the growth of food crops. In overwintering or starved

Lemnaceae plants, starch accumulation is comparable to corn kernels, up to 60%–75% of dry weight. We propose to design new strains of *Lemnaceae* to divert a substantial portion of accumulated carbon from starch to oil and alkane metabolism, as these products possess an energy density approximately eightfold higher than that of starch. We have sequenced the genome of *Lemna gibba*, and a database of gene annotation and a genome browser are available (http://www.lemna.org). Duckweed species have been reported to contain lipid up to 9% of dry weight, but there is limited information about composition of neutral lipids and enzymes responsible for their synthesis and degradation. We have characterized the proteome of lipid droplets in order to identify structural proteins and enzymes for synthesis and degradation of triacylglycerol in vegetative tissues of duckweeds. The composition of triacylglycerol (TAG) was analyzed using liquid chromatography–tandem mass spectrometry (LC-MS/MS) with isolated lipid droplets (LDs). We also tested whether environmental stresses increase expression of genes associated with TAG synthesis and LD production. We are currently developing methods to knock down expression of genes encoding enzymes involved in starch synthesis and TAG hydrolysis using artificial miRNA and transcriptome data.

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PLANT DEVELOPMENTAL GENETICS

M. Timmermans A. Benkovics, M. Dotto, C. Fernandez-Marco, A. Husbands, M. Javelle, M. Lodha, K. Petsch, E. Plavskin, D. Skopelitis

Specification of adaxial–abaxial (upper/lower) polarity in the developing leaf drives the flattened outgrowth of the leaf blade and directs the differentiation of distinct cell types within the leaf's adaxial/top and abaxial/bottom domains. Both are important innovations in the evolution of land plants that maximize photosynthesis while minimizing water loss to the environment. In addition to being a key developmental process, adaxial–abaxial patterning is of particular interest from a mechanistic point of view and has proven to be an excellent model to study small RNA-mediated gene regulation. We previously showed that patterning of this developmental axis involves a cascade of opposing small RNAs, in which microRNA (miRNA) miR390 triggers the biogenesis of the *TAS3*-derived 21-nucleotide tasiR-ARF on the adaxial side of developing leaves. These confine the accumulation of abaxial determinants, including AUXIN RESPONSE FACTOR3 (ARF3) and miR166, to the lower side of leaves. miR166, in turn, demarcates the abaxial side by repressing expression of class III homeodomain leucine zipper (HD-ZIPIII) transcription factors that specify adaxial fate.

Importantly, our analysis of the *TAS3 trans*-acting short interfering RNA (tasiRNA) pathway in *Arabidopsis* demonstrated that tasiR-ARF functions as a mobile positional signal in adaxial–abaxial patterning. Movement of this small RNA from its defined source of biogenesis in the two uppermost cell layers of leaves creates a gradient of accumulation that dissipates abaxially. The tasiR-ARF gradient generates a sharply defined expression domain of the abaxial determinant ARF3 on the bottom side of leaf primordia, suggesting that small RNAs can establish pattern through a morphogen-like activity. This work provided the first direct evidence that small RNAs are mobile and can function as instructive signals in development, thereby revealing a novel patterning activity of small RNAs. We are currently studying the role of this specific small RNA pathway and the properties of mobile small RNAs, in general, in maize, *Arabidopsis,* and the moss *Physcomitrella patens*.

Dissecting Small RNA Mobility in Plants

D. Skopelitis, C. Fernandez-Marco

Given the scope of miRNA-regulated gene networks, the cell-to-cell movement of small RNAs has important implications with respect to their potential as instructive signals in development or in response to physiological and stress stimuli. We are using artificial miRNAs targeting easy-to-score reporter genes to study parameters of miRNA movement, such as tissue specificity, directionality, dose dependence, and the kinetics of movement. To investigate miRNA mobility in multiple distinct developmental contexts, we are expressing an artificial miRNA targeting a cell-autonomous green fluorescent protein (GFP) reporter (miR-GFP) from a number of promoters with distinct spatiotemporal patterns of expression. Expression of miR-GFP from the epidermis-specific *ATML1* promoter, mesophyll-specific *RBCS* promoter, or vasculature companion cell-specific *SUC2* promoter showed that miRNAs move bidirectionally between all three cell layers and that the number of cells across which a small RNA moves is determined in part by its abundance. Moreover, analysis of GFP fluorescence in the vasculature supports the idea that miRNA-mediated gene regulation is a dose-dependent process and depends on the relative abundance of the miRNA versus the target mRNA. No GFP silencing was observed in the symplastically isolated stomata, suggesting that miRNA movement occurs via plasmodesmata, small channels that connect most plant cells.

Interestingly, production of miR-GFP in young leaf primordia surrounding the shoot apical meristem (SAM) resulted in silencing of GFP inside the SAM, demonstrating that miRNAs traffic from determinate organs into the shoot stem-cell niche. In addition, miR-GFP was able to move from the vasculature below the SAM into the niche. These findings suggest that miRNAs may function as mobile signals between differentiated tissues and stem cells, possibly

integrating environmental/physiological cues and plant development. Analyses of miRNA mobility in other developmental contexts are still ongoing. In addition, with the knowledge that miRNAs can traffic from the epidermis into underlying tissue layers, we are carrying out forward genetic screens to identify factors influencing this process.

Small RNA Gradients Create Stable Developmental Boundaries

A. Benkovics, D. Skopelitis, C. Fernandez-Marco

Mathematical modeling of the tasiR-ARF–ARF3 interaction predicts that small RNA gradients resulting from mobility are uniquely suited to generate sharply defined boundaries of target gene expression. To test this, we are using a line expressing a miR166-insensitive HD-ZIPIII reporter (PHB*-YFP) that is active throughout the leaf and induces an adaxialized leaf phenotype. In this background, we expressed a modified version of miR166 (miR166*) that specifically cleaves the PHB*-YFP transcripts. Given the importance of miRNA dosage to gene silencing, we are expressing miR166* in an inducible manner in the adaxial or abaxial epidermis. This allows us to quantitatively measure the relationship between miRNA gradients and the spatial patterning of their targets. The first results show that miR166* expression from the abaxial epidermis is sufficient to suppress the PHB*-YFP leaf defects, indicative of movement of this small RNA. Efforts to visualize the PHB*-YFP and miR166* expression domains are ongoing.

This question is also being addressed using a different reporter system. The *pARF3:ARF3-GUS* reporter in an *rdr6* mutant background, which lacks the tasiR-ARF gradient, is expressed throughout the developing leaf. In this background, we are expressing an artificial miRNA targeting *ARF3*, again from different leaf-specific promoters and in an inducible manner, to modulate the position, direction, and steepness of the small RNA gradient. Target gene expression is being monitored by GUS histochemistry, and *miR-ARF3* expression patterns will be determined by in situ hybridizations. The first observations suggest that small RNA gradients indeed generate sharply defined domains of target gene expression and provide evidence for dose dependence in the miRNA gradient–target gene interaction.

Considering that patterning of the adaxial–abaxial axis involves two opposing small RNA gradients, these might serve to confer robustness onto the leaf development program by stabilizing the adaxial–abaxial boundary throughout organogenesis and under a range of environmental conditions. Our preliminary results suggest that the adaxial–abaxial boundary as visualized by an abaxial reporter is less sharp in *sgs3* mutants, which lack the tasiR-ARF gradient, than in wild-type plants. We are also comparing variability in leaf parameters under normal and mild stress conditions between wild-type and *sgs3* mutant plants. The outcome of these experiments will reveal whether small RNA gradients provide robustness to the leaf developmental program under a range of environmental conditions.

The START Domain Regulates HD-ZIPIII Activity and Organ Polarity

A. Husbands [in collaboration with V. Yong and H. Djaballah, Memorial Sloan-Kettering Cancer Center, New York]

Based on our previous observations regarding the expression and function of miR166 and tasiR-ARF, it is evident that adaxial–abaxial patterning involves a cascade of positional signals. The mobile signals that pattern the newly formed leaf are distinct from those that maintain polarity during subsequent development. This project aims to identify additional signals in adaxial–abaxial patterning.

The adaxial promoting HD-ZIPIII transcription factors contain a predicted START lipid-binding domain. Modeling of the START domain of the HD-ZIPIII member PHABULOSA (PHB) suggests structural similarity to human PC-TP, a phosphatidylcholine-binding START domain. This model and sequence conservation with other START domains was used to predict amino acids in PHB critical for START domain ligand binding. To assess the importance of the START domain to PHB function, we generated transgenic *Arabidopsis* lines that express START domain variants of a PHB-YFP fusion protein. These PHB-YFP reporters also carry silent mutations in the miR166-target site, which normally leads to the formation of severely adaxialized leaves. Lines expressing a PHB-YFP variant in which several residues lining the ligand-binding pocket of the

START domain are mutated show a wild-type phenotype, as do lines that express a variant in which the START domain is deleted entirely. These findings reveal positive regulation of HD-ZIPIII activity by an unknown ligand and show that the START domain is required for proper function of HD-ZIPIII proteins.

Experiments to determine the mechanism through which the START domain controls HD-ZIPIII function are ongoing. Ligand binding does not appear to affect the subcellular localization of these transcription factors, as confocal imaging showed that the PHB-YFP derivatives correctly localize to the nucleus. We are currently investigating whether ligand binding affects PHB's ability to bind DNA, activate transcription, or to interact with required protein partners. As part of these analyses, we developed a yeast-based assay and conducted a high-throughput screen for chemical compounds that bind the HD-ZIPIII START domain and modulate protein activity. Using yeast growth as a readout, we identified compounds that reduced yeast growth (antagonists) and others that promoted growth (agonists). Validation experiments in yeast and plants are under way to eliminate false positives and demonstrate that these chemicals act via modulation of HD-ZIPIII activity. Finally, we have generated plants expressing a biotin-tagged form of the START domain and will be performing immunoprecipitations and mass spectrometry to identify the endogenous ligand bound by HD-ZIPIII proteins. These latter experiments will be carried out in early 2013 in collaboration with the CSHL proteomics facility.

tasiRNA Pathways in Maize and New Players in Leaf Polarity

M. Dotto [in collaboration with M. Hammell, Cold Spring Harbor Laboratory; M. Aukerman, M. Beatty, R. Meeley, DuPont-Pioneer]

Small RNAs control the activities of a wide variety of genetic elements, including genes that encode proteins, transposons, and viruses. These diverse functions are achieved by distinct classes of small RNAs generated through discrete genetic pathways. tasiRNA biogenesis is triggered when *TAS* transcripts are targeted by either a single 22-nucleotide miRNA (one-hit model) or two 21-nucleotide miRNAs (two-hit model). These miRNA-guided cleavage events trigger conversion of the tasiRNA precursor transcript into long double-stranded RNAs (dsRNAs) by RDR6 and SGS3/LBL1 and set the register for the subsequent production of phased 21-nucleotide tasiRNAs by DCL4. Only the *TAS3* pathway is conserved across land plant evolution. The binding of two miR390-loaded AGO7 complexes triggers the biogenesis of *TAS3*-derived tasiRNAs, a subset of which are the tasiARFs. We previously identified four *TAS3* loci in the maize genome; however, additional *TAS* loci described in other species are not conserved in maize. We used a deep-sequencing and bioinformatics approach to perform a genome-wide search for clusters of phased small RNA in combination with a comparison of the small RNA content between wild-type and *lbl1* mutant apices. This approach identified five novel *TAS* loci, all belonging to the *TAS3* family. No *TAS* loci triggered by 22-nucleotide miRNAs were identified. In-depth target analyses indicate that the tasiARFs are the only functional tasiRNAs in the maize vegetative apex, where they regulate expression of *ARF3* homologs. This study also revealed unexpected contributions for LBL1 in the regulation of miRNAs and 24-nucleotide transposon- and retrotransposon-derived small interfering RNAs (siRNAs), illustrating substantial crosstalk among small RNA pathways. The mechanisms underlying these regulatory networks remain to be elucidated.

In a separate approach, we are characterizing small RNAs associated with diverse AGO proteins. Using a specific peptide antibody, we analyzed the small RNAs associated with ZmAGO10 by deep sequencing the small RNAs present in immunoprecipitates. We found that the immunoprecipitation fraction was enriched for just a subset of small RNAs: miR394, miR166, and tasiARFs. We had previously shown the function of the latter two in the specification of maize leaf polarity. However, miR394 remains poorly characterized. In situ hybridization showed that this miRNA also accumulates in a polar pattern, establishing a gradient on the adaxial side of developing leaves. This presents the possibility that organ polarity is regulated also by a fourth small RNA signal. We are currently characterizing the maize single and double mutants in components of this pathway: *mir394a* and *mir394b*, the two F-BOX proteins predicted as targets, and both *ZmAGO10* genes. It was reported that AGO10 in *Arabidopsis* associates exclusively with

miR166. We are currently analyzing how structural differences between these miRNA precursors and the AGO10 proteins in both species account for the differential AGO10/miR394 binding in maize.

Effects of Natural Variation Present in Maize Inbreds on the tasiRNA Pathway

K. Petsch

Mutants that disrupt tasiRNA biogenesis exhibit defects in adaxial–abaxial patterning. Interestingly, the severity of these phenotypes is greatly dependent on the inbred background. tasiRNA pathway mutants, as well as other small RNA biogenesis mutants, typically exhibit weaker phenotypes when introgressed into B73 as opposed to other inbred backgrounds, such as W22, Mo17, and A619. To understand how inbred background influences expressivity of tasiRNA pathway mutants, we are using the severe *ragged1* allele of *leaf-bladeless1 (lbl1-rgd1)*. In the B73 inbred background, *lbl1-rgd1* conditions a classical leaf polarity phenotype with most leaves developing as radial, abaxialized organs. In W22, however, *lbl1-rgd1* embryos lack a shoot meristem and germinate with just a root. We have generated an F_2 population to map loci that contribute to the variable expressivity of *lbl1-rgd1* in the two inbreds. This identified a major modifier locus on chromosome 8, which we are fine-mapping in collaboration with DuPont. In addition, we are generating NIL populations to further define the effect of this locus on the *lbl1-rgd1* phenotype and to identify the causative gene.

In a complementary approach, we performed a transcriptome analysis of *lbl1-rgd1* embryos and nonmutant siblings in B73 and W22. This identified 214 genes that are differentially expressed in *lbl1-rgd1* embryos in both backgrounds. These include known tasiRNA targets and genes showing differential expression across the adaxial–abaxial axis, as well as genes predicted to function in the embryonic shoot meristem. Most notably, ~30% of genes that are downregulated in W22 mutant embryos have "meristem-associated" functions. Further investigation into specific genes is ongoing. Importantly, this study identified 40 genes within the chromosome-8 modifier region that are expressed in the developing embryo and show differential expression between the inbreds or between *lbl1-rgd1* and nonmutant siblings. By combining these approaches, we hope to gain a better understanding of the factors contributing to natural variation in tasiRNA pathway function.

Ancestral Role of the tasiRNA Pathway

E. Plavskin [in collaboration with R. Quatrano, Washington University, St Louis, Missouri; M. Hasebe, National Institute for Basic Biology, Okazaki, Japan]

The diversity of multicellular organisms raises the question of how so many varied morphologies evolved. A number of studies have demonstrated that novel structures often arise through the hijacking of existing developmental pathways for new functions. The pathways controlling leaf development seem to be no exception, as many of them have been shown to be conserved in the moss *Physcomitrella patens*, whose ancestors diverged from the lineage of flowering plants ~100 million years before leaves first evolved in the latter. Thus, studying the role of leaf polarity pathways in moss provides a unique opportunity to explore the evolution of complex novel structures.

We are focusing on the miR390-dependent tasiRNA pathway. The genes involved in biogenesis of tasiRNAs as well as the tasiRNA targets are conserved between maize, *Arabidopsis*, and *P. patens*. Elucidating the function of this pathway in *Physcomitrella* may lead to an understanding of its ancestral role in plant development. For this, we are taking advantage of the unique ability of this moss to be transformed by homologous recombination and characterizing knockouts of genes involved in tasiRNA biogenesis, as well as the targets of the moss tasiR-ARFs. Our results indicate a role for tasiRNA regulation in plant architecture during the filamentous stage of growth. *sgs3* loss-of-function mutants display increased branching and decreased formation of caulonema, long runners that allow the moss plants to rapidly spread (Fig. 1). These phenotypes are consistent with a defective response to the plant hormone auxin, which is evolutionarily more ancient than the land plant lineage itself. Further experiments demonstrated that tasiRNAs are important for setting a threshold for auxin response and that deregulation of their targets results in a perturbed response to nitrogen deprivation.

Much like filamentous runners in moss, formation of lateral roots in dicots is regulated by auxin and modulated by nitrogen levels in the substrate; in addition, a link between lateral root outgrowth and

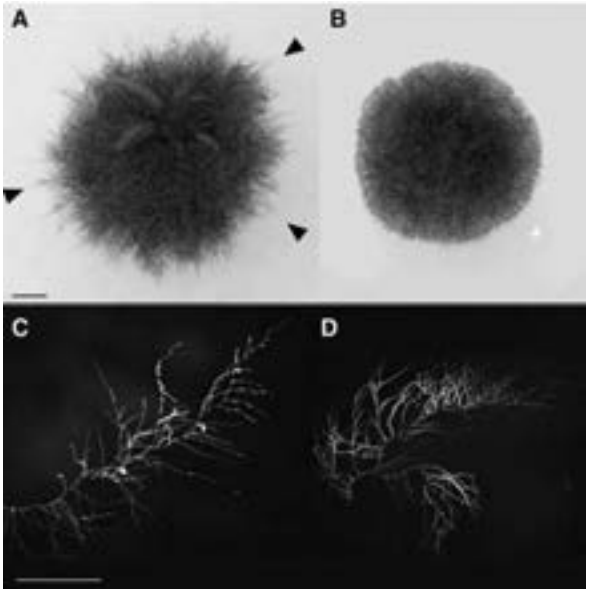

Figure 1. *Physcomitrella sgs3* mutants show altered filamentous tissue architecture. (*A,B*) Wild-type and *sgs3* plants after 15 days of growth on solid media. Caulonemal runners (arrowheads) are visible in wild-type plants (*A*) but absent in *sgs3* (*B*). Wild-type primary filaments (*C*) have dramatically fewer branches than in *sgs3* (*D*). Both the lack of caulonema and the failure to suppress branching in this tasiRNA biogenesis mutant suggest a perturbed auxin response. Scale bar, 1 mm.

tasiRNA biogenesis has recently been demonstrated. It is exciting to hypothesize that tasiRNAs and their targets may be part of an ancient mechanism for regulating plant architecture in response to auxin signaling and environmental inputs such as soil nutrient content. To address this experimentally, we are now exploring lateral root formation in *Arabidopsis* mutants with perturbed tasiRNA biogenesis.

High-Resolution Gene Expression Atlas for the Maize Shoot Apex

M. Javelle [in collaboration with M. Scanlon, Cornell University, Ithaca, New York; G. Muehlbauer, University of Minnesota, Minneapolis; J. Yu, Kansas State University, Manhattan; P. Schnable, Iowa State University, Ames]

Plants have the distinctive ability to form new organs throughout their lifetime, which can span hundreds or even thousands of years. The growing tip of a plant contains a population of stem cells that are located within a specialized niche, termed the shoot apical meristem (SAM). These stem cells divide to maintain the SAM and to generate daughter cells from which lateral organs, such as leaves and flowers, arise. The gene regulatory networks controlling meristem indeterminacy and organogenesis remain largely obscure. To gain insight into such networks, we have generated a high-resolution gene expression atlas for the maize shoot apex. Functional domains of the meristem were isolated by laser microdissection and analyzed by RNA deep sequencing. These include the whole meristem, the stem-cell-containing SAM tip, the newly initiating leaf (P0), the epidermal layer of the SAM (L1), and the subepidermal region (L2). Because gene networks controlling meristem maintenance and leaf development are largely interconnected, we further compared expression profiles in an ontogenic series of leaf primordia, P1, P2, and P3. We identified 502 genes that show strong differential expression between the L1 and the L2. Using CAST clustering, we further identified genes that specifically mark the meristem, the P0 or developing leaf primordia. Most notably, this analysis identified 177 genes that specifically mark the stem cell domain of the meristem, and several genes that mark the presumptive organizing center. This comprehensive data set allows us to precisely predict genes involved in meristem maintenance, leaf initiation, and/or leaf patterning and to assess the distinct contributions of the L1 and L2 to these processes. Reverse genetic resources available for maize are being used to directly test the function of select genes in these processes.

Several known regulators of meristem indeterminacy and organ initiation are under the control of miRNAs. To gain insight into small RNA-controlled gene networks required for SAM function, we characterized the precise expression patterns of eight highly conserved plant miRNAs by in situ hybridization. Each shows a distinct expression pattern suggesting diverse contributions of small RNAs and the pathways they target to the regulation of SAM function. miRNAs appear to function predominantly during leaf initiation and leaf development, and only one miRNA has been shown to function in the stem-cell region of the SAM. Using the gene expression atlas, we analyzed the expression domains of individual precursor genes. Only 41 out of 144 precursor genes are expressed within the shoot apex, and their level of expression generally shows a good correlation with the level of the corresponding mature miRNA. However, a few exceptions highlight instances of more complex

regulation of miRNA processing, stability and/or mobility. To complete our understanding of miRNA action within the shoot apex, we are currently overlaying these small RNA expression patterns with expression data from validated miRNA target genes. This rich resource will be used to guide functional genomics experiments to address the role of new candidate genes in meristem function.

Establishment of Determinacy during Lateral Organ Development

M. Lohda

Stem cell activity in the SAM is maintained in part by the class I *KNOX* homeobox genes. To give rise to differentiating structures, such as leaves, *KNOX* expression needs to be maintained in a stable "off" state throughout lateral organ development. We had previously shown that this process is mediated by the transcription factors AS1 and AS2, which form heterodimers that bind specific sites in the *KNOX* promoters. We have now uncovered the mechanism via which AS1-AS2 maintain the stable repression of *KNOX* genes during leaf development.

We found that *KNOX* gene silencing also requires the activities of the Polycomb repressive complexes, PRC2 and PRC1. PRC2 has histone H3K27 trimethylation (H3K27me3) activity, and we have shown that this repressive chromatin mark is enriched at the *BP* and *KNAT2* loci in leaves of *Arabidopsis*. Mutations in the PRC2 component CURLY LEAF (CLF) lead to reduced levels of this repressive H3K27me3 mark at the *KNOX* loci and to ectopic *KNOX* expression in developing leaves. PRC1 is a downstream component in the Polycomb pathway that maintains long-term silencing. In plants, this complex contains LIKE HETEROCHROMATIN PROTEIN1 (LHP1), which recognizes the H3K27me3 signature deposited by PRC2. We found that LHP1 is present at the silenced *KNOX* loci and that mutations in *LHP1* lead to *KNOX* misexpression in developing leaves. Importantly, levels of H3K27me3 and LHP1 at the *KNOX* loci are dramatically reduced in *as1* and *as2* mutants, identifying these DNA-binding proteins as upstream components in the *KNOX* silencing pathway. We were further able to show that AS1 and AS2 interact physically with PRC2 and that the AS1-AS2 complex mediates the recruitment of PRC2 to the *KNOX* promoters.

Genome-wide H3K27me3 profiling studies predict that as many as 20% of *Arabidopsis* genes are marked with H3K27me3 and regulated by PRC2. However, how PRC2, which does not bind DNA specifically, recognizes defined targets remained a major outstanding question in the field. This work showed that PRC2 is recruited to select targets by the sequence-specific DNA-binding factors AS1 and AS2. This recruitment mechanism resembles the Polycomb response element (PRE)-based recruitment of PRC2 originally defined in flies and provides the first such example in plants. Moreover, these data provide a framework for the cellular memory system underlying the somatically heritable repression of stem-cell-promoting homeobox genes that is required for cellular differentiation in leaves.

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GENOMICS

Thomas Gingeras and colleagues study where and how functional information is stored in genomes. These efforts help explain the biological and clinical effects of disease-causing gene mutations in humans and other organisms. Gingeras is a leader of the ENCODE (ENCyclopedia of DNA Elements) and the mouseENCODE and modENCODE (model genome ENCODE) projects of the National Institutes of Health. His research has altered our understanding of the traditional boundaries of genes, revealing that almost the entire lengths of genomes in organisms ranging from bacteria to humans can be transcribed into RNA (pervasive transcription) and that most RNA products made by a cell are not destined to be translated into proteins (noncoding, or ncRNAs). In fact, ncRNAs are proving to be involved in a variety of other important biological functions. Some have been shown to be critical components in the pre- and posttranscriptional and translational processes*, as scaffolds upon which large protein complexes are assembled and as extracellular signals*. The initial studies that led to these observations were this year extended to cover the entire human genome.

There has been a growing appreciation in recent years that gene function is frequently context-dependent, with a large part of that context provided by the activities of other genes. But trying to understand how genes interact to produce function is a hugely complicated problem and one that appears likely to become more so as genomic information becomes more detailed. **Jesse Gillis** and colleagues are computational biologists who are presently challenging an oft-taken approach to the problem in which the functions of genes are interpreted in the context of networks derived from gene association data. Such networks consist of millions of interactions across thousands of genes, derived from protein-binding assays, RNA coexpression analysis, and other sources. Historically, many attempts to understand gene function through networks have leveraged a biological principle known as "guilt by association." It suggests that genes with related functions tend to share properties (e.g., physical interactions). In the past decade, this approach has been scaled up for application to large gene networks, becoming a favored way to grapple with the complex interdependencies of gene functions in the face of floods of genomics and proteomics data. Gillis' work centers on identifying the limits of the approach and making fundamental improvements to its operation, as well as applying those improvements to neuropsychiatric gene network data.

Gholson Lyon's lab focuses on analyzing human genetic variation and its role in severe neuropsychiatric disorders and rare diseases, including Tourette syndrome, ADHD, obsessive compulsive disorder (OCD), intellectual disability, autism, and schizophrenia. By recruiting large groups of related individuals living in the same geographic location, for example, Utah, his lab can study the breadth and depth of genetic variants in a similar environmental background. Utilizing the exome, the parts of the genome that code for protein, and whole-genome sequencing, his lab looks for mutations that segregate with syndromes in the various populations. A second focus of the Lyon lab is to study the mechanistic basis of a new rare disease that they described in 2011. This is the first human disease involving a defect in the amino-terminal acetylation of proteins, a common modification of eukaryotic proteins carried out by amino-terminal acetyltransferases (NATs). The team has been using several different cellular model systems to better understand the disease pathophysiology and the basic process of amino-terminal acetylation.

The insights of **W. Richard McCombie** and colleagues have led to the introduction and optimization of novel methods of high-throughput genome sequencing. His team has made it possible to catalog variation among individual organisms in a way that would have been unthinkable 5 years ago. They have brought online a new generation of Illumina sequencers and optimized their

function to a level at which eight to 10 trillion DNA bases can be sequenced in a month. McCombie's team has been involved in international efforts culminating in genome sequences for maize, rice, and this year for bread wheat, three of the world's most important food crops. They have also had an important role in projects to sequence the flowering plant *Arabidopsis thaliana*, the fission yeast *Schizosaccharomyces pombe,* as well as *Homo sapiens* and other important genomes. McCombie's group is currently involved in several important projects to resequence genes in patient samples that are of special interest to human health, including *DISC1*, a strong candidate gene for schizophrenia, looking for genetic variants implicated in bipolar illness and major recurrent depression. With Sloan-Kettering Cancer Center, they are using a method called hybrid selection, developed with Greg Hannon, to look at mutations in samples collected from patients with prostate cancer.

Using multidisciplinary approaches that combine computational analysis, modeling and prediction with experimental verification, **Doreen Ware**'s lab seeks a deeper understanding of the evolution of genome sequences in plants and their implications for agricultural improvement. By looking comparatively across the genomes of plants in the same lineage, they seek answers to the following questions: How are genes conserved and lost over time? What are the fates of duplicated genes? What is the impact of structural variation on phenotypic variation? Ware's team also studies gene regulation in plants, focusing on gene regulatory networks, targeting transcription factors and microRNA genes with the objective of understanding how these parts of the plant genome work together in determining spatial and temporal expression of genes. The lab had an important role in the project to produce a haplotype map reference genome of maize, spearheading the most comprehensive analysis of the crop yet. This has provided important information on the variation of the reference genome, as well as comparative data showing changes in the genome acquired through domestication and breeding. They have devoted special attention to examining diversity within maize, grape, and tomato, aiming to accelerate the development of strategies to introduce new germplasm that is needed to meet demands of increasing population and a changing environment. The lab also has brought fully sequenced genomes into an integrated data framework, to enhance the power of their comparative studies. This past year, Ware was named as its principal investigator for the National Science Foundation-funded Gramene project, a comparative genomics resource for agriculturally important crops and models to support sustainable food and fuel production. Ware, as principal investigator for plants, has also helped lead an effort funded by the Department of Energy to the create out of many separate streams of biological information a single, integrated cyber-"knowledgebase" for plants and microbial life.

GENOME ORGANIZATION AND REGULATION AND FUNCTIONAL ROLES OF NONCODING RNAS

T.R. Gingeras P. Batut, S. Chakrabortty, C. Davis, A. Dobin, J. Drenkow, M. Fastuca, S. Jha, W. Lin, F. Schlesinger, L.-H. See, C. Xue, C. Zaleski

Human-ENCODE Project

P. Batut, S. Chakrabortty, C. Davis, A. Dobin, J. Drenkow, M. Fastuca, S. Jha, W. Lin, F. Schlesinger, L.-H. See, C. Xue, C. Zaleski

Living cells make many types of primary and processed RNAs that are found either in specific subcellular compartments or throughout cells. A complete catalog of these RNAs is not yet available and their characteristic subcellular localizations are also poorly understood. Because RNA represents the direct output of the genetic information encoded by genomes and a significant proportion of a cell's regulatory capabilities are focused on its synthesis, processing, transport, modification, and translation, the generation of such a catalog is crucial for the identification and characterization of functional genomic regions.

Our laboratory has been involved in all three phases of the Encyclopedia of DNA Elements (ENCODE) project (2002–present). This project endeavors to identify and characterize the functional elements present in the human genome sequence. The raw and mapped data and elements for the 5 years of phase II of ENCODE are available at the ENCODE Data Coordination Center, DCC (www.genome.ucsc.edu/ENCODE/dataSummary.html). These data, as well as additional data on all intermediate processing steps, are available on the RNA Dashboard: www.genome.crg.cat/encode_RNA_dashboard. This genome-wide compilation of subcellular localized and product-precursor-related RNAs has recently been published (see *Nature* 2012) and also can be found in the four manuscripts listed below. These data serve as a public resource and reveal new and detailed facets of the RNA landscape that include the following:

- A total of 62.1% and 74.7% of the human genome is transcribed into either processed or primary transcripts, respectively, with no cell line showing more than 56.7% of the union of the expressed transcriptomes across all cell lines. The consequent reduction in the length of intergenic regions leads to a significant overlapping of neighboring gene regions and prompts a redefinition of a gene.
- Isoform expression by a gene does not follow a minimalistic expression strategy, resulting in a tendency for genes to express many isoforms simultaneously, with a plateau at ~10–12 expressed isoforms per gene per cell line.
- Coding and noncoding transcripts are predominantly localized in the cytosol and nucleus, respectively, with a range of expression spanning six orders of magnitude for polyadenylated RNAs and five orders of magnitude for nonpolyadenylated RNAs.
- Approximately 6% of all annotated coding and noncoding transcripts overlap with small 5-capped and -uncapped RNAs and are likely to be precursors to these small RNAs. The subcellular localizations of both annotated and unannotated short RNAs are highly specific.
- We have contributed to the GENCODE consortium a most complete human long noncoding RNAs (lncRNA) annotation to date, produced within the framework of the ENCODE project and comprising 9277 manually annotated genes producing 14,880 transcripts. Our analyses indicate that lncRNAs are generated through pathways similar to those of protein-coding genes, with similar histone modification profiles, splicing signals, and exon/intron lengths. In contrast to protein-coding genes, however, lncRNAs display a striking bias toward two-exon transcripts; they are predominantly localized in the chromatin and nucleus, and a fraction appear to be preferentially processed into small RNAs.
- Analysis of the RNA from subcellular fractions obtained through RNA-Seq in the cell line K562 revealed that, consistent with cotranscriptional

spliceosome assembly and splicing, we have found significant enrichment of spliceosomal small nuclear RNAs (snRNAs) in chromatin-associated RNA compared with other cellular RNA fractions and other nonspliceosomal snRNAs. These data are in agreement with a ''first-transcribed, first spliced'' rule, yet more downstream exons carry other characteristics, favoring rapid cotranscriptional intron removal.

Model Genome (mod-) ENCODE: Human–Fly–Worm Comparative Analyses of Transcriptomes

C. Davis, A. Dobin, C. Xue, C. Zaleski

A major effort to compare the transcriptional characteristics of *Drosophila* and *Caenorhabditis elegans* to those of the human transcriptome has been under way during the last year. In collaboration with members of the modENCODE groups, detailed analyses have resulted in a significant set of insights on the similarities and differences observed in the transcriptomes of each of the three species. The results of these comparative analyses are currently being summarized in a manuscript that is in preparation.

Mouse-ENCODE Project: Human–Mouse Comparative Analyses of Transcriptomes

C. Davis, A. Dobin, C. Xue, C. Zaleski

Approximately 80% of the human genome can be associated with specific and individual biochemical activities, but it is still unclear which of these activities is specific to human biology. The mouse ENCODE consortium has endeavored to determine how much of these activities has been conserved in orthologous and syntenic regions. Genomic analyses for more than 100 mouse cell and tissue types have been conducted. As a result, as with the human genome, a large fraction of the mouse genome is associated with transcriptional activities, transcription factors, or a chromatin state indicative of transcriptional regulatory function. Comparative analyses of the functional annotations in the human and mouse genomes show that there has been both considerable conservation and a surprisingly large degree of divergence in gene expression patterns, RNA splicing, chromatin organization, and transcriptional regulatory mechanisms since the last common ancestor of these two mammals. Interestingly, different groups of genes have a distinct rate of conservation in their expression patterns and a corresponding degree of divergence in their *cis*-regulatory sequences. A number of unannotated intergenic potentially enhancer regions are clearly conserved.

Software and Database Development

A. Dobin, C. Zaleski

Recent advances in sequencing technologies have made transcriptome analyses at the single-nucleotide level almost routine. Accurate alignment of high-throughput RNA-Seq data is a challenging and heretofore unsolved problem because of the noncontiguous transcript structure, relatively short read lengths, and constantly increasing throughput of the sequencing technologies. Several critical problems have been found to afflict previously published approaches, such as high mapping error rate for contiguous and especially spliced alignments; mapping biases (some types of alignments are artificially favored over others); low sensitivity for unannotated transcripts; poor scalability with the read length; restrictions in the number of junctions, mismatches and indels per read; inability to detect nonlinear transcripts such as chimeric RNAs; and, crucially, very high demands on computational resources owing to the low mapping throughput.

To align our large (exceeding 80 billion reads) ENCODE transcriptome RNA-Seq data set, we developed and published the spliced transcripts' alignment to a reference (STAR) software based on a previously undescribed RNA-Seq alignment algorithm that uses sequential maximum mappable seed search in uncompressed suffix arrays, followed by a seed clustering and stitching procedure. Unlike many other RNA-Seq mappers, STAR is not an extension of a short-read DNA mapper; it was developed as a stand-alone C^{++} code. STAR is capable of running parallel threads on multicore systems with close to linear scaling of productivity with the number of cores. STAR is very fast: On a modern but not overly expensive 12-core server, it can align 550 million 2×76-nucleotide reads per hour with the human genome, surpassing all other existing RNA-Seq aligners by a factor of 50. At the same time, STAR exhibits better alignment precision and sensitivity than other RNA-Seq aligners for both

experimental and simulated data. In addition to unbiased de novo detection of canonical junctions, STAR is capable of discovering noncanonical splices and chimeric (fusion) transcripts. Using Roche 454 sequencing of reverse transcriptase–polymerase chain reaction (RT-PCR) amplicons, we experimentally validated 1960 novel intergenic splice junctions with an 80%–90% success rate, corroborating the high precision of the STAR mapping strategy.

Importantly, STAR can align the full range of sequence lengths currently attainable by emerging third-generation sequencing technologies. To demonstrate STAR's ability to align long reads, we have mapped the long (0.5–5 kb) human mRNA sequences from GenBank. The accuracy of STAR alignments is similar to or higher than that of BLAT, which is the de facto standard expressed sequence tag (EST)/mRNA aligner. At the same time, STAR outperforms BLAT by more than two orders of magnitude in alignment speed, which is important for high-throughput sequencing applications.

The algorithm extensibility to long reads shows that STAR has a potential to serve as a universal alignment tool across a broad spectrum of emerging sequencing platforms. STAR can align reads in a continuous streaming mode, which makes it compatible with novel sequencing technologies such as the one recently announced by Oxford Nanopore Technologies. As these sequencing technologies and protocols evolve, new mapping strategies will have to be developed, and the STAR core algorithm will be able to provide a flexible framework to address emerging alignment challenges.

The infrastructure of the Gingeras' laboratory analyses has been dependent on the continued development and maintenance of the large databases collected for each of the ENCODE projects. Notable achievements in these areas include the following:

- Implemented a new database system to track sequencing samples and subsequent data files. TDM (transcriptome data manager) is a MySQL database with a web-based interface utilizing HTML, Javascript, Flash, and Apache web services.
- Continued management and organization of sequencing data consisting of "raw" fastq files and genome-mapped data files, including bam, bigwig, contig, and other formats in a growing repository almost 30 TB in size.
- Update of lab website to a "Google Site." Content was updated, and the Google Site implementation allows anyone (with permission) to access and update the site without programming knowledge.
- Migration of our sequencing production pipeline to the new CSHL compute cluster. All codes within the pipeline were updated to accommodate new resources and restrictions and fully tested with live data.

Novel Exon Detection by Hierarchical RNA-Seq Clustering

F. Schlesinger

The transcriptome, the collection of all RNAs, of human cells contains a complex population of RNAs in addition to protein-coding mRNAs and known structural noncoding RNAs. RNA-Seq—the analysis of the transcriptome using high-throughput DNA sequencing—is a powerful method to unravel this complexity and to study the whole transcriptome, which represents the crucial molecular intermediate between genome sequence and function. However, due to the interleaved and overlapping nature of RNA transcripts and isoforms in the genome, gaining an accurate picture of complex genomic regions from short-read sequencing is a major challenge.

To address this, we developed a signal segmentation method based on maximum likelihood read count statistics, hierarchical segmentation, and model selection. It can identify individual, potentially overlapping transcriptional elements (exons, transcription start sites, splice junctions, etc.) from different types of RNA-Seq data by finding nested regions of significant signal enrichment, without arbitrary conditions on length, expression level, or location in the genome. Applying these and other methods to the large data collections generated by ENCODE allowed them to identify a large number of new transcripts, especially in samples of nuclear and nonpolyadenylated RNAs. We are following up on these predictions by using high-throughput single-molecule full-length cDNA sequencing on the PacBio RS platform in combination with custom hybridization capture probes. This allows us to avoid the error-prone assembly of short reads and observe the full structure of individual transcripts directly.

In addition to identifying these novel transcripts themselves, a key question is what regulatory elements in the genome sequence control their transcription. To this end, we focused on the transcription start sites (TSS) of novel RNAs, outside of known promoter regions. We analyzed chromatin immunoprecipitation (ChIP) data and found cell-type-specific enrichment of histone marks and DNA-binding proteins, associated with active transcription, around the predicted TSS of the novel RNAs, confirming the predictions. Interestingly, the chromatin profiles at these TSS fall into two general classes: The first, showing the canonical promoter histone signature, includes many internal and divergent antisense transcripts as well as thousands of long intergenic RNAs. The others show a more enhancer-like chromatin state and often overlap predicted or experimentally confirmed enhancers. These transcripts, from a subset of enhancer RNA (eRNA) regions, occur specifically in cell types in which the enhancer is active and are linked to differential DNA methylation of these intergenic regulatory elements. By integrating the different types of ENCODE RNA data, we could characterize in detail the properties of eRNA transcription start sites, which are specifically and directionally positioned at cryptic promoter-like sites within the enhancer region, as well as the properties of the RNA itself. In collaboration with the Hannon lab here at CSHL, we analyzed how this intergenic transcription contributes to cell-type-specific DNA hypomethylation of the enhancers and their epigenetic state during cell differentiation.

Transposons and Evolution of Transcriptional Regulation in the *Drosophila* Clade

P. Batut, A. Dobin

Regulatory changes are thought to have a central role in the evolution of development and cellular physiology, as well as in disease. Consistent with this, the regulatory circuitry underlying biological processes is very fluid on evolutionary timescales. This has been illustrated by multispecies studies of transcription factor binding, which have revealed strikingly fast dynamics of binding-site evolution. The evolution of other types of regulatory elements and the impact of such changes on transcriptional programs are currently the focus of intense investigation.

To explore transcriptional regulation in an evolutionary framework, we have developed RAMPAGE (RNA annotation and mapping of promoters for the analysis of gene expression), a high-throughput approach for genome-wide promoter discovery, quantitative promoter expression profiling, and transcript characterization. On the basis of 5′-complete cDNA sequencing, RAMPAGE offers exceptional promoter specificity, large-scale multiplexing capabilities, and accurate transcript quantification. Promoter activity profiling throughout the full *Drosophila melanogaster* life cycle has revealed widespread use of alternative promoters implementing distinct regulatory programs. Indeed, more than 40% of all expressed genes were found to have at least two promoters, and these generally had very distinct expression dynamics. Transposable elements, long proposed to have a central role in the evolution of their host genomes through their ability to regulate gene expression, contribute at least 1300 promoters shaping the developmental transcriptome of *D. melanogaster*. The co-option of transposons to distribute stereotyped regulatory modules throughout genomes is a clear example of what may be called regulatory recycling mechanisms.

Transcriptional profiling throughout embryogenesis, at high resolution and in multiple species, has expanded our view of recycling processes by revealing that species-specific transcription start sites are often created by conserved promoters that become bidirectional. Together, transposons and bidirectional promoters account for ~25% of *D. melanogaster* promoters that are not shared with *D. erecta*. Current analyses of two additional species (*D. simulans* and *D. pseudoobscura*) are shedding further light on the evolutionary dynamics of these phenomena. Although the internal organization of core promoters is well conserved across the *Drosophila* clade, there is significant plasticity in the genomic landscape of promoters: The birth and death of these elements are both very active evolutionary processes. The developmental expression profiles of individual genes are highly plastic as well, which is in agreement with recent studies in *Drosophila* and mammals. Strikingly, these analyses have revealed more than 1000 promoters driving the expression of noncoding RNAs that are functionally conserved in all four species, which last shared a common ancestor

more than 25 million years ago. As a class, these promoters also show clear hallmarks of purifying selection at the sequence level, which unambiguously establishes their functionality and biological relevance.

RNA-Mediated Intercellular Signaling

S. Chakrabortty, A. Dobin

Intercellular transference of RNA through exosomes has recently emerged as a possible novel mode of communication between cells and tissues of multicellular organisms. Exosomes are small 40–100-nm vesicles of endocytic origin that are secreted into the extracellular milieu upon fusion of multivesicular bodies (MVB) to the plasma membrane. Not only are exosomes secreted by many cultured mammalian cells investigated, they also appear to be abundantly present in various body fluids such as serum, plasma, urine, saliva, and cerebrospinal fluid. Recently, exosomes have been shown to contain RNA molecules, including mRNA and microRNA (miRNA).

We have characterized short RNAs isolated from exosomes, and their parent K562 whole cells were sequenced in duplicate. Approximately 1000 and 1250 small RNA genes from exosomes and whole cells, respectively, can be detected with 90% or higher reproducibility. One particular category, called miscellaneous RNA, was more abundant in exosomes than in their source cells. Miscellaneous RNA comprises mainly four classes of RNA, namely, 7SK RNA, 7sl RNA, Vault RNA, and Y RNA. Y RNA, more specifically, Y5 RNA, was determined to be the dominant component of miscellaneous RNA present in exosome. Together, Y RNAs and miRNA are the two most enriched classes of short RNAs in exosomes, constituting ~70% of the enriched small RNA population in exosomes. A second class of RNAs enriched in exosomes is miRNAs, which map as an exact copy of the mature 21-nucleotide miRNA (Guide) sequence. Interestingly, many of the exosomal miRNAs have been previously identified as regulating oncogenes (e.g., Mir17-92 cluster) and tumor suppressors (e.g., Mir193b) with established roles in cancer.

The capability of exosomes to mediate intercellular RNA transfer has been unambiguously demonstrated by two different approaches. First, lipid labeling of cell membranes followed by live-cell time-lapse microscopy was performed using dyes PKH67 and PKH26 (Sigma). Live-cell time-lapse imaging revealed the presence of red vesicles (from donor cells) within green recipient cells, consistent with exosome transfer. Confocal microscopy combined with Z-stacking analysis was performed to confirm that the red vesicles were internalized by the recipient cell and not merely sticking to the cell membrane. Second, a RNA-Seq-based approach was used to examine the ability of exosomes to transfer RNA into another cell. Human K562 cells were cocultured with mouse B-lymphocyte cells (HB-84, ATCC) in a transwell culture across a membrane of 1-μm pore size for 24 and 48 h (in duplicate per time point). As a parallel approach, preisolated K562 exosomes were directly added to mouse HB-84 cells and incubated for 24 and 48 h (in duplicate per time point). Short RNAs were extracted from the recipient mouse cells and analyzed by RNA-Seq. The data were mapped to a combined genome of human and mouse. Reads that mapped to the mouse genome as well as to both the human and mouse genomes were discarded, and only those reads that were uniquely mapped to human genome were used for further downstream analysis. A total of 464 and 892 annotated human short RNA genes were reproducibly detected in the mouse cells after 24- and 48-h coculture, respectively. Although all the categories of annotated short RNA genes that were previously identified in exosomes were detected in each assay and time points, transfer RNAs were found to be most abundant among them.

Although several annotated classes of short RNAs were found to be enriched in exosomes compared to their source cells, the regulatory machinery in the cell involved in sorting RNA to these extracellular vesicles is unknown. We hypothesized that specific RNA-binding proteins may be involved in this enrichment process. To address this question, mass spectrometric analyses of purified K562 exosomes (two bio-reps) were performed. A total of 462 proteins were reproducibly detected between the replicates, of which 101 are characterized as having RNA-binding protein properties along with other functional associations. These proteins constitute an interesting collection of candidates for further analyses to determine their possible role in RNA enrichment in exosomes or as convenient biomarkers for the extracellular vesicles.

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Sudipto Chakrabortty

GENE NETWORK ANALYSIS

J. Gillis

My research centers on using computational methods to understand gene function. Computational biology has taken up the challenge of determining gene function mainly by attempting to interpret the activities of genes in the context of networks derived from gene association data. As data sets characterizing genes grow in size and complexity, it seems self-evident that computation can assist in inference as to gene function. Gene network analysis intended to provide insight into complex disorders is a dominant interest in the field. Gene associations (of various sorts) are believed to encode functional interaction, and this interaction is frequently shown to be able to substantially predict gene function across all functions. This approach, commonly called "guilt by association" (GBA), is embedded in everything from prioritization of de novo variants to uncovering novel molecular phenotypes or mechanisms of disease. My research focuses on identifying limitations in the GBA approach and making fundamental improvements to its operation for the interpretation of neuropsychiatric genomics data. I joined CSHL in June 2012. Postdocs Wim Verleyen and Sara Ballouz will be joining my lab in early 2013.

"Guilt by Association" Is the Exception Rather Than the Rule in Gene Networks

In the past 10 years, GBA has been scaled up for application to large gene networks, becoming a favored way to grapple with the complex interdependencies of gene functions in the face of floods of genomics and proteomics data. However, there is a growing realization that scaled-up GBA is not a panacea. In this study, we report a precise identification of the limits of GBA and show that it cannot provide a way to understand gene networks in a way that is simultaneously general and useful. Our findings indicate that the assumptions underlying the high-throughput use of gene networks to interpret function are fundamentally flawed, with wide-ranging implications for the interpretation of genome-wide data. We show that functional information within gene networks is typically concentrated in only a very few interactions whose properties cannot be reliably related to the rest of the network. In effect, the apparent encoding of function within networks has been largely driven by outliers whose behavior cannot even be generalized to individual genes, let alone to the network at large. Although experimentalist-driven analysis of interactions may use prior expert knowledge to focus on the small fraction of critically important data, large-scale computational analyses have typically assumed that high-performance cross-validation in a network is due to a generalizable encoding of function. Because we find that gene function is not systemically encoded in networks, but dependent on specific and critical interactions, we conclude it is necessary to focus on the details of how networks encode function and what information computational analyses use to extract functional meaning. We explore a number of consequences of this and find that network structure itself provides clues as to which connections are critical and that systemic properties, such as scale-free-like behavior, do not map onto the functional connectivity within networks.

Assessing Identity, Redundancy, and Confounds in Gene Ontology Annotations Over Time

The Gene Ontology (GO) is a key means by which systems biologists operationalize gene function, making it a heavily relied upon tool in innumerable analyses and data interpretation exercises. GO annotations are often used as a gold standard, but the approach has widely appreciated imperfections. Ironically, it is very difficult to assess the properties of GO itself, since there is no other comprehensive gold standard against which to hold it. Broadly speaking, assessment of GO has focused on three distinct attributes: the accuracy of annotations assigned to GO, GO's structure independent of annotation, and the utility of GO and its annotations for the interpretation of data. Despite misgivings about the incompleteness of GO annotations, the use of GO "sets"

as representing "functions" is now endemic. This is put to use in numerous applications such as "gene group enrichment," gene network analysis, and gene function prediction. It is essential to understand the extent to which such applications are valid.

The statement "the differentially expressed genes were enriched for genes with functions in cell growth" does not necessarily mean the same thing today as it did 5 years ago, because the definition of "cell growth genes" has changed in GO. Valid experimental results often become obsolete over time, but the reported facts of the experiment should not. But that is what happens when GO changes. This is of course to be expected, and the problem can be ameliorated by reporting which version of the gene annotations was used. We find that genes can alter their "functional identity" over time, with 20% of genes not matching to themselves (by semantic similarity) after 2 years. We consider ensuring independence of GO from the data sets to which it is being applied as an absolute minimum standard, and our results show that at least some protein interaction data do not meet this standard. We discovered that many entries in protein interaction databases are due to the same published reports that are used for GO annotations, with 66% of assessed GO groups exhibiting this confound. In our experience, among systems biologists, there seems to be a broadly appreciated disjunction between the true utility of GO and how often it is used, even if this is rarely acknowledged in the peer-reviewed literature. The use of GO annotations is often regarded as a minimally interesting validation of results, but not safe to use for discovery purposes. We believe the problems we have identified are among the underlying sources of these mixed feelings about GO. If it is "too easy" to obtain interesting results using GO, and those results do not consistently hold up, then GO's use for such purposes is limited.

Assessing the First Computational Gene Function Prediction Assessment

The computational assignment of gene function remains a difficult but important task in bioinformatics. The establishment of the first critical assessment of functional annotation (CAFA) was aimed at increasing progress in the field. We present an independent assessment of the results of CAFA, aimed at identifying challenges in assessment and at understanding trends in prediction performance. We found that well-accepted methods based on sequence similarity (i.e., BLAST) have a dominant effect. Many of the most informative predictions turned out to be either recovering existing knowledge about sequence similarity or "post-dictions" already documented in the literature. These results indicate that deep challenges remain in even defining the task of function assignment, with a particular difficulty posed by the problem of defining function in a way that is not dependent on either flawed gold standards or the input data itself. In particular, we suggest that using the GO (or other similar systematizations of function) as a gold standard is unlikely to be the way forward.

Schizophrenia Coexpression to Prioritize Candidate Variants

RNA coexpression data is commonly used to construct gene networks, but it is often considered to be more difficult to interpret than protein interactions. This is in part due to the lack of consensus on methods for constructing networks from expression profiles and the relatively poor performance of coexpression for function prediction, as measured by its ability to recapitulate data with GO, KEGG, or other databases. On the other hand, coexpression affords a major advantage over current large-scale protein interaction databases: It can be used to create "condition-specific" networks. Our preliminary results show that by appropriate consideration of data pretreatment, aggregation, and network construction, coexpression networks become a powerful tool for gene function analysis, on par with or better than protein interaction networks in terms of overall properties, while providing condition specificity. We leverage these properties to examine the role of de novo schizophrenia variants in the most comprehensive analysis to date of coexpression patterns in schizophrenia, combining seven studies of prefrontal cortex in affected individuals and unaffected controls.

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HUMAN GENETICS AND GENOMIC MEDICINE

G. Lyon M. Doerfel J. O'Rawe
P. Kota Y. Wu

Our laboratory focuses on analyzing human genetic variation and its role in severe idiopathic neuropsychiatric disorders. We do this by studying large pedigrees living in the same geographic location, where one can study the expressivity and segregation of variants in a similar environmental background and with fewer population stratification concerns. Toward this end, we collect pedigrees in Utah and elsewhere, and then use exome and whole-genome sequencing to find mutations that segregate with syndromes in the pedigrees. We focus on the discovery of families with rare diseases and/or increased prevalence of syndromes such as intellectual disability, autism, and schizophrenia.

X-Linked Malformation and Infantile Lethality Syndrome

M. Doerfel, Y. Wu [in collaboration with T. Arnesen, Norway]

We have previously identified a lethal X-linked disorder of infancy comprising a combination of distinctive craniofacial features producing an aged appearance, growth failure, hypotonia, global developmental delays, cryptorchidism, and acquired cardiac arrhythmias. The first family was identified in Ogden, Utah, with five affected boys in two generations of family members. A mutation was identified as a c.109T>C (p.Ser37Pro) variant in *NAA10*, a gene encoding the catalytic subunit of the major human amino-terminal acetyltransferase (NatA). This same mutation was identified in a second unrelated family, with three affected boys in two generations. This X-linked malformation and infantile lethality syndrome has provisionally been named Ogden Syndrome, in honor of the hometown where the first family resides. This is the first human disease likely involving a defect in the amino-terminal acetylation of proteins, a common (yet vastly understudied) modification of eukaryotic proteins carried out by amino-terminal acetyltransferases (NATs). There is significantly impaired biochemical activity of the mutant hNaa10p, suggesting that a reduction in acetylation of some unidentified proteins by hNaa10p might contribute to this disease. There is currently very limited knowledge on the functional importance of Nt-acetylation at the protein level and at the organismal level. We have begun to study these processes in mammalian cell culture and yeast, along with setting up collaborations involving other model organisms.

The Characterization and Analysis of an Idiopathic Intellectual Disability Syndrome via Whole-Genome Sequencing Analysis

J. O'Rawe, Y. Wu [in collaboration with A. Rope and J. Swensen, University of Utah]

We have delineated a new idiopathic syndrome with intellectual disability and distinctive facial dysmorphology. The propositi are two affected male brothers, aged 10 and 12, respectively, with severe intellectual disability, autism-like behavior, attention deficit issues, and very distinctive facial features, including broad, upturned nose, sagging cheeks, downward-sloping palpebral fissures, relative hypertelorism, high-arched palate, and prominent ears. Their parents are nonconsanguineous and are both healthy, and family history does not demonstrate any other members with anything resembling this current syndrome. X-chromosome inactivation assays reveal skewing in the mother, suggesting the possibility of an X-linked disorder. High-density genotyping arrays in the mother, father, and two sons have not revealed previously known copy-number variants (CNVs) that might contribute to the phenotype. Whole-genome sequencing has led to the identification of several rare variants that are currently being characterized further, including in other unaffected members of the extended family.

Expansion of Collection Efforts in Utah

Work done in collaboration with R. Robison and C. Johnson, Utah Foundation for Biomedical Research and K. Wang, University of Southern California

I have worked during the past year to collect dozens of additional families in Utah with severe neuropsychiatric disorders, as part of an Institutional Review Board (IRB)-approved protocol at the Utah Foundation for Biomedical Research (UFBR). We are collecting blood for DNA, RNA, and peripheral blood mononuclear cells, with these cells to be used in future experiments to make and characterize pluripotent stem cells. For now, we are obtaining genotyping on genomic DNA, and we have recently initiated whole-genome sequencing of ~50 samples to a depth of >30× on the Illumina HiSeq platform, in an effort to understand the genetic basis of these disorders. The illnesses we are studying include intellectual disability, autism, schizophrenia, and other childhood-onset neuropsychiatric disorders.

Toward More Accurate Variant Calling for "Personal Genomes"

J. O'Rawe, Y. Wu [in collaboration with K. Wang, University of Southern California]

To facilitate the clinical implementation of genomic medicine by next-generation sequencing, it will be critically important to obtain accurate and consistent variant calls on personal genomes. Multiple software tools for variant calling are available, but it is unclear how comparable these tools are or what their relative merits in real-world scenarios might be. We sequenced 15 exomes from four families using the Illumina HiSeq 2000 platform and Agilent SureSelect v.2 capture kit, with ~120× mean coverage. We analyzed the raw data using near-default parameters with five different alignment and variant calling pipelines (SOAP, BWA-GATK, BWA-SNVer, GNUMAP, and BWA-SAMTools). We additionally sequenced a single whole genome using the Complete Genomics (CG) sequencing and analysis pipeline, with 95% of the exome region being covered by 20 or more reads per base. Finally, we attempted to validate 919 single-nucleotide variants (SNVs) and 841 indels, including similar fractions of GATK-only, SOAP-only, and shared calls, on the MiSeq platform by amplicon sequencing with ~5000× average coverage. SNV concordance between five Illumina pipelines across all 15 exomes is 57.4%, whereas 0.5%–5.1% variants were called as unique to each pipeline. Indel concordance is only 26.8% between three indel calling pipelines, even after left-normalizing and intervalizing genomic coordinates by 20 bp. Eleven percent of CG variants that fall within targeted regions in exome sequencing were not called by any of the Illumina-based exome analysis pipelines. On the basis of targeted amplicon sequencing on the MiSeq platform, 97.1%, 60.2%, and 99.1% of the GATK-only, SOAP-only, and shared SNVs can be validated, but only 54.0%, 44.6%, and 78.1% of the GATK-only, SOAP-only and shared indels can be validated. Additionally, our analysis of two families, one containing four individuals and the other containing seven, demonstrates additional accuracy gained in variant discovery by having access to genetic data from a multigenerational family. Our results suggest that more caution should be exercised in genomic medicine settings when analyzing individual genomes and that more scrutiny should occur when interpreting positive and negative findings, especially for indels.

Identifying Disease Mutations in Genomic Medicine Settings: Current Challenges and How to Accelerate Progress

Work done in collaboration with K. Wang, University of Southern California

The pace of exome and genome sequencing is accelerating, with the identification of many new disease-contributory mutations in research settings, and it is likely that whole-exome or whole-genome sequencing could have a major impact in the clinical arena in the relatively near future. However, the human genomics community is currently facing several challenges, including phenotyping, sample collection, sequencing strategies, bioinformatics analysis, biological validation of variant function, clinical interpretation and validity of variant data, and delivery of genomic information to various constituents. In light of this, I worked with my collaborator Kai Wang on a comprehensive review of these challenges and summarized the bottlenecks for the clinical application of exome

and genome sequencing. We also suggested ways for moving the field forward, including the need for clinical-grade sample collection, high-quality sequencing data acquisition, digitalized phenotyping, rigorous generation of variant calls, and comprehensive functional annotation of variants. Additionally, we suggested that a "networking of science" model encouraging much more collaboration and online sharing of medical history, genomic data, and biological knowledge, including among research participants and consumers/patients, will help establish how certain mutations may contribute to the development of certain phenotypes.

A Proposal for an Analytic-Interpretive Split (or a So-Called "Distributive Model") Across Both Clinical and Research Genomics

Work done in collaboration with J. Segal, New York Genome Center

I have been working on a policy piece related to the field of clinical genomics. In brief, the United States federal government mandates that any laboratory performing tests on human specimens "for the purpose of providing information for the diagnosis, prevention, or treatment of any disease" must satisfy the conditions set forth in the Clinical Laboratory Improvement Amendments (CLIA) of 1988. Most laboratories in academic research settings do not have sufficient standards in place to qualify them for CLIA approval. At the time CLIA was enacted, the separation of the clinical and research worlds seemed a fairly straightforward proposition. But today, the issues we face from a regulatory and ethical standpoint around genomics stem from the simple question: What do we do when it becomes difficult to draw a clear line of distinction between these two types of laboratory practices, particularly when researchers are working directly with families? Families afflicted with rare genetic disorders now have a reasonable expectation of definitive and potentially actionable results on the order of days to months, and all such families regardless of diagnosis are candidates for a relatively standardized genomic (rather than disease-specific mechanistic) analysis.

Clearly, history has shown that the quality of unregulated diagnostics is susceptible to perverse market forces, as many prior poor practices can be tied directly to economic conflicts of interest. At a minimum, CLIA erects standards to protect laboratories from these forces and provides an enforcement structure. The field would be prudent to embrace this protection, in light of the ongoing commoditization of sequencing and its associated potential for severe price competition.

As a solution, we are proposing an analytic-interpretive split (or a so-called "distributive model") across clinical genomics. This split model simply means that one laboratory performs sample processing/sequencing and then downstream laboratories can perform the interpretation and reporting. Thus, together, the laboratories perform all the functions that make up a laboratory test. The practical effect of this split would be to turn an exome or genome sequence into a discrete deliverable clinical unit that could be used for multiple purposes by downstream labs. The raw data, if individually approved, could be uploaded to electronic health records or other online sources, allowing its use in downstream investigative analysis. Another overlooked benefit of performing more standardized genome sequencing would be its effect on resultant data sets, and the community's resultant confidence therein. Enacting the type of split described herein allows organizations to leverage their strengths in sequencing or bioinformatics, thus lowering costs across the entire process. It will also help to provide guidance to the many rapidly proliferating laboratories devoted to clinical genomic data mining. However, it is essential that we think of these companies as laboratories, even if they may be separate from the wet lab. Their postanalytic processing would simply be an extension of a prior wet-lab process, and thus included as a core component of a medical test.

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DEVELOPMENT OF NEXT-GENERATION SEQUENCING TECHNOLOGY

W.R. McCombie E. Antoniou, N. Azamy, M. Bell, Y. Berstein, G. Cheang, A. DeSantis, P. Deshpande, S. Ethe-Sayers, E. Ghiban, M. Gierszewska, M. Kolli, M. Kramer, S. Lata, D. Lewis, J. Marchica, S. Mavruk, S. McCarthy, P. Mocombe, S. Muller, J.S. Parla, R. Solomon, S. Teng, J.C. Yao

In 2012, we continued to expand our sequencing capacity, but we focused primarily on improving efficiency, analyzing data, and developing new technologies to meet the challenges we have found in our work to date. In the cognitive genomics area, we completed the analysis and submitted for publication the results of our first large-scale study: the sequencing of the *Disrupted-In-Schizophrenia 1* (*DISC1*) gene in more than 1500 individuals. We have also sequenced ~62 genes that interact with *DISC1* in the same samples, and analysis of these data is ongoing. We continued the analysis of several extended pedigrees that were highly burdened with psychiatric disorders. In collaboration with scientists at Trinity College, Dublin, we also carried out a project to sequence the exomes of families that have a schizophrenic child and unaffected parents.

In all of these projects, we anticipate the need for a very efficient way to test a small number of genes for variants in a large number of people and have made significant strides this year in developing a method to carry this out.

In cancer genetics, we submitted our study of Barrett's esophagus and esophageal cancer for publication, and in plant genomics, we made a significant contribution to the first version of the gene content of wheat, which was published in 2012.

Targeted Resequencing of the Human *DISC1* Gene Using Long-Range PCR and Illumina Sequencing

J.S. Parla, M. Kramer, S. McCarthy, J.C. Yao, E. Ghiban, W.R. McCombie [in collaboration with D. Blackwood, D. Porteous, P. Thompson, and I. Deary, University of Edinburgh; P. Visscher and A. McRae, University of Queensland]

DISC1 has been an important gene in understanding the genetics of psychiatric disorders. In previous annual reports, we described the sequencing of this region in more than 1500 individuals (221 bipolar disorder, 240 schizophrenia, 192 major depression, and 889 controls).

In 2012, our collaborators used TaqMan assays to check for segregation of interesting, rare single-nucleotide variants in extended family members. There was evidence of segregation of the noncoding variant rs16856199 with depression in family members of the probands, but the result was not replicated in a second population sample set. Samples from the Lothian Birth Cohort (LBC) had quantitative measures of mood, intelligence, and cognitive aging. Our collaborators found nominal associations in the LBC sample for burden of minor alleles in conserved transcription-factor-binding sites with measures of depression, as well as burden of minor alleles in coding or CpG regions with cognition. Finally, pairwise epistatic analysis of *DISC1* single-nucleotide polymorphisms (SNPs) indicated nominally significant interactions in cases versus controls.

There were two main findings of the study. A relatively common SNP in an intron of the *DISC1* gene (rs16856199) is nominally associated with major depression. In addition, we identified several extremely rare variants that appear to be seen only or almost exclusively in psychiatric patients. One of these, R37W, has been seen by another group of investigators in another patient and not in more than 10,000 controls in a variety of projects. Several of these variants appear to largely segregate with illnesses in other members of the family of the individual we originally sequenced. We completed final analysis of the data in 2012 and the manuscript was submitted to *Molecular Psychiatry*.

Targeted Resequencing of the *DISC1* Interactome Using Solution-Based Hybrid Selection and Illumina Sequencing

S. Teng, S. McCarthy, M. Kramer, W.R. McCombie [in collaboration with D. Blackwood, D. Porteous, P. Thompson, and I. Deary, University of Edinburgh; A. McRae, University of Queensland]

The *DISC1* Interactome is a term we use to describe the network of genes that interact with *DISC1*. These

genes converge on pathways critical for neuronal signaling and genes important in the treatment of schizophrenia, bipolar disorder, and major depression. Structural rearrangements in several genes that interact with *DISC1*, such as *PDE4B and NDE1*, have been implicated in psychiatric and cognitive disorders; therefore, it is possible that mutations in other genes constituting the *DISC1* interactome may also be of large effect and significantly increase the risk of psychiatric disorders. In collaboration with the University of Edinburgh, we resequenced, in 1554 samples, the exons, promoters, and conserved regions of 262 genes that are known to directly interact with *DISC1* or that are part of functional biological pathways related to *DISC1* interactome activity.

To specifically enrich and resequence the 262 genes of the *DISC1* interactome, in the previous year, we worked with Roche NimbleGen to develop a custom solution capture probe set that took advantage of a balanced probe-target selection. This probe set selectively targeted 13 Mb (0.4%) of the human genome representing each exon, promoter, and conserved region 20 kb proximal and distal of all *DISC1* interactome genes. Exons of every isoform were obtained from Ref-Seq and the UCSC Gene List (hg18). Promoters were defined as 2 kb upstream of each gene, whereas conserved regions were defined as runs of 10 bp or greater with an average score equal to or greater than 0.3 based on phastCons 44-way nucleotide-wise (Siepel et al., *Genome Res, 15*: 1034 [2005]) conservation values.

During fall 2012, we finished sequencing the *DISC1* interactome in 1554 individuals representing 241 patients with schizophrenia, 222 patients with bipolar disorder, 192 cases with unipolar depression, and 899 healthy controls. About one-third of these were done in 2011 and the rest were carried out in 2012. Given the target size and HiSeq 2000 sequencing capacity, we sequenced 16 barcoded samples across three lanes of a HiSeq 2000 flowcell and achieved greater than 20× coverage for 80% of the target in more than 95% of the samples.

We took advantage of the sample overlap between the *DISC1* sequencing and interactome projects and used our previous Sanger sequencing validations in the *TRAX/DISC1* region to determine sensitivity and specificity and to establish variant filtering criteria. In total, we have detected 317,807 single-nucleotide variants (SNVs), of which 248,415 have a minor allele frequency of less than 1%. Approximately 1.8% of these rare variants are nonsynonymous. We are currently evaluating additional data-quality measures and implementing several association approaches to analyze the frequency distribution of variants between cases and controls. In addition to burden and main effect analyses, we aim to analyze epistatic interactions between genes, perform multivariant analysis of available cognitive information, and use expression data to prioritize variants and genes for functional follow-up.

Investigation of Bipolar Disorder Genetics Using Exome Capture and Resequencing

J.S. Parla, S. Muller, G. Cheang, M. Kramer, E. Ghiban, W.R. McCombie [in collaboration with J. Potash, University of Iowa; P. Zandi, F. Goes, R. Karchin, and A. Chakravarti, Johns Hopkins School of Medicine]

Bipolar disorder I (BP) is a major mental illness with a significant genetic component. Approaches that are capable of effectively identifying and studying rare variants associated with BP are necessary. We are applying exome capture and massively parallel sequencing toward finding rare genetic variants that contribute to BP. In 2012, we scaled up exome capture and sequencing in this project to ~100 samples per week and brought our sample data set totals to 62 family subjects, 497 bipolar cases, and 470 controls. Our production workflow was predominantly two samples per exome capture and four exome-captured samples per HiSeq 2000 lane, but the end of 2012 marked our positive validation of a higher-throughput workflow with six exome-captured samples per HiSeq 2000 lane. In 2013, we anticipate further developments in our production sample preparation and sequencing workflow to support increased sample pooling at both the exome capture and sequencing levels. Our goal is to increase sample multiplexing while still maintaining a minimal exome coverage threshold of 80% at 20× sequencing depth per sample.

Our production workflow also involves a standardized GATK-based sequence analysis pipeline that produces capture performance metrics and SNV and small indel calls. Once the sequence data are fully processed through our analysis pipeline, we will maintain a shared repository enabling all three institutions to access the full genotype and variant call data. With the close of 2012, we started reviewing the changes and updates made to the GATK package by the Genome Sequencing and Analysis (GSA) group at the Broad Institute to determine which of the changes we should

apply to our standardized analysis pipeline. Once the pipeline updates and parameter adjustments are validated for this project's aims and the full study sample set, the updated pipeline will go into use immediately. In addition to the sequence analysis pipeline for identifying high-confidence genetic variants, we have implemented a standard sample data QC workflow that involves gender validation, principal component analysis to test for any population or otherwise broad inconsistencies, and Mendelian testing for the samples known to be genetically related. Another QC measure we plan to add to our sample QC is testing for Hardy-Weinberg equilibrium of observed allelic frequencies.

The bioinformatics analysis of the genetic variants identified to date in this study has been more family-focused due to our need for significantly greater numbers of case-control subjects to perform association analysis. We began with a variant segregation analysis with the family samples to identify candidate genes. To further investigate our family linkage findings, additional families and extended pedigrees were chosen to genotype 37 SNVs contributing to the linkage signals we observed for the 10 top-ranked candidate genes we identified. The results of those genotyping assays did not support significant association of those genes with BP, thus underscoring the need for greater numbers of study subjects and further refinement of our analysis strategy. Those candidate genes were also assessed in the case-control subjects for possible corroboration of phenotype association, using several variant weighting and pathway analysis approaches, and the findings have not supported association. We expect to obtain more robust results with increased data sets, which we plan to finalize in 2013. However, this is a challenge faced by the field as a whole. It is likely that very large numbers of samples will need to be analyzed to find significant signals. With this in mind, we have been focusing considerable effort on developing ways to sequence relatively small regions of the genome, such as ~100 genes in thousands of people.

Highly Multiplexed Targeted Resequencing of Sub-Million Base Genomic Targets

J.S. Parla, P. Deshpande, S. Ethe-Sayers, J. Marchica, M. Kramer, E. Ghiban, W.R. McCombie

We and other investigators are finding potentially important candidate genes for complex traits such as schizophrenia, bipolar disorder, and autism through genome and exome sequencing projects. However, there is a great remaining challenge in the field to demonstrate the significance of variants in these genes or genomic regions in very large numbers of individuals. As of now, technology does not exist to allow the sequencing of a small part of the genome (0.5 million to 5 million bases) in large numbers of individuals (1000s to 10,000s). We are making a focused effort to develop this capability.

To address the apparent incompatibility between the genetic discovery and large-scale population study phases, we have been developing a method that adapts the highly successful solution exome capture methodology to significantly smaller genomic targets. Our goals for developing our microtargeting technique include the minimal modification of existing commercial solution exome capture techniques and the establishment of maximal sample multiplexing capability at the capture level, which should support a straightforward experimental design and candidate validation strategy. We have initiated the development of our method with the commercial capture platform with which we have had the most success (namely, SeqCap EZ from Roche NimbleGen), and a selection of five human genes that were identified in a preliminary analysis of pilot exome data we produced for one of our bipolar disorder projects. The final approved probe design targeted a cumulative 637,760 bp of the five full-length genes and was predicted to capture 92.6% of the target given a 100-base offset window accounting for library insert length. In 2012, we successfully validated significant sample multiplexing at the capture level, with up to 96 uniquely barcoded samples per NimbleGen custom capture and one 96-plex capture per HiSeq 2000 lane. Our workflow produced ~80% of sequence reads mapping on or near the target probe regions, ~90% of the target covered at ≥20× sequence depth, and ~200× mean target depth per sample per capture. These results indicated that the HiSeq 2000 data capability per lane supported even greater numbers of samples than 96. Thus, by the end of 2012, we started considering additional ways to increase sample multiplexing, beyond just maximizing our use of available sample library barcodes. One possible method we started testing involves sample pooling prior to barcoding and then barcoding each pool of samples. This layered approach allows the pooling of greater numbers of samples in capture and sequencing while

still maintaining barcode uniqueness in the sample pools. This method is dependent on intuitive sample pooling (e.g., pooling cases and controls separately) and a modified sequence data analysis pipeline with parameters adjusted for input data containing more than one sample. An alternative sample layering approach we hope to evaluate in 2013 also involves prebarcode sample pooling, but the pooling is performed in a two-dimensional matrix-dependent manner that supports the direct pairing of an observed genotype with a specific sample, granted that the variant is observed uniquely from each dimension of the matrix (i.e., from one row and one column only). Notably, the latter method is more intuitively relevant for studying rare genetic variants, and if we are able to validate both of the sample-layering methods described above, then we could choose among them accordingly, depending on the aims of specific projects.

Exome-Based Sequencing of Parent Offspring Trios with Schizophrenia

S. McCarthy, J. Gillis, M. Kramer, W.R. McCombie
[In collaboration with D. Morris and A. Corvin, Trinity College, Dublin]

During the past 6 years, there has been a resurgence of interest in understanding the impact of rare variation underlying disease risk and causality, especially for psychiatric conditions such as autism and schizophrenia. This rapidly growing interest has been influenced by the below-expected contribution of common variation to the genetic risk of diseases prevalent in the population and by the work done here at CSHL, which has shown that the rate of new novel mutations, in the form of copy-number variants (CNVs), is significantly greater in autism, bipolar disorder, and schizophrenia. In addition, advances in genomic technologies and DNA sequencing here at the Laboratory have enabled us to search for de novo mutations at an unprecedented throughput and at a resolution that allows us to identify single genes that can provide significant insight into the pathobiology of all genetic diseases.

In collaboration with Trinity College Dublin, we used hybrid selection to target and resequence the human exome in 42 parent-offspring patients with schizophrenia in search of de novo mutations that could affect the function of protein-coding genes potentially underlying the pathobiology of the disease. The exomes of each proband and parent were captured using Roche NimbleGen SeqCap EZ v2 probes and sequenced to a mean depth of 20x over 80% of the exome target using Illumina's HiSeq 2000. We identified 47 de novo single-nucleotide mutations with, on average, 1.12 de novo events per trio, which is not different from our expectations. Interestingly, a prominent feature of our data was the overlap between genes with de novo mutation and genes implicated in autism and intellectual disability ($p < 10^{-3}$). The overlap with autism was the greatest of 203 Neurocarta phenotypes (defined by 20 or more associated genes) and determined by de novo mutations that possibly affect the function of *CHD8, MECP2, MKL2, AUTS2, HUWE1,* and *MLL2*. This finding supports previous genetic and epidemiological studies that suggest a shared genetic component between schizophrenia and autism. However, in contrast to CNVs, this work highlights single genes for in-depth functional analysis.

Notably, several of the genes with de novo mutations have a significant role in reading and editing of chromatin and methylated DNA, for example, *CHD8, MECP2,* and *MLL2*. We therefore tested the hypothesis that genes with likely functional de novo variants were enriched in chromatin remodeling. Five genes—*CHD8, MECP2, MLL2, TDRD5,* and *NUP98*—significantly clustered ($p = 8.5 \times 10^{-3}$) among a list of 433 curated chromatin remodelers (provided by Chris Vakoc), suggesting that perturbations of epigenetic mechanisms that control gene expression could be significant risk factors in the neurodevelopment of psychiatric illnesses. In light of studies demonstrating that novel antidepressants target the epigenetic mechanisms controlling the expression of genes associated with depression, our results suggest possible avenues for developing novel antipsychotics and therapeutics for improving the cognitive deficits in mental illness.

Finally, we combined the power of next-generation sequencing in schizophrenia trios with a meta-analysis of gene expression data to analyze the coexpression patterns of our genes with likely functional de novo variants. Surprisingly, we found that our genes demonstrated significantly lower coexpression with other genes in the prefrontal cortex compared to the other brain and nonbrain regions. This reduced coexpression is lower than that of randomly selected genes and supports hypotheses that genes with loss-of-function mutations express lower connectivity. Our novel analysis

demonstrates the potential of combining orthogonal and complementary genomic analyses to elucidate the function of genes for which little is known. Going forward, we aim to expand this study of de novo variants in additional trios with schizophrenia and prioritize genes based on their role in chromatin remodeling and the neurodevelopment of psychiatric diseases using neurons derived from patient-specific induced pluripotent stem cells.

Discovery of Genetic Variation Underlying Major Depressive Disorder and Co-Morbid Edema

W.R. McCombie, S. McCarthy, M. Kramer, E. Ghiban [In collaboration with D. Blackwood, M. Ayub, D. Porteous, and P. Thompson, University of Edinburgh]

Major depressive disorder (MDD) is a debilitating disorder that affects more than 10% of the US population. Despite having a genetic component, uncovering genetic variants in MDD has been limited. We are analyzing four families burdened with MDD and a crippling co-morbidity, idiopathic edema, which is an unexplainable swelling of the face, extremities, breasts, and abdomen.

A previous linkage analysis by our collaborators of four pedigrees co-morbid for MDD and idiopathic edema identified three potential regions of interest on chromosomes 7, 8, and 14. Of the 47 affected individuals examined, 28 had both MDD and unexplained swelling, whereas eight had MDD only and 11 had unexplained swelling only.

Beginning in late 2011 and continuing in 2012, we sequenced the entire genome of 19 members across these four families to discover genetic variation that may increase the risk of MDD and pinpoint variants that could be implicated in the causality of idiopathic edema. Of these 19 individuals, 12 have both major depression and edema, three have edema but not depression, one has depression but not edema, and three are controls with neither depression nor edema. Illumina libraries were constructed and 100-bp paired-end sequences were obtained on the HiSeq 2000. Reads were aligned to the human reference (UCSC hg19) and variants relative to the reference were detected. For each of the samples sequenced, the mean coverage is greater than 30×, and more than 80% of the genome is represented by at least 20 reads. As a first step, we determined the intersection of variants in affected individuals within each family, eliminating variants found in unaffected relatives. We are continuing to analyze these data by prioritizing genes with segregating variants for validation and looking for any overlap among families. We are also expanding the analysis by grouping individuals across families by diagnosis.

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PLANT GENOME ORGANIZATION, EVOLUTION, AND FUNCTION

D. Ware J.-M. Chia, A. Eveland, Y. Jiao, M. Karey Monaco, S. Kumari, Y.K. Lee, C. Liseron-Monfils, Z. Lu, C. Noutsos, A. Olson, S. Pasternak, J. Stein, J. Thomason, P. Van Buren, L. Wang, S. Wei, K. Youens-Clark, L. Zhang, Z. Zhang

Our lab has dual goals of conducting plant genomics research and developing genomics resources and discovery tools for use by the broader research community. Research includes a broad range of activities in physical, statistical, and functional genomics in both model systems and crop plants, with applications in agriculture. Our lab also contributes to two large-scale multi-institutional cyberinfrastructure collaboratives that are designed to serve broader research and educational communities. During the past year, we were joined by visiting scientist Zhiming Zhang and postdoctoral researchers Yinping Jiao and Christophe Liseron-Monfils. Jer-Ming Chia has advanced his career, taking a position in the industry sector.

Gramene

Y. Jiao, S. Kumari, M. Karey Monaco, S. Pasternak, J. Stein, J. Thomason, S. Wei, K. Youens-Clark [in collaboration with P. Jaiswal, Oregon State University; P. Kersey and H. Parkinson, EMBL-European Bioinformatics Institute; L. Stein, Ontario Institute of Cancer Research; C. Taylor, American Society of Plant Biologists; E. Buckler, USDA, Cornell University; S. McCouch, Cornell University]

Gramene is a collaborative project that leverages sequence and functional information from plant reference genomes to promote research and translational genomics in agriculture and its various applications, including food and biofuel production. The Gramene website (http://www.gramene.org) serves as a portal to multiple genome browsers and manually curated databases of genes, proteins, biochemical pathways, quantitative trait loci (QTL), ontologies, germplasm, and genetic diversity data. In the last year, our group completed several milestones on the Gramene project, including its 35th and 36th releases since 2012. Of the many improvements made, we increased the number of partial and complete sequenced genomes in our Ensembl genome browser to 23 scientifically and economically important species including rice, corn, tomato, potato, sorghum, barley, soybean, poplar, grape, banana, and the unicellular green alga *Chlamydomonas reinhardtii*. Gramene is also home to reference sequences for three cultivated and eight wild species of rice, making it the single most comprehensive genus-level (*Oryza*) resource in plants. Gramene is also positioning itself as a principal resource for conducting population-based genetics research. We added several new diversity/phenotype data sets covering *Arabidopsis*, rice, and maize, and have added new tools for querying single-nucleotide polymorphisms (SNPs) and visualizing genome-wide association studies. In addition, we used the Ensembl 69 infrastructure to include multi-species views from whole-genome alignments, synteny maps, browsable phylogenetic gene trees highlighting Gene Ontology functional descriptors, and SNP views that display diversity data in the context of functional impacts on gene structure. All of this work has contributed to significantly increased traffic on the Gramene website over the last year from users all over the world. Our databases are created in partnership with the European Bioinformatics Institute (EBI), and they are now also available through the Ensembl Plants portal. In the next year, we will continue to add new reference genomes, data sets, and functionalities to this resource.

PLANT GENOME RESEARCH

In the last decade, the decoding of complete plant genomes has borne fruit for scientists who seek to understand the complexities of plant function and evolution, and to affect economically important traits. The generation of reference genomes spans many disciplines, starting in the laboratory with scientists who generate the raw sequence data. From there, it is the responsibility of computational biologists and bioinformaticians, like those in our lab, to help interpret the output. This includes assembly of raw sequence reads into overlapping segments, or contigs, and scaffolding

these to properly represent their order and orientation within chromosomes. Another step is annotation—the discovery and description of genes and other functional elements, as well homologies to other genomes. In addition, this information must be faithfully communicated and visualized, for example, in web-based platforms such as Gramene. All of these activities are rapidly evolving with the advent of new sequencing technologies, algorithms, and data-handling requirements. For example, high-depth sequencing of RNA transcripts at low cost is providing new evidence that informs genome annotation, and it is spurring the development of new software to model and perform this task. Low-cost sequencing is also transforming the types of questions that can be asked, moving beyond the generation of a single reference for a given species. Ongoing projects within the maize, rice, and *Arabidopsis* research communities are now sequencing hundreds and even thousands of genotypic backgrounds within species, gathered from carefully constructed populations, wild populations, and breeding germplasms. Information on genetic variation is helping scientists to understand the genetic basis of phenotypic traits and questions about the origins of domesticated crop species. New technologies are also driving research in epigenetics, the study of heritable traits that are not caused by changes in underlying genome sequence. Epigenetic mechanisms include modification of DNA by methylation and various forms of histone modifications, which can cause changes in gene expression and other phenotypes. Both of these modification types can be studied with new sequence technologies and analysis methods. Our lab is engaged in many of these areas of inquiry. For some species such as maize, we are beginning to see how the provision of genome sequence, annotation, genetic variation, epigenetic variation, and transcript expression is converging to bring about deeper understanding of genome function.

Updating the Maize Reference Genome and Annotation

A. Olson, S. Pasternak, J. Stein [in collaboration with J. Glaubitz, Cornell University; E. Buckler, USDA, Cornell University; R. Fulton and R. Wilson, Washington University; The Maize Genome Sequencing Consortium]

Work continues on refining the assembly and annotation of the B73 reference sequence. The RefGen_v2 assembly and annotations are hosted on Gramene and these have been deposited in GenBank along with contigs assembled from Roche/454 sequencing of a whole-genome shotgun library. RefGen_v3 incorporates some of these contigs in order to increase coverage of missing gene space. Contigs were selected based on alignment to FLcDNA sequences and inserted into gaps in the RefGen_v2 assembly guided by a genetic map and synteny to rice and sorghum. Approximately 500 genes were added or improved with new annotation. A number of unplaced physical map contigs were also anchored based on these maps. The RefGen_v3 assembly will be released following acceptance by GenBank. Work on RefGen_v4 is also under way. To improve the maize genome sequence, we are adding additional depth to the existing capillary sequence using the Illumina 2000. We are resequencing 17,173 BACs (bacterial artificial chromosomes) that constituted the minimal BAC tiling path from the original B73 sequencing project by pooling the BACs in pools of 96 and generating Illumina data to greater than 150× coverage per clone. This increased depth, density of read pairs, and differential bias compared to capillary sequencing will serve as an excellent resource to further improve the maize sequence. These data, once completed, will be made available through public databases prior to completion of the RefGen_v4 assembly.

Tackling Wheat

J. Stein, S. Pasternak, A. Olson [in collaboration with J. Dvorak and M.C. Luo, University of California, Davis; O. Anderson, University of California, Davis/USDA-ARS, Albany; B.S. Gill, Kansas State University; W.R. McCombie and M. Schatz, Cold Spring Harbor Laboratory]

One of the great frontiers in plant genomics is to determine the complete sequence of bread wheat, *Triticum aestivum*. From its early domestication in the Fertile Crescent, large-scale cultivation of wheat enabled the rise of human civilization and the formation of city-based societies. Today, it remains one of the most important food crops worldwide, grown on more land area than any other crop, and is a leading source of calories and protein in the human diet. Better understanding of the wheat genome will enhance future breeding efforts to perpetuate its critical role in feeding the world. Consistent with its superlative nature, wheat also represents the most challenging plant genome to be attempted to date by scientists.

At 16,000 million base pairs, its nuclear genome is twice the size of human, eight times the size of maize, and 40 times the size of rice. One explanation for its imposing size is that wheat is hexaploid—i.e., it contains not one but three genomes, termed A, B, and D, that were brought together through hybridization of closely related diploid species during domestication. Because the three genomes are actually quite closely related, it is difficult to assign genes to their appropriate genome. Making matters more complex, the wheat genome is also one of the most repetitive, with ~90% of its content bloated with transposable elements that obscure the discovery of functioning genes. These facts have necessitated multiple international initiatives to sequence wheat, overseen by the International Wheat Genome Sequencing Consortium (IWGSC), wherein individual chromosomes have been divvied up for work by individual labs. Our lab is a collaborator on the National Science foundation (NSF)-funded project to physically map chromosomes 1D, 4D, and 6D of bread wheat (cultivar Chinese Spring) and all seven chromosomes of the closely related species *Aegilops tauschii*. This latter species is highly informative because it is the diploid progenitor of the wheat D genome. Now complete, the project has produced physical maps containing almost three-quarters of a million BACs, positioned relative to one another by a restriction enzyme fingerprinting method. The project has also produced the first high-resolution genetic map of *Ae. tauschii*, with more than 7000 sequenced markers that are anchored to the physical map. These maps are scheduled for public release this year via the Gramene web portal. Gramene will also display comparative maps between the D genome and other grass reference genomes to enable study of gene function and evolution (Fig. 1). Together, the information and physical reagents produced by this project will help lead the way for future research in wheat genomics. Funded by a second NSF grant, CSHL is pioneering the discovery of wheat genes by using ultra-high-throughput sequencing technologies and rapidly releasing these data to the scientific community. In this whole-genome shotgun approach, enormous quantities of sequence data can be generated in a cost-effective manner. To date, the McCombie lab has deposited more than 850 gigabases (~200-fold coverage) of *Ae. tauschii* to the NCBI Sequence Read Archive (SRA). Similar numbers have been deposited for *Triticum monococcum*, the diploid progenitor of the wheat AA genome. Efforts are under way in our lab to assemble these sequences and build scaffolds to describe the gene content of these genomes. Using physical/genetic maps such as those described above, and by comparative mapping to other grass species, we have positioned many of the genes to their location in the genome. Results will be released via a browser within Gramene. Work by CSHL complements extensive research efforts taking place worldwide that together will ultimately tackle the wheat genome.

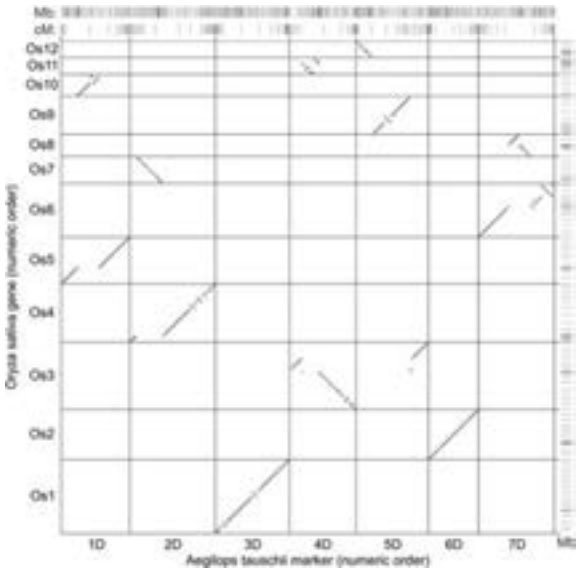

Figure 1. Pairwise comparative map between genomes of *Aegilops tauschii* (the wheat D genome) and rice (*Oryza sativa*). The matrix shows collinear genes based on the integrated genetic and physical map for *Ae. tauschii* produced by the NSF-funded wheat D genome-mapping project (PI Jan Dvorak; co-PIs, Doreen Ware and others). Genetic distances for *Ae. tauschii* are shown with each tick-mark corresponding to 10 cM. Physical distances are plotted with each tick-mark corresponding to 10 Mb for Ae. tauschii and 2 Mb for rice.

The Maize Methylome

Z. Lu, A. Olson, S. Pasternak [in collaboration with R. Martienssen, J. Hicks, W.R. McCombie, M. Regulski, and J. Kendall, Cold Spring Harbor Laboratory; Scott Tingey and Antoni Rafalski, Pioneer/DuPont]

DNA methylation has an important role in the regulation of gene expression and control of transposable elements. The patterns of DNA methylation, referred to as the methylome, must be faithfully propagated

for proper development in plants and animals. We are collaborating with the Martienssen lab and DuPont to sequence the methylome of two maize inbred lines, B73 and Mo17 (Fig. 2). The genomic DNA is treated with bisulfite, which converts unmethylated cytosine to thymine. Sequencing using next-generation Illumina GA2 paired-end reads, followed by mapping back to the maize genome, resulted in identification of the methylome in single-base resolution. We have generated 20×–30× coverage over the mappable portion of the maize genome. Alignment with RNA sequences indicates that the methylation patterns are correlated with gene expression, small RNA, and alternate splicing. Diversity in cytosine methylation patterns was observed in transposable elements and especially in genes and was found to be largely heritable in recombinant inbred lines (RILs). However, significant deviations from heritability were observed, many of which were conserved in different RILs. In the future, this will help us to reveal the roles of DNA methylation in gene regulation and other biological functions.

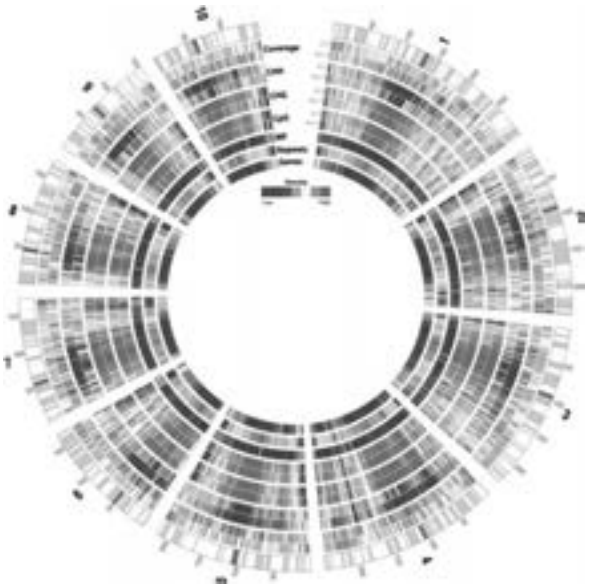

Figure 2. Distribution of methylated sites within the maize nuclear genome. Feature density is given in 1 Mb bins. Coverage tracks show average read depth. CpG/CHG/CHH tracks show average methylation level. MF track is the methylation filtration density. Repeats track shows repeat density. Gene track represents gene density.

Maize HapMap

J.-M. Chia [in collaboration with E. Buckler, USDA, Cornell University; M. McMullen, USDA, University of Missouri, Columbia]

Maize breeders have long exploited the diversity in maize for improving its yield through selective breeding programs, contributing to its current position at the apex of the production crop list. We are in an era in which traditional crop breeding techniques are being supplemented, and in some cases supplanted, by molecular breeding approaches that speed up the breeding process. Understanding the diversity in maize and how it impacts agronomic traits is crucial in implementing these new approaches and will be an important platform for sustainable agriculture in the future.

In the previous 2 years, we sequenced more than 100 inbred lines of the *Zea mays* genus and wild relatives. The volume of sequences generated per inbred line is approximately five times the size of the genome, and from these sequences, we have identified variant loci. Given the complexity of the genome, these variations were scored using carefully designed population genetics-based filters that leverage the carefully constructed maize breeding populations. In total, more than 55 million SNPs were identified. This work culminated in two journal publications in *Nature Genetics* that give insight into the fluidity of the maize genome and factors contributing to the domestication and improvement of maize as a crop. This extensive variation data set is also displayed and annotated in the Gramene browser.

Tracking the Wild Tomato

Jer-Ming Chia, Shiran Pasternak [in collaboration with Z. Lippman, Cold Spring Harbor Laboratory]

This work also contributed to the Tomato Genome Sequencing Project under the direction of principal investigator Giovanni Giuliano (ENEA CR Casaccia, Italy), which included multiple coprincipal investigators and researchers from the United States and internationally.

To explore variation between the tomato and its nearest red-fruited relative, together with the Lippman lab, we sequenced, analyzed, and assembled a *Solanum pimpinellifolium* genome using Illumina short reads. We focused on identifying SNP and insertion/

deletion (indel) variation by aligning the quality-trimmed reads to the pseudomolecule reference of the cultivated tomato, *S. lycopersicum* cv. Heinz. This revealed surprisingly low genome-wide diversity, supporting an extremely close evolutionary relationship with Heinz that is consistent with *S. pimpinellifolium* serving as the foundation for tomato domestication. Assembling the reads into sequence contigs, we see no large structural variations between the two genomes. This extensive molecular marker database, which is now available, makes it possible to access *S. pimpinellifolium* variation data for crop improvement. In particular, the diversity in the undomesticated species for adaptation and disease resistance will be particularly valuable.

Computational Prediction and Analysis of Core Promoter Elements in Plant Genomes

S. Kumari

Transcription initiation, essential to gene expression regulation, involves recruitment of the basal transcription factors to the core promoter elements (CPEs). The distribution of currently known CPEs across plant genomes is largely unknown. We have developed high-throughput in silico methods for motif prediction using the publicly available CREAD suite of tools. Our computational pipeline systematically identified transcription regulation motifs at the whole-genome level, including TATA, CCAAT, INR, BRE, CG-box, DPE, MTE, and Y-Patch motif-containing genes across plant genomes. DNA free-energy profiles of eight plant genome promoters suggested that monocot core promoters have lower DNA free energy than dicot core promoters. Functional classification of TATA-containing and TATA-less genes based on annotated gene ontologies showed enrichment in distinct molecular functions and biological processes. Studies on the prediction of core promoter elements will help us to understand the architecture of core promoters and the mechanisms by which basal transcription machinery function. This study contributes to our long-term goal of elucidating the gene regulatory networks that act at the interface of development and response to the environment.

PLANT SYSTEMS BIOLOGY

Exploring the Gene Regulatory Network Guiding *Arabidopsis* Stele miRNA Expression

L. Zhang, S. Kumari, Y. Koung Lee, C. Liseron-Monfils, C. Noutsos [in collaboration with S. Brady, University of California, Davis]

Plant roots not only serve to physically anchor plants to the soil, but also are responsible for uptake of water and critical nutrients, and therefore must respond quickly to various environmental stresses such as drought, waterlogging, and heavy metal pollution. microRNAs (miRNAs) have a central role in plant development and in the response to environmental stress. We are interested in studying the gene regulatory network (GRN) that regulates miRNA expression in the root. Most miRNAs are transcribed by RNA polymerase II, and their transcription process is regulated by transcription factors (TFs). To systematically resolve points of cross-talk among TFs, miRNAs, and their targets in a comprehensive GRN, we make use of a gene-centered yeast one-hybrid (Y1H) experimental system. The roots of the model plant *Arabidopsis* have rotational symmetry: The stele is the central part of the root and is important for nutrient transfer. We have developed an automated, enhanced Y1H (eY1H) system and a near-complete TF library from the *Arabidopsis* stele (Fig. 3). Using this system, we have screened more than 100 promoters of stele-expressed miRNAs, their targets, and some highly connected TFs. The resulting network, together with published protein–protein interaction data, enabled us to uncover several interesting subnetworks for cytokinin signaling and heavy metal homeostasis. To validate in vivo the protein and DNA interactions that resulted from the eY1H data set, we screened more than 250 T-DNA insertion mutants from *Arabidopsis* and obtained 80 homozygous gene-perturbed lines for TFs, miRNAs, and miRNA targets. From these, we characterized their molecular phenotype by quantifying TF expression by quantitative real-time PCR (polymerase chain reaction) and miRNA expression by Taqman miRNA assays. Our results showed that perturbation in planta of ~80% of TFs and 70% of miRNAs tested displayed molecular phenotypes in the *Arabidopsis* root. The GRN derived from the root stele

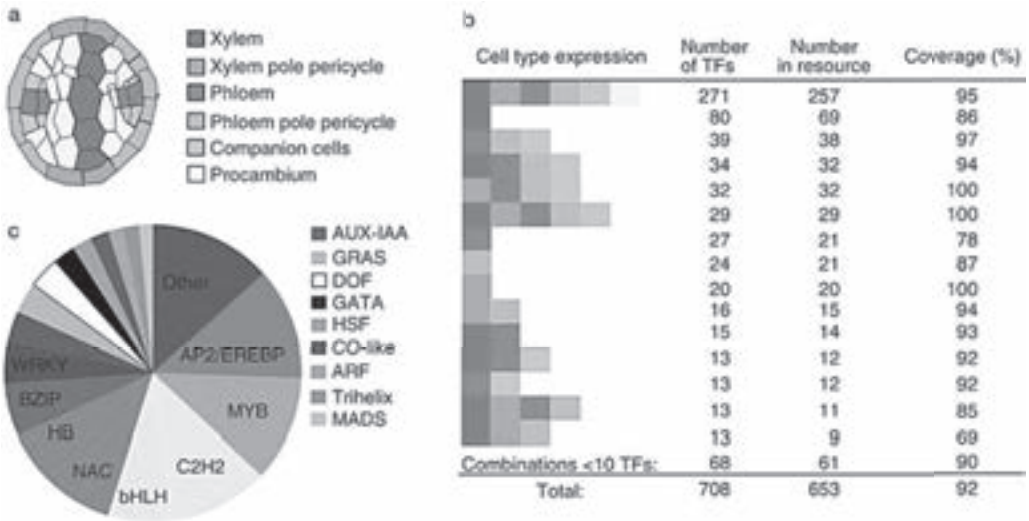

Figure 3. An *Arabidopsis* eY1H transcription factor resource. (*a*) Schematic of root stele cell types. (*b*) Stele cell-type expression profiles of the transcription factors present in our collection. (*c*) Family categorization of transcription factors within the resource. "Other" represents families with fewer than eight members. (GRAS) Gibberellic acid–insensitive, repressor of GA1 and scarecrow; (DOF) DNA-binding with one finger; (GATA) GATA DNA motif; (HSF) heat shock factor; (CO-like) Constans-like; (ARF) auxin response factor; (MADS) MCM1, Agamous, Deficiens, and SRF box; (HB), homeobox; (BZIP), basic leucine zipper. (From Gaudinier et al. 2011. *Nature Methods* **8:**1053–1055.)

will provide a framework for modeling adaptive responses to environmental conditions at the whole-plant level. This will facilitate identification of candidate genes for improving germplasm that can sustain more detrimental conditions, thus addressing global food security and growing demand for renewable energy resources.

Developmental Networks Controlling Inflorescence Architecture in Maize

A.L. Eveland (with contributions by S. Kumari)
[in collaboration with D. Jackson, Cold Spring Harbor Laboratory]

The goal of this work is to integrate genetics and genomics data sets to elucidate molecular networks that determine the morphology (architecture) of maize inflorescences. Because inflorescences bear the fruits and grains that we eat, understanding the genetic and regulatory basis for how these structures are formed has clear relevance to important agronomic traits such as grain yield and harvesting ability. Our data sets represent maize inflorescence primordia sampled during key developmental transitions and in perturbed genetic backgrounds. The latter includes loss-of-function mutants in three important regulators of the *RAMOSA* (*RA*) pathway, which controls stem cell fate decisions and ultimately the decision to branch. We have established a robust system to investigate the networks that modulate branching, including characterization of precise timing of developmental events and associated spatiotemporal changes in gene expression. We integrated genome-wide mRNA-Seq data to resolve coexpression networks during key stages of maize inflorescence development and are working to expand these networks by incorporating additional data sets, such as genome-wide TF occupancy profiles and *cis*-regulatory information.

Primary objectives for this project included the following: (1) Establish a comprehensive pipeline for mRNA-Seq and chromatin immunoprecipitation sequencing (ChIP-Seq) data analysis in maize by evaluating and optimizing available software for mapping and quantification. This also included testing various statistical methods to extract biological relevance. (2) Characterize genome-wide expression signatures specific to a given developmental event or branching phenotype. We made use of known developmental marker genes and their spatiotemporal transcriptional responses to genetic perturbation in order to test our experimental system and establish a proxy for

developmental staging. (3) Evaluate and implement clustering approaches to identify candidate genes that are coexpressed with key regulators and/or coincide with specific developmental events. Our results from this included identification of candidate genes (specifically, developmentally regulated TFs and novel genes of unknown function) that are potentially involved in stem cell maintenance and determinacy. We continue to interrogate coexpression clusters for enrichment of functional processes and *cis*-regulatory motifs that lie within proximity to the transcriptional start site of coexpressed genes (with S. Kumari). (4) Identify targets of the *RA1* TF using ChIP-Seq and integrate results with data from parallel mRNA-Seq experiments. Based on this approach, we showed that one-third of genes with altered expression levels in the *ra1* mutant are also bound by *RA1*. We are incorporating additional ChIP-Seq data sets as they become available to investigate combinatorial binding of TFs associated with the branching pathway. The ChIP-Seq data also provide in vivo confirmation for binding sites of developmental regulators in maize, information that is being leveraged in efforts to resolve *cis*-regulatory modules across the maize genome (S. Kumari, D. Ware).

In the next phase, we will further prioritize candidates from this work by overlaying the *Arabidopsis* regulatory network information (see previous section). The resulting hypotheses can be tested in *Arabidopsis*, for example, responses to stress, and ultimately translated to agronomic systems. Additionally, candidate genes that are maize- and/or grass-specific are of high priority as they may contribute to the unique morphology of maize inflorescences and/or features shared among other grasses. We will further use comparative genomics approaches, including both computational and integration of analogous RNA-Seq data sets from closely related grasses such as sorghum, to identify candidate genes that may contribute to grass-specific aspects of inflorescence architecture.

CYBERINFRASTRUCTURE PROJECTS

The iPlant Collaborative

L. Wang, J. Lu, C. Noutsos, J. Stein, Y. Koung Lee

This project is in collaboration with Cold Spring Harbor Laboratory and employs more than 100 staff. It is headquartered at the University of Arizona, under the direction of principal investigator Stephen Goff. Dozens of collaborators are located at more than 20 institutions.

Driven by the large-scale generation of data, ranging from genomics to imaging, research in biology is increasingly information-based. Lagging behind is the ability of individuals to make efficient use of this information due to limitations in data access and storage, computational infrastructure, and available tools. To tackle big questions in biology, researchers also need effective ways to collaborate that reach across institutional and disciplinary lines. The iPlant Collaborative (http://iplantcollaborative.org) is an NSF-funded cyberinfrastructure project that provides public access to high-performance computing, data storage, and tools via customized web-based interfaces. Having completed the first 5-year grant, the iPlant Collaborative has made extensive progress toward meeting these goals and has been recommended for renewing for another 5 years. Work in the last year has culminated in the development of multiple platforms that are now being used by the research and educational communities. Staff at CSHL directly contributed to some of these cyberinfrastructure platforms or have built upon them to provide ready access to needed software and analysis tools by plant scientists and educators. Within our lab, these platforms include the Discovery Environment (DE), Atmosphere, and the Taxonomic Name Resolution Service (TNRS). The DE is perhaps the most visible portal to iPlant tools and services. This web-based platform supports an "app store" model of user-extensible tools, automated workflows, and data storage. Users can take advantage of existing tools integrated by iPlant staff and user community or add their own tools to use privately or share. Although users may not be aware, the underlying infrastructure is providing access to iPlant's massive data store at the University of Arizona and the Texas Advanced Computing Center (TACC). Computationally intensive tasks are handled by supercomputers located at TACC and other centers within the Extreme Science and Engineering Discovery Environment (XSEDE). So far, well over 300 tools have been integrated into the DE that enable a broad range of research activities, including genome/transcriptome assembly, annotation, RNA-Seq quantification, variant detection, genome-wide association studies (GWAS), and phylogenetics. Members of our lab have had important

roles in contributing to workflow design, tool integration, validation, and documentation. Atmosphere is iPlant's cloud infrastructure platform that addresses the growing need for configurable and cloud-enabled computational resources by the plant research community. From Atmosphere's web interface, users can launch a virtual machine (VM) with preconfigured working environments and ready-to-use software precustomized. Users can also create their own applications and environments as VMs and share with others via Atmosphere. As with the DE, Atmosphere is a gateway to access iPlant's core infrastructure resources, such as the high-performance and grid computing environment and big data storage system. Using the Atmosphere platform, we created a VM to be used in the fields of ecological and functional genomics. The VM includes various binary tools and R statistics packages used in ecology and genetics research and for plotting complex data in graphs. Our lab contributed directly to the development of the Taxonomic Name Resolution Service (TNRS), a platform to help standardize taxonomic names for all plant species—a nontrivial task. Erroneous and synonymous taxonomic names are a major challenge for virtually every field of plant biology. Large organismal databases (GBIF, SpeciesLink, VegBank, SALVIAS, TraitNet, GenBank, TreeBASE) are plagued by taxonomic error and uncertainty. In some databases, up to 30% of names do not match to any published name. Furthermore, 5%–20% of published names may be synonymous. Correcting and harmonizing taxonomy is the time-consuming and ad hoc responsibility of the individual researcher. The TNRS tool overcomes this barrier, enabling higher-quality comparative biodiversity science. The TNRS is available to other researchers wishing to perform similar taxonomic name resolutions on their data sets, enabling a wider community to expand the public scientific knowledge base. The TNRS is currently available at http://tnrs.iplantcollaborative.org and is described in a recent publication in *BMC Bioinformatics.* A major mission of iPlant is to promote adoption of the cyberinfrastructure through training workshops and outreach at academic institutions and scientific meetings. In 2012, members of our lab participated as instructors in several tools and services workshops focused on transcriptomics, GWAS, and phylogenetics using the DE, Atmosphere, and Data Store platforms.

KBase, the Department of Energy Systems Biology Knowledgebase

S. Pasternak, J. Thomason, S. Kumari, J.-M. Chia

The Department of Energy Systems Biology Knowledgebase (KBase, www.kbase.us) is a cyberinfrastructure collaborative led by principal investigator Adam Arkin of Lawrence Berkeley National Laboratory (LBNL), with co-principal investigators Rick Stevens of Argonne National Laboratory (ANL), Robert Cottingham of Oak Ridge National Laboratory (ORNL), and Sergei Maslov of Brookhaven National Laboratory. In addition to Doreen Ware at CSHL, participating investigators include Pamela Ronald of the University of California, Davis, Matthew DeJongh of Hope College in Michigan, Gary Olsen of the University of Illinois at Urbana-Champaign, and Mark Gerstein of Yale University. KBase is a software and data environment designed to accelerate large-scale collaborative research in fields that impact bioenergy. KBase leverages the power of high-performance and cloud-based computing resources across the DOE system of labs. Built on this foundation is a community-driven, extensible, and scalable open-source software framework and application system. This free and open-access infrastructure will handle the anticipated rapid growth in data volumes and computing requirements necessary for predictive modeling in microbes, microbial communities, and plants. Future functionality will focus on metabolic engineering, regulatory modeling, improved annotation, complex querying of microbial communities, assembly, and network-based functional ortholog prediction. Doreen Ware serves as the Plants Science Team Lead for KBase. In addition to providing domain expertise in plant genomics, members of our lab are making significant contributions to software development for this project. The Genotype Phenotype Workbench provides a platform for users to explore various data sets stored in KBase using genome-wide association studies as a starting point. The workbench is designed for seamless visual interaction across stored "omics" data sets. The Network Workbench enables users to explore KBase networks using dynamic visualizations. Users navigate among sets of genes and clusters over a variety of network data sets available for microbes, plants, and metagenomic communities. The IRIS interface allows users to run all of the KBase command line scripts and useful UNIX tools from a web-based interface without downloading any software.

The interface allows users to keep track of results and history by means of the KBase IRIS server. The next release of KBase will offer a unified environment enabling users to view, query, and download data for microbes, plants, and microbial communities. Users will also be able to apply a series of powerful demonstration workflows, including genome annotation, metabolic modeling, and phenotype analysis.

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QUANTITATIVE BIOLOGY

Gurinder S. "Mickey" Atwal and colleagues are applying insights from the physical and computational sciences to the study of population genetics and human disease. In collaboration with colleagues at the Institute for Advanced Study, Princeton, Atwal has modeled the process by which genetic variants, or alleles, have evolved in the last 100,000 years of human history. This has recently led to surprising insights about the role in female fertility played by p53, a master tumor suppressor gene, and furthered our understanding of how complex gene networks evolve. Recently, Atwal has analyzed the physical organization of the cancer genome and its role in mediating tumorigenesis across numerous tissue types.

Ivan Iossifov focuses on the development of new methods and tools for genomic sequence analysis and for building and using molecular networks, and he applies them to specific biomedical problems. He studies the genetics of common diseases in humans using two main tools: next-generation sequencing and molecular networks representing functional relationships among genetic loci. These approaches in combination enable the kind of large-scale studies necessary for furthering our understanding of the complex etiology of disorders such as autism, bipolar disorder, and cancer.

Alexander Krasnitz and colleagues use mathematical and statistical tools to discover key genetic elements involved in cancer and to understand how cancer cells evolve. Array-based comparative genome hybridization, a technique honed in the Wigler lab, and, more recently, sequencing experiments, have revealed subtle patterns of frequent and widespread aberration in cancer genomes. Krasnitz hypothesizes that aberrant genomic loci observed to recur in a range of cancer types are under selection and therefore are enriched in important cancer genes. He has developed a novel, comprehensive methodology to discover such "epicenters" and has used it to analyze multiple genome data sets in breast, liver, ovarian, and prostate cancer. The results have been shared with cancer biology labs across CSHL, and they have been a key enabling agent of functional studies using mouse models and RNA interference. Krasnitz has begun to apply advanced statistical methods to the latest generation of experimental data, which have characterized tumor samples down to the level of single cells. With such data, he and colleagues seek to learn how specific tumors evolve and how cancer cells migrate in metastasis.

There is increasing evidence that rare and unique mutations have a significant role in the etiology of many diseases such as autism, congenital heart disease, and cancer. **Dan Levy**'s group develops algorithms to identify these mutations from large, high-throughput data sets comprising thousands of nuclear families. After earlier working with high-resolution CGH arrays, Levy's group now uses targeted sequence data. Levy has developed methods for identifying de novo mutations (i.e., those seen in a child but not in his or her parents) by simultaneously genotyping the entire family; the team is currently focused on building algorithms to detect copy-number variants and multiscale genomic rearrangements. Although their copy-number methods are based on "read" density, there are classes of mutations that require analysis at the level of the read. Thus, they are developing algorithms to identify insertions, deletions, inversions, transpositions, and other complex events. Other projects in the Levy lab include analysis of single-cell RNA, phylogenetic reconstruction from sparse data sets, and disentangling haplotypes from sperm and subgenomic sequence data.

Michael Schatz is a computational biologist and an expert at large-scale computational examination of DNA sequencing data including the alignment, assembly, and analysis of next-generation sequencing reads. These methods have been used to reconstruct the genomes of previously

unsequenced organisms, probe sequence variations, and explore a host of biological features across the tree of life. Recent improvements in sequencing technologies are challenging our capacity to store and analyze the huge volume of DNA sequence data being generated. Consequently, Schatz is particularly interested in capitalizing on the latest advances in distributed and parallel computing, especially cloud computing technologies, to advance the state of the art in bioinformatics and genomics. In a recent breakthrough, Schatz was able to create a hybrid software-based solution to eliminate errors in so-called third-generation sequencing. This makes it remarkably easier to compile, align, and analyze full-genome sequences.

QUANTITATIVE BIOLOGY

G.S. Atwal R. Aboukhalil W. Liao
Y. Cai Y. Mo
B. Fendler S. Thomain
J. Homburger

Fueled by data generated from recent technological developments in DNA sequencing, our lab is primarily focused on population genetics, cancer biology, and high-performance computing. We often tackle scientific questions analytically and computationally by invoking theoretical concepts from statistical physics and machine learning. Previous work in our lab, in collaboration with colleagues at the Cancer Institute of New Jersey and Weill Cornell Medical College, has established the association between single-nucleotide polymorphisms (SNPs) in the p53 tumor suppressor pathway and female infertility in mice and humans. This hypothesis was first generated through computational investigations of haplotypic diversity and positive selection in genes in the TP53 pathway, where we detected signatures of recent positive selection in SNPs present at high frequencies in various human populations. Selected alleles in SNPs in the *LIF, Mdm2, Mdm4*, and *Hausp* genes, each of which lies in the p53 molecular network, were found to be enriched in women undergoing in vitro fertilization (IVF) treatment. Moreover, some of these SNPs have been demonstrated to be associated with estrogen-driven cancer risk, highlighting the pleiotropic character of genetic variants in the p53 pathway. More recently, we have begun to investigate the association of SNPs in p63 and p73, and the initial results are encouraging, although we await an increase in sample numbers before the results can be deemed to be statistically significant. Following up on these earlier investigations, we have begun exploring the contribution of multiple alleles from both a population genetics and human disease perspective. This systems biology approach has led to the development of new computational tools that can address the vast complexity of genomics data. In addition, our lab has continued research in the use of information theory and other machine learning tools to address the tsunami of data generated by next-generation sequencing. Suyash Shringapure, a former visiting researcher from Carnegie Mellon, now at Stanford University, continues to collaborate with us and Bud Mishra (New York University). Bernard Fendler is a postdoctoral associate with training in theoretical physics. Willey Liao, Yifan Mo, and Ying Cai are graduate students from the Applied Mathematics and Statistics department at Stony Brook. Yifan Mo graduated in December and took a postdoctoral position at Memorial Sloan-Kettering Cancer Center. Julian Homburger was an URP student during the summer of 2012 and continues to work with us remotely. Sophie Thomain carried out a rotation in our lab during the summer. Robert Aboukhalil, a graduate student in the Watson School, joined the lab, following up on his earlier research as an URP student in the same lab.

Conserved Colocalization of Tumor Suppressor Genes

B. Fendler, R. Aboukhalil [in collaboration with S. Lowe, Memorial Sloan-Kettering Cancer Center; S. Powers, D. Esposito, and W. Xue, Massachusetts Institute of Technology]

During the last decade, several studies have demonstrated, across many eukaryotic species, that genomes contain chromosomal regions in which functionally related genes physically cluster. Although it is well known that operons, ubiquitous in prokaryotes, allow multiple genes to be transcribed at once into a polycistronic mRNA, operons are rare in eukaryotes. However, there is evidence to suggest that genes within the same biological pathway may be clustered more than expected by random rearrangements (possibly because of coregulation), although the extent to which genes colocalize in eukaryotes is largely unknown.

Tumor suppressor genes (TSGs) are central to our understanding of human tumorigenesis. However, our knowledge of the physical distribution of TSGs

throughout the genome, and the implications that this has for tumor development, is poor. We carried out an integrative analysis of the known TSGs and demonstrated significant colocalization of these genes into conserved clusters throughout the genome. We provide evidence of natural selection enforcing this colocalization in 46 species, ranging from mammals to worms. Analysis of 5659 human expression arrays reveals that proximal TSGs exhibit significant positively correlated coexpression, after correcting for batch effects by singular value decomposition. Finally, we analyzed copy-number variation from 1199 tumor samples across 11 different tissue types and showed that the probability of TSG deletion grows with the number of TSGs in the cluster. These results demonstrate that the evolution of the physical distribution of TSGs bears significantly on the risk of cancer development.

Web Tool for Multispecies Gene Colocalization Analysis

R. Aboukhalil, B. Fendler [with technical assistance from P. Andrews, Cold Spring Harbor Laboratory]

The evolutionary pressures that underlie the large-scale functional organization of the genome are not well understood in eukaryotes. Recent evidence suggests that functionally similar genes may colocalize (cluster) in the eukaryotic genome, suggesting the role of chromatin-level gene regulation in shaping the physical distribution of coordinated genes. However, few of the bioinformatics tools currently available allow for a systematic study of gene colocalization across several evolutionarily distant species. Furthermore, most tools require the user to input manually curated lists of gene position information, DNA sequence, or gene homology relations between species. With the growing number of sequenced genomes, there is a need to provide new comparative genomics tools that can address the analysis of multispecies gene colocalization. We built Kerfuffle, a web tool designed to help discover, visualize, and quantify the physical organization of genomes by identifying significant gene colocalization and conservation across the assembled genomes of available species (currently up to 47, from humans to worms). This is the first such web tool from the newly installed web server set up in the Quantitative Biology group. Kerfuffle only requires the user to specify a list of human genes and the names of other species of interest. Without further input from the user, the software queries the e!Ensembl BioMart server to obtain positional information and discovers homology relationships in all genes and species specified. Using this information, Kerfuffle performs a multispecies clustering analysis, presents downloadable lists of clustered genes, performs Monte Carlo statistical significance calculations, estimates how conserved gene clusters are across species, plots histograms and interactive graphs, allows users to save their queries, and generates a downloadable visualization of the clusters using the Circos software. These analyses may be used to further explore the functional roles of gene clusters by interrogating the enriched molecular pathways associated with each cluster.

Cell-Type-Specific Spatiotemporal Gene Expression Dynamics Reflects the Cerebellar GABAergic Circuit Development

Y. Cai [in collaboration with Huang Lab, Cold Spring Harbor Laboratory]

We have analyzed the temporal gene expression dynamics of two GABAergic neuron subtypes in developing mice. The objective was to unravel the cell-type-specific molecular correlates of a developing circuit—counter to the prevailing wisdom that expression landscape remains relatively static throughout postnatal development. We developed methods based on random matrix theory to determine the significance of principal component analyses of temporal expression profiles. A number of distinct temporal patterns, from day 0 to day 56, were found for a large subset of genes corresponding to distinct biological pathways. In addition, the research (1) uncovered groups of novel cell-type-specific genes; (2) captured the phasic expression dynamics of ion channels, receptors, cell adhesion molecules, gap junction proteins, and transcription factors; (3) revealed the active developmental modulation of different molecular pathways involved in circuit formation; and (4) yielded evidence of hundreds of genomically clustered and developmentally coexpressed transcripts, suggesting the involvement of chromatin regulatory forces in early postnatal development, as well as maintenance of cell type identity in the adult stages.

Maximally Informative Models and Diffeomorphic Modes

This work was done in collaboration with J. Kinney, here at Cold Spring Harbor Laboratory.

Motivated by data-rich experiments in transcriptional regulation and sensory neuroscience, we consider the following general problem in statistical inference. A system of interest, when exposed to a stimulus S, adopts a deterministic response R of which a noisy measurement M is made. Given a large number of measurements and corresponding stimuli, we wish to identify the correct "response function" relating R to S. However, the "noise function" relating M to R is unknown a priori. Here, we show that maximizing likelihood over both response functions and noise functions is equivalent to simply identifying maximally informative response functions, those that maximize the mutual information I[R;M] between predicted responses and corresponding measurements. Moreover, if the correct response function is in the class of models being explored, maximizing mutual information becomes equivalent to simultaneously maximizing every dependence measure that satisfies the data processing inequality. We note that experiments of the type considered are unable to distinguish between parametrized response functions lying along certain "diffeomorphic modes" in parameter space. We show how to derive these diffeomorphic modes and observe, fortunately, that such modes typically span a very low-dimensional subspace. Therefore, given sufficient data, maximizing mutual information can pinpoint nearly all response function parameters without requiring any model of experimental noise.

Recent Coselection in Human Populations

This work was done in collaboration with S. Shringapure at Stanford University and B. Mishra at New York University.

A variety of selective forces, including diverse habitats and diseases, have been acting on human populations since their earliest migration out of Africa. Studying recent natural selection through statistical analysis of human genetic polymorphisms is therefore an important way of illuminating this recent human history. In the last few years, a number of methods have been proposed for detecting recent natural selection. These methods have found a large of number of genes in human populations showing strong signals of recent natural selection, but they are incapable of detecting networks of genes (not necessarily syntenic) that might have responded to shared selective forces. For instance, because many of these genes are involved in various common biological pathways and networks, we hypothesize that selection pressure on one gene in a network will result in a simultaneous selection pressure on other genes in the network. We have analyzed SNP data from the HapMap project to find candidates for recent coselection in human populations.

Our methodology is built upon a novel "entropic test," based on haplotype statistics and extended to the joint analysis of pairs of SNPs. The underlying idea, formulated using information theoretic entropy concepts, is that the joint genetic diversity, or inverse linkage disequilibrium, around coselected alleles would be reduced with respect to the rest of the genome. We demonstrate the efficacy of the coselection test by analyzing large-scale computer simulations of Wright-Fisher models, incorporating the effects of neutral drift arising from finite sampling in each generation, and joint positive selection of pairs of alleles in large populations. We analyzed SNP data from the HapMap project (Phase 3) to find candidate SNP pairs that exhibit statistical signatures of recent coselection in the 11 HapMap populations. Our results indicate that a number of functionally coherent gene pairs have undergone coselection in the HapMap populations. We also observe that the selected gene pairs are population specific, suggesting that these pairs may be involved with local adaptation.

Exome Studies of Rare Variants in Neuropsychiatric Disorders

J. Homburger [in collaboration with D. Lewis and D. McCombie, Cold Spring Harbor Laboratory]

Large genome-wide association studies have so far failed to identify genetic variants that explain much of the estimated heritability of complex human traits. Next-generation sequencing technologies have made whole-exome studies feasible, allowing the study of rare variants in the human population. The common disease rare variants hypothesis argues that much of the heritability of diseases could be due to multiple rare variants of moderate to large effect. However, novel statistical methods will be needed to find associations

between rare variants and disease. Studies must deal with issues such as locus heterogeneity and the excess of rare variants in the humane genome due to recent population expansions. We determined the efficiency of two commonly used statistical tests for rare variants (the weighted sums approach and variable threshold approach) and attempted to develop new statistical techniques with increased power. Toward this goal, we simulated a whole-exome data set based on parameters observed in the Exome Sequencing Project. We ran simulations under multiple experimental conditions, changing the parameters of samples chosen and number of disease-causing variants. Both the weighted sums test and variable threshold test performed poorly as the number of disease-causing variants increased. To deal with the issue of locus heterogeneity, we also proposed a new test based on the mutual exclusion of disease-causing variants.

Novel Foxo1-Dependent Transcriptional Programs Control T_{reg} Cell Function

Y. Mo, W. Liao [in collaboration with M. Zhang, University of Texas at Dallas; and M. Li, Memorial Sloan-Kettering Cancer Center]

Regulatory T (T_{reg}) cells, characterized by expression of the transcription factor forkhead box P3 (Foxp3), maintain immune homeostasis by suppressing self-destructive immune responses. Foxp3 operates as a late-acting differentiation factor controlling T_{reg} cell homeostasis and function, whereas the early T_{reg}-cell-lineage commitment is regulated by the Akt kinase and forkhead box O (Foxo) family of transcription factors. However, whether Foxo proteins act beyond the T_{reg}-cell-commitment stage to control T_{reg} cell homeostasis and function remains largely unexplored. The Li lab showed that Foxo1 is a pivotal regulator of T_{reg} cell function. T_{reg} cells express high amounts of Foxo1 and display reduced T-cell-receptor-induced Akt activation, Foxo1 phosphorylation, and Foxo1 nuclear exclusion. Mice with T_{reg}-cell-specific deletion of Foxo1 develop a fatal inflammatory disorder similar in severity to that seen in Foxp3-deficient mice, but without the loss of T_{reg} cells. Yifan Mo and Willey Liao conducted a systematic genome-wide analysis of Foxo1 binding sites, revealing 300 Foxo1-bound target genes, including the proinflammatory cytokine Ifng, that do not seem to be directly regulated by Foxp3. These findings show that the evolutionarily ancient Akt–Foxo1 signaling module controls a novel genetic program indispensable for T-cell function.

Modeling Morphogenesis in Leaf Polarity

S. Thomain [in collaboration with the Timmermans Lab, Cold Spring Harbor Laboratory]

The adaxial-abaxial polarity is essential for leaf functioning in plants. The polarity is sharply defined by a precise establishment of gene expression patterns with cells across the adaxial-abaxial axis. Previous work in the Timmermans Lab has shown that two short RNAs (miR166 and tasiR-ARF) may act as morphogens in establishing the leaf polarity. However, it is not clear whether these two opposite gradients of small RNA are sufficient to explain the sharp spatial expression pattern in leaves. We first modeled the dynamic behavior of the morphogen using a minimal network of the relevant genes, whereby tasiR-ARF inhibits ARF3/4 and miR166 inhibits HD-ZIPIII. A fixed-point analysis indicated that the system is stable with respect to small perturbations, although the influence of stochastic noise has yet to be determined. We next investigated the positional information carried by the morphogens using tools from information theory in an effort to determine how a cell optimally predicts its position in the adaxial-abaxial axis using the two opposing morphogens. The analysis from simulations of the stochastic concentration gradients indicated that the two RNA gradients were indeed sufficient to convey enough information to induce two cell fates. Further work remains to supplement the simulations with actual RNA-Seq data from each cell layer.

Network Inference in Cancer

B. Fendler, Y. Cai

An important challenge in cancer systems biology is to uncover the complex network of interactions between genes (tumor suppressor genes and oncogenes) implicated in cancer. Next-generation sequencing provides unparalleled ability to probe the expression levels of the entire set of cancer genes and their transcript isoforms. However, there are onerous statistical and computational issues in interpreting

high-dimensional sequencing data and inferring the underlying genetic network. In this study, we analyzed RNA-Seq data from lymphoblastoid cell lines derived from a population of 69 human individuals (Yoruban population) and implemented a probabilistic framework to construct biologically relevant genetic networks. In particular, we used a graphical lasso analysis, motivated by considerations of the maximum entropy formalism, to estimate the sparse inverse covariance matrix of RNA-Seq data. Reverse engineering the network of genetic isoforms revealed a layer of genetic regulatory complexity not exhibited by traditional microarrays. Gene ontology, pathway enrichment and protein–protein path length analysis were all carried out to validate the biological context of the predicted network of interacting cancer gene isoforms.

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DE NOVO MUTATION IN AUTISM

I. Iossifov E. Dalkic

We study the genetics of common diseases in humans, using two main tools: next-generation sequencing and molecular networks representing functional relationship among genetic loci. These tools in combination enable the large-scale studies that are necessary for furthering our understanding of the complex etiology of disorders such as autism, bipolar disorder, and cancer. We focus on both developing new methods (for sequence analysis and for building and using molecular networks) and applying them to specific biomedical problems.

Exome Sequencing Reveals De Novo Gene Disruptions in Children on the Autistic Spectrum

The genetic component of autism can be transmitted or acquired through de novo mutation. Previous studies have focused on large-scale de novo copy-number events, either deletions or duplications, and have identified a large number of autism candidate genes. Because copy-number events often span many genes, discerning which of the genes in the target region contribute to the disorder requires complex network analysis and inference. In contrast, with high-throughput DNA sequencing, we can readily search for de novo single-nucleotide and small-insertion or deletion mutations that affect a single gene. Such mutation is fairly common, ~100 new mutations per child, but with only a few—on the order of one per child—falling in coding regions.

We are collaborating in an ongoing large project for sequencing the exomes of 2800 families from the Simons Simplex Collection (SSC). Our preliminary analysis (Iossifov et al. 2012) of ~350 of these families, as well as the reports of three other groups, demonstrated the power of identifying de novo mutations through exome sequencing to implicate autism genes. The following were among the major results: (1) strong evidence for the role of likely gene-disrupting (LGD) mutations (nonsense, splice site, and frame shifts), with affecteds having twice as many LGDs compared to unaffected siblings; (2) the identification of 127 de novo LGD mutations across the four reports with five genes (*CHD8*, *DYRK1A*, *KATNAL2*, *POGZ*, *SCN2A*) having two de novo mutations in unrelated individuals ("double hits"); (3) estimation of a total number of ~400 autism target genes, with a prediction of ~100 double hits after the entire set of 2800 families had been processed; and (4) the discovery of a strong association between the target LGD mutations in autism and in vivo targets of the RNA-binding translational regulator FMRP (encoded by *FMR1*, which results in fragile-X syndrome when silenced or mutated). Our preliminary assessment has not seen evidence for a role of de novo missense mutations or inherited LGD variants in autism pathogenesis, but with a greater sample size, we hope to detect such effects (if present). We expect to present the results of the joint analysis of ~1000 families by November 2012.

Ongoing Projects

- ***Whole-exome sequencing of the SSC.*** The SSC comprises 2800 families with one child with autism spectrum disorder diagnosis and with at least one unaffected child. Such a collection is ideally suited for identification of de novo mutations with a strong effect on the disorder. The recent improvements of the techniques for enrichment of coding genomic sequences and of the new-generation sequencing technology made whole-exome sequencing for large numbers of samples feasible. The generated data are of high quality and can be used to identify de novo single-nucleotide, short-indel, and copy-number mutations. This project is being done in collaboration with Michael Wigler and Richard McCombie here at CSHL.
- ***Analysis of the overall sharing of parental genome by concordant and discordant autistic siblings.*** The goal is to test the hypothesis that a substantial proportion of the inherited autism is caused by a dominant mutation inherited from the mother,

with different mothers having different affected loci. So far, we see a suggestive increase of the sharing of the mothers' genome in a cohort of ~300 multiplex families from the AGRE collection with microsatellite markers. The next step is to see if the signal is preserved and hopefully statistically significant in a larger set of 700 families genotyped with a 10K single nucleotide polymorphism (SNP) array. This project is being done in collaboration with Michael Wigler and Kenny Ye.

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Iossifov I, Ronemus M, Levy D, Wang Z, Hakker I, Rosenbaum J, Yamrom B, Lee Y, Narzisi G, Leotta A, et al. 2012. De novo gene disruptions in children on the autistic spectrum. *Neuron* **74:** 285–299.

IN SILICO CANCER GENOMICS

A. Krasnitz E. Glassberg
M. Kato
G. Sun

Research in our group is focused on in silico cancer genomics. In the last few years, there has been explosive growth in the volume, as well as the quality and detail, of cancer-related genomic data available for analysis. This development came about through rapid accumulation of public data sets generated by individual laboratories worldwide, through large-scale cancer genome projects such as TCGA and ICGC, and with the advent of new experimental methodologies, especially next-generation sequencing and single-cell genomics. We see our goal in channeling this flood of data into a number of clinically relevant applications, including pinpointing and prioritizing targets for functional analysis, discovery of genomic markers for clinical outcome and molecular classification of cancer, and elucidating the clonal structure of tumors and its relation to progression, invasion, metastasis, and response to treatment. All of our work is done in close coordination with experimental studies performed by the Wigler, Hicks, Powers, Lowe (presently at the Memorial Sloan-Kettering Cancer Center), and Stillman laboratories at CSHL.

Recurrence Analysis of Genomic Interval Data

Large collections of intervals are a common form of data in high-throughput genomics. In the context of ChIP-Seq (chromatin immunoprecipitation–high-throughput sequencing) or CLIP-Seq (cross-linking immunoprecipitation–Seq) analysis, these intervals represent fragments of DNA or RNA. DNA copy-number analysis yields intervals of the genome corresponding to gains or losses of copy number. In all cases, a common goal is inference of contiguous genomic regions covered by the intervals with a high degree of recurrence. We call such regions cores. In cancer, genomes display complex patterns of DNA copy-number alteration, but recurrent aberrations are observed within a given cancer type. Detection and quantitative characterization of the cores in a given malignancy are potentially beneficial in two ways. First, it is plausible that at least some of the recurrence owes to selective pressure on regions harboring cancer-related genes. Focusing on these regions may therefore facilitate cancer gene discovery. Second, once the cores are known, the genome of a tumor can be described in a drastically simplified fashion by indicating the presence or absence of copy-number alteration in any given core. This simplified form of the data is better suited for further analysis, such as finding subtypes in a given type of cancer or discovering associations between genomic properties and clinical parameters.

We designed and implemented a method for identifying cores in large collections of genomic interval data. A descriptive name for the method is cores of recurrent events (CORE). Central to CORE is the notion of explanatory power. We say that a core explains an event and quantify its explanatory power by means of a numerical value between 0 and 1. The explanation is a measure of how closely the event is matched by the core. We then seek a set of cores that jointly provide the best possible explanation of the data, subject to additional criteria of statistical significance. Figure 1 shows an example of CORE analysis applied to a set of 257 copy-number profiles of breast tumors. Cores delineate regions of recurrent somatic copy-number variation in breast cancer, such as whole-arm amplification of chromosome 1 and deletion of chromosome 16 or narrow amplification containing the ERBB2 oncogene on chromosome 17. In the course of 2012, we created a software implementation of CORE as an R package and applied CORE to a number of problems in genomics, as discussed below.

Structure of Tumor Cell Populations from Single-Cell Sequencing

Recent studies of cancer genomes on a single-cell level have revealed the complexity of the disease and the

Figure 1. Regions (cores) of recurrent copy-number aberration identified in a published set of 257 breast tumor samples. The 44 amplification (22 deletion) cores are shown above (below) the corresponding chromosomes. Darker shades of gray correspond to higher degrees of recurrence. Genomic positions of four validated driver genes (MYC, CCND1, IGF1R, and ERBB2) are indicated by vertical bars.

presence of multiple genealogically related cell populations in a tumor. Detailed knowledge of the clonal structure of a cancer potentially is of high clinical value: Multiplicity of clones or of lesions in most advanced clones is a possible measure of progression; spatial pattern of clone dispersal in a tumor may signal elevated propensity to invade; and lesions observed in individual clones but not in the bulk tissue may point to targets for therapy. DNA copy-number profiling of cells from low-coverage sequencing is an accurate, economically feasible technological approach to the study of cancer subpopulation structure. Novel multiplex sequencing techniques, developed by the Wigler lab at CSHL, permit simultaneous sequencing of dozens of single-cell DNA specimens and their subsequent copy-number profiling at up to 50-kb resolution. Optimal use of these data forms for robust reconstruction of cancer cell phylogenies is a challenging computational problem requiring new and robust informatics and statistical tools. At present, no such robust algorithmic methods and software tools exist for computational reconstruction of tumor population structure and genealogy from such single-cell copy-number data. Indeed, phylogenetic analysis can only be performed if

the biological entities to be analyzed are specified in terms of a well-defined set of characters. If the genome sequence of each entity is known in detail, the set of characters is formed by residues at individual genomic positions. However, no obvious choice of a character set for phylogeny exists if the entities are given as DNA copy-number profiles. As a remedy, we used CORE to transform copy-number profiles of single cells into a form suitable for phylogeny. As a result of this transformation, each profile is characterized in terms of presence or absence in it of archetypal copy-number events recurrent in the single-cell populations. The entire set of copy-number profiles of single cells sampled from a tumor is summarized as an incidence table, essentially a matrix with cores as columns, single cells as rows, and elements quantifying, on a scale between 0 and 1, how well a given core is matched by a copy-number event in a given cell. Distances among cells as rows of this matrix can be readily computed and used for distance-based phylogeny. This methodology was used successfully to reconstruct the cell population structure in a number of samples from breast and prostate cancers. In particular, in one of the cases of breast cancer, we observed that aneuploid cells from the primary site and those from the liver metastasis form separate branches of a phylogenetic tree. A similar analysis, applied to a case of prostate cancer, shows that some of the cells from an area with pathological characteristics of carcinoma in situ bear close genomic similarity to those from a region affected by frank carcinoma.

Markers of Response to Anthracycline Therapy in Breast Cancer

Anthracyclines are a class of chemotherapeutic drugs that are effective and widely used in treatment of a number of cancers, in particular those of the breast. Sensitivity to these agents varies widely across the entire population of patients and likely depends on the pattern of genomic alterations in the tumor. Knowledge of genomic biomarkers of response to anthracyclines will help tailor treatment options to the needs of individual patients. With this goal in mind, we recently applied CORE to DNA copy-number profiles derived by the Hicks lab at CSHL from breast tumors of ~250 patients enrolled in a clinical study. The study was conducted by the Bartlett group at the Ontario Institute for Cancer Research. To discover markers of sensitivity to anthracyclines, we performed CORE analysis of the set of profiles and computed the incidence table. Next, we evaluated each core as a prospective marker of sensitivity and found dramatically higher benefit from treatment in patients positive for a narrow amplified region of chromosome 8. None of the marker-positive patients treated by anthracyclines have suffered a relapse in 5 years past treatment, whereas the marker-positive patients who were not treated displayed a markedly higher rate of relapse than the remainder of the cohort. This marker will be further examined as part of a clinical study being planned by our collaborators.

CORE and Nucleosome Positioning in Yeast

Nucleosomes—octamers of basic histone proteins wrapped with ~147 bp of DNA and connected by a short stretch of linker DNA—form the primary level of chromatin architecture. Chromatin serves to condense DNA by ~10,000-fold, allowing large molecules of DNA to be contained within the nuclei of eukaryotic cells. Chromatin also modulates the accessibility of varying regions of DNA, thus regulating gene expression, DNA replication, recombination and repair, chromosome segregation, and other critical cellular processes. Thus, there is significant biological interest in elucidating nucleosome positions within the genome. In some species, such as yeast, nucleosomes tend to occupy fixed positions across the entire population, facilitating the determination of these positions. A method of choice for this purpose exploits protection by nucleosomes of DNA against digestion by micrococcal nuclease (MNase). With a suitable choice of digestion regime, DNA fragments left intact will roughly correspond to nucleosomes. Once the fragment boundary coordinates have been determined by paired-end sequencing, nucleosome positions must be inferred from this input set of genomic intervals. CORE—as a method to identify accumulations of intervals with roughly coincident boundaries—lends itself naturally to nucleosome-positioning analysis. Moreover, an important advantage of CORE over other approaches to this problem is that the sizes of the core regions delineating the nucleosomes are entirely data-driven rather than fixed at 147 bp. Thus, CORE is better suited to discover partial unwinding of nucleosomes, a phenomenon closely related to gene regulation. With this motivation, we initiated, in collaboration with the Ptashne

lab at Memorial Sloan-Kettering Cancer Center and the Hicks lab at CSHL, CORE analysis of two massive sets of fragments of MNase-digested yeast DNA, one sequenced on the Illumina, and the other on the SOLiD platform. Our initial results show ample promise to justify further investigation. We find, in particular, that the spacing between consecutive nucleosome centers averages to 165 bp, corresponding to the expected 18 bp of linker DNA per nucleosome. We further see strong evidence for nucleosome phasing downstream from transcription start sites (TSS) in genic regions, and substantially less in intergenic regions upstream of TSS, where regulatory elements tend to be located. Our ongoing work aims at optimizing CORE as a tool to study this important aspect of chromatin structure.

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COMPUTATIONAL GENETICS

D. Levy

The data-rich environment at CSHL generates a wealth of opportunities for the application of mathematics and computation to further our understanding of biology and genetics. The primary activities of our lab are algorithm development, data analysis, and genetic theory, with a focus on sporadic human diseases such as autism, congenital heart disease, and cancer.

Collaborations

The work on autism genetics was done in collaboration with Michael Wigler, Ivan Iossifov, and Michael Ronemus. Copy number from exome sequence is the work of Kith Pradhan in collaboration with Chris Yoon. Peter Andrews implemented methods for exact sequence matching, haplotype phasing, and data processing. Single-cell methods for the haplotype study are the work of Ravi Kandasamy. Swagatam Mukhopadhyay contributed the implementation and analysis in our genetic modeling of autism.

Autism Genetics and New Mutation

Our efforts in autism, in collaboration with Michael Wigler and Ivan Iossifov, bridge between genetic theory and large-scale data analysis. In 2007, Zhao et al. (*Proc Natl Acad Sci 104:* 12831 [2007]) proposed a genetic theory of autism where new (or "de novo") mutations serve as an entry point for highly deleterious genetic lesions. This theory was supported by early studies showing an excess of de novo copy-number variations (CNVs) in autistic children and suggested a strategy for uncovering the set of genes whose disruption may result in autism. The Simons Simplex Collection (SSC) includes genetic samples and phenotypic data from families with exactly one child affected with an autism spectrum disorder (ASD). All 3000 families have samples collected from the mother, the father, the affected child, and typically at least one unaffected sibling. By identifying mutations that are present in the autistic child but absent in the parents and comparing to the observed rate in the unaffected siblings, we can infer a contributory rate for new mutations and identify likely genetic targets.

From 2008 to 2011, we generated and analyzed thousands of high-resolution CGH (comparative genome hybridization) arrays with 2.1 million data points per individual and, in the process, developed a set of tools to reduce system noise and uncover rare and de novo CNVs. The resulting study, published in 2011 by Levy et al. (*Neuron 70*: 886 [2011]), included 1000 SSC families and established a rate of de novo CNV of 8% in autistic children compared to 2% in their unaffected siblings. De novo CNVs were more frequent and larger in affected girls than affected boys, and we found evidence for excess transmission of rare variants to the affected children. In collaboration with Dorothy Warburton at Columbia University Medical Center, we conducted a similar study of 300 children with congenital heart disease (CHD) and found strong evidence of a causal role for de novo CNVs, with 10% of affected children having a de novo variant.

Although copy-number variants provide an excellent signal to background (4:1), even with the high resolution of the microarrays and the sophisticated informatics for denoising and analysis, most observable CNVs disrupt more than one gene. This necessarily complicates the effort of identifying causal genetic targets. With the availability of low-cost targeted sequencing methods, the SSC data analysis effort switched to identifying de novo point mutations and small insertion or deletion events (indels) in the exome. Our results from the first 350 families, published in Iossifov et al. (2012), are in agreement with the results of the other groups studying exomic mutation: de novo "loss-of-function" variants, those that severely impair gene function, occur in 20% of affected children and in only 10% of their siblings. Both the CNV and exome studies estimate that ~500 genes are likely involved in ASD.

Copy Number from Exome Sequence

Although we are no longer generating copy-number microarrays, we do have more than 1000 families with greater than 40x sequence coverage on the exome. The principal utility of these data is for its sequence content, but there is evidence that we can also extract copy-number variants. The primary challenge in identifying CNVs from exome capture data is that the capture process distorts coverage in ways that vary from sample to sample. Our initial expectation was that capturing and sequencing the family together would result in the same systemic distortion for all members in the family. That proved to not be the case. Even samples captured together showed variability in their coverage distortion, likely resulting from differences in sample handling prior to applying the capture protocol (sample acquisition, DNA extraction, etc.).

Fortunately, the variability expressed in an individual sample is often recapitulated on a population level, and we can use our library of samples to identify the major elements of systemic noise. Our normalization procedure is coupled to a hidden Markov model (HMM) that integrates over all the copy-number possibilities for a given sample and returns a probability for each copy state for each region of the genome. We then modify the expected coverage based on the results of the HMM, repeat our normalization procedure, and iterate until the results are unchanged. After identifying likely regions of CNV, we apply statistical tests over the segmented events in each family to determine the plausibility of the CNV. These results include tests for goodness of fit, rarity of the event in the population, and its pattern of inheritance.

There are four objectives to our present copy-number study. The first is to increase the number of de novo copy-number variants associated with ASD. The second is to look for an imbalance in small de novo CNVs—events that were too small to detect using CGH technology, but might be within our reach using the sequence data. The third is to test the claims with respect to rare inherited copy-number events and their unequal distribution to autistic children. And finally, we would like to combine the CNV analysis in the larger context of sequence data, for example, identifying SNPs that occur opposite a deletion and establishing parent of origin for de novo copy-number changes.

Insertions and Deletions by Exact Match

We are also developing an algorithm for identifying large genomic rearrangements—deletions, insertions, translocations, and inversions—by cataloging discontinuities in the mapping of reads against the reference genome. The majority of existing methods for finding such events approach the problem one read at a time, typically identifying a target region by anchoring a mate-pair and then fitting the misaligned read using a scoring algorithm with a mismatch/gap penalty. Elaborations on this method make a secondary correction to obtain consensus among gapped mappings per sample. These methods are reasonably good provided the event is small and occurs in an uncomplicated region.

Our algorithm uses all the reads over the whole population to make inferences about genomic rearrangements. Thanks to Moore's law, computational methods that were impractical at the dawn of genomic analysis are now feasible, fast, and useful. Suffix arrays require vast amounts of memory but perform very fast lookups for a query sequence of all its unique maximal exact matches (MEMs) to the human genome. By cataloging and indexing all reads in a sample by their MEMs, in particular those with two distinct matches into the reference genome, we identify and label recurrently observed discontinuities. Integrating this information across all samples, we can recognize common events, spurious rearrangements resulting from sequence homology, and global discrepancies within the reference. With all reads indexed and anchored to the reference genome, we reassemble rare and de novo discontinuities within an individual or family. Early versions of this method using fixed width exact matching and applied to the SSC exome data successfully identified large deletions, inversions, and insertions, and small indels, as well as rare and de novo pseudogenes.

Genetic Models of Autism

Autism is often described as the most highly heritable neurological disorder. Concordance for autism among identical twins is as high as 90%. There are several other interesting statistics in the epidemiology of autism that are less often noted. If a family has a single child with autism, the risk to the next born male child is ~20%. This elevated risk has led to an explanation

that autism is a multifactorial disorder and that several gene disruptions are needed to produce an autism phenotype. That claim, however, is belied by another statistic: Given two children in the family with autism, the risk to a third-born male child is 50%.

One model that fits these data is to postulate two risk classes for autism: Nearly all families have a very low risk of having a child with autism, whereas a small percentage of families have a very high risk. We have been exploring simple genetic models that recapitulate the observed risk statistics, both for boys and girls, as well as observations that stem from genetic analyses of ASD families. Our model includes parameters such as de novo mutation rates, variability of penetrance, assortative mating, and the number and type of genetic targets. Combinatorics allows for fast simulations of infinite populations, providing a means for quickly exploring the space of possible models consistent with the observable measures. One result is that a simple model with two gene classes and different selection profiles for males and females is sufficient to match observable risk and mutation rates.

Phasing from Haploid Cells and Subgenomic Data

Single nucleotide variants (SNVs) occur in the human genome at a rate of about one every 1000 bp. Two SNVs that are 1000 bp apart may both reside on the maternal copy of the chromosome, both on the paternal copy, or one on each. This information is known as the *phase* of the variants. Current high-throughput sequencing technologies typically require that the DNA be fragmented into pieces significantly smaller than 1 kb. This makes it difficult to obtain phase information because no single sequence read will contain both positions. Recent developments in sequencing small quantities of DNA invite new methods for obtaining this information. The two methods we are exploring include sequencing single haploid cells (sperm or egg) and genomic dilutions of large DNA fragments. By sequencing many such samples in independent wells, we can reconstruct a picture of the original genome with information about variant phasing.

The problems of phasing from small quantities of DNA are many. Single-cell sequencing methods are still in their infancy, and the resulting coverage is sparse and uneven. Recombination in the haploid cells scrambles phase information, occasionally confounded by more than one haploid cell entering a sequencing well. Furthermore, there are genomic positions that appear heterozygous due to copy-number polymorphism in a single parental haplotype.

We have constructed an algorithm for integer haplotype phasing (INHAPH) that simultaneously solves the problem of phasing the genome and reconstructing the identity of the fragments in each sample well. INHAPH is an expectation maximization algorithm that treats the observations of each well as the output of an HMM. The transitions of this model are determined by the type of data: For haploid cells, transitions depend on recombination rates; for subgenomic sequencing, transitions are determined by the expected number of fragments and their average length. The emission probabilities are drawn from the latest guess of parental haplotype phasing. Having analyzed each well in this way, we then update the parental haplotype phasing and repeat. For the case of single-cell sperm sequencing, INHAPH reconstructs the parental haplotypes, finds recombination points in each sperm, and determines whether each well contained one or two sperm cells.

We are currently running INHAPH on a few hundred sperm cells from a single individual. Results are good: At positions where we can determine phase and make a comparison, there is near perfect agreement between the INHAPH phasing and that inferred from family information. This provides us with confidence in the second half of the output—the detailed information about recombination observable in our samples. From these data, we are examining the personal recombination map against recombination maps drawn from large populations, identifying copy-number variants, and testing for gene conversions and segregation distortion.

Iossifov I, Ronemus M, Levy D, Wang Z, Hakker I, Rosenbaum J, Yamrom B, Lee Y-H, Narzisi G, Leotta A, et al. 2012. De novo gene disruptions in children on the autistic spectrum. *Neuron* **74:** 285–299.

COMPUTATIONAL SEQUENCE ANALYSIS

M.C. Schatz E. Biggers, A. Gupta, R. Gupta, J. Gurtowski, H. Lee, S. Marcus, G. Narzisi, D. Nettem, A.H. Wences

Our lab develops novel computational analysis methods to study the information content in biological sequences and the dynamics of biological processes. These include methods for assembling sequence data into complete genomes, for aligning sequences to discover variations or measure transcription levels, and for mining these data to discover relationships between an organism's genome and its traits. We then apply these methods to study several biological systems, for example, to discover mutations associated with human diseases such as cancer and autism or to reconstruct the genomes of important agricultural crops and biofuels. In the past year, our lab has grown by several new members. Dr. Shoshana Marcus started as a computational postdoctoral researcher after completing her Ph.D. in computer science at the City University of New York. Her thesis was on developing novel algorithms for advanced sequence alignment, and she is currently researching methods for genome assembly using emerging third-generation sequencing technology. Avijit Gupta, Rushil Gupta, and Deepak Nettem joined as master-degree students in computer science at Stony Brook University to research methods for large-scale genome assembly using cloud computing. Over the summer, Eric Biggers joined as an undergraduate researcher from Macalester College to develop new algorithms for analyzing heterogeneous genomes, focusing on the assembly of the pineapple genome. Alejandro Wences was a year-long visiting undergraduate student from the Genomics Institute of the University of Mexico in Cuernavaca, and he is researching methods for improving genome assembly by merging multiple assemblies into a single consensus sequence. In the past year, our lab has continued working on several projects studying the genetics of autism and of several important biofuel species and developing algorithms for genome assembly and large-scale sequence analysis. In addition, CSHL Assistant Professor Zachary Lippman and I were awarded a 3-year, $850,000 grant from the National Science Foundation (NSF) to study the genes and gene networks responsible for flower development in tomato and other related species. I was also funded to be a member of the NSF-sponsored iPlant consortium to develop algorithms and systems to empower plant research. Finally, CSHL Professor Greg Hannon and I were awarded a $500,000 instrumentation grant from the National Institutes of Health (NIH) to fund a major upgrade to the CSHL computing capacity, and along with funds from the Stanley Institute, we purchased a 1600-core cluster "Black-n-Blue." This cluster is substantially faster and more powerful than the previous cluster "BlueHelix" and will be a critical asset for CSHL in managing the flood of biological data created on campus. In May, I was awarded for the second time the CSHL Winship Herr Award for Excellence in Teaching for contributions to the CSHL course in Quantitative Biology, co-taught by M. Atwal. In the past year, I have been interviewed by the editors of *Nature, Nature Biotechnology, Nature Methods, Wired Magazine*, and *The New York Times*, especially to describe my work applying third-generation sequencing technology from Pacific Biosciences to human and plant genetics.

Hybrid Error Correction and De Novo Assembly of Single-Molecule Sequencing Reads

Single-molecule sequencing instruments can generate multi-kilobase sequences with the potential to greatly improve genome and transcriptome assembly. However, the error rates of single-molecule reads are high, which has limited their use thus far to resequencing bacteria. To address this limitation, we introduced a correction algorithm and assembly strategy that uses short, high-fidelity sequences to correct the error in

single-molecule sequences. We demonstrated the utility of this approach on reads generated by a PacBio RS instrument from phage, prokaryotic, and eukaryotic whole genomes, including the previously unsequenced genome of the parrot *Melopsittacus undulatus*, as well as for RNA-Seq reads of the corn (*Zea mays*) transcriptome. Our long-read correction achieves >99.9% base-call accuracy, leading to substantially better assemblies than current sequencing strategies: In the best example, the median contig size was quintupled relative to high-coverage, second-generation assemblies. Greater gains are predicted if read lengths continue to increase, including the prospect of single-contig bacterial chromosome assembly.

Genomic Dark Matter: The Reliability of Short-Read Mapping Illustrated by the Genome Mappability Score

Genome resequencing and short-read mapping are two of the primary tools of genomics used for many important applications. The current state of the art in mapping uses the quality values and mapping quality scores to evaluate the reliability of the mapping. These attributes, however, are assigned to individual reads and do not directly measure the problematic repeats across the genome. Here, we present the genome mappability score (GMS) as a novel measure of the complexity of resequencing a genome. The GMS is a weighted probability that any read could be unambiguously mapped to a given position, and thus, it measures the overall composition of the genome itself. We have developed the Genome Mappability Analyzer to compute the GMS of every position in a genome. It leverages the parallelism of cloud computing to analyze large genomes and enabled us to identify the 5%–14% of the human, mouse, fly, and yeast genomes that are difficult to analyze with short reads. We examined the accuracy of the widely used BWA/SAMtools polymorphism discovery pipeline in the context of the GMS and found discovery errors are dominated by false-negatives, especially in regions with poor GMS. These errors are fundamental to the mapping process and cannot be overcome by increasing coverage. As such, the GMS should be considered in every resequencing project to pinpoint the "dark matter" of the genome, including known clinically relevant variations in these regions.

De Novo Gene Disruptions in Children on the Autistic Spectrum

Exome sequencing of 343 families, each with a single child on the autism spectrum and at least one unaffected sibling, reveal de novo small indels and point substitutions, which come mostly from the paternal line in an age-dependent manner. We do not see significantly greater numbers of de novo missense mutations in affected versus unaffected children, but gene-disrupting mutations (nonsense, splice site, and frame shifts) are twice as frequent, 59 to 28. On the basis of this differential and the number of recurrent and total targets of gene disruption found in our and similar studies, we estimate between 350 and 400 autism susceptibility genes. Many of the disrupted genes in these studies are associated with the fragile X protein, FMRP, reinforcing links between autism and synaptic plasticity. We find FMRP-associated genes are under greater purifying selection than the remainder of genes and suggest that they are especially dosage-sensitive targets of cognitive disorders.

Answering the Demands of Digital Genomics

The continuing revolution in DNA sequencing and biological sensor technologies is driving a digital transformation to our approaches for observation, experimentation, and interpretation that form the foundation of modern biology and genomics. Whereas classical experiments were limited to thousands of hand-collected observations, today's improved sensors allow billions of digital observations and are improving at an exponential rate that exceeds Moore's Law. These improvements have made it possible to monitor the dynamics of biological processes on an unprecedented scale, but they have proportionally greater quantitative and computational requirements. The exponentially growing digital demands have motivated extensive research into improved algorithms and parallel systems. Recently, a great deal of research has been focused on applying emerging scalable computing systems to genomic research. One of the most promising is the Hadoop open-source implementation of MapReduce: It is specifically designed to scale to very large data sets, its intuitive design supports rich parallel algorithms, and it is naturally applied to analysis of many biological assays. There has also been success accelerating

numerically intensive genomics applications using heterogeneous processors such as graphics processing units (GPUs) and field-programmable gate arrays (FPGAs). These are promising early results, but it is clear that continued computational research will become even more important in the years to come.

The Rise of a Digital Immune System

Driven by million-fold improvements in biotechnology, biology is increasingly shifting toward high-resolution, quantitative approaches to study the molecular dynamics of entire populations. One exciting application enabled by this new era of biology is the "digital immune system." It would work in much the same way as an adaptive, biological immune system: by observing the microbial landscape, detecting potential threats, and neutralizing them before they spread beyond control. With the potential to have an enormous impact on public health, it is time to integrate the necessary biotechnology, computational, and organizational systems to seed the development of a global, sequencing-based pathogen surveillance system.

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Alejandro Hernandez Wences

QUANTITATIVE BIOLOGY FELLOW

Quantitative Biology Fellows are independent researchers who enter this position soon after receiving a Ph.D. They come from the fields of mathematics, physics, engineering, or computer science and spend 3–5 years at CSHL applying techniques from these disciplines to important questions in biology.

Justin Kinney completed his Ph.D. in Physics at Princeton University in 2008 and began his term as a Quantitative Biology Fellow in 2010. His research focuses on developing next-generation DNA sequencing as a tool for dissecting the structure and function of large macromolecular complexes. Of particular interest to his lab is the biophysical basis of transcriptional regulation—how simple interactions between proteins and DNA allow promoters and enhancers to modulate genes in response to physiological signals.

In 2010, Kinney and colleagues published a paper demonstrating Sort-Seq, a novel sequencing-based method that can measure the functional activity of hundreds of thousands of slightly mutated versions of a specific DNA sequence of interest. Using a novel information-theoretic analysis of the resulting data, Kinney et al. were able to quantitatively measure, in living cells, the protein–DNA and protein–protein interactions controlling mRNA transcription at a chosen promoter. Kinney continues to develop this approach using a combination of theory, computation, and experiment. From a biological standpoint, Sort-Seq allows researchers to investigate important but previously inaccessible biological systems. Kinney's lab is currently using Sort-Seq to address open problems in transcriptional regulation, DNA replication, and immunology. These experiments also present new challenges for the field of machine learning, and a substantial fraction of Kinney's efforts are devoted to addressing the theoretical and computational problems relevant to the analysis of Sort-Seq data.

Transcriptional Regulation, Biophysics, Machine Learning

J.B. Kinney

My lab pursues a combination of experiments, computation, and theory centered on the question of how elementary interactions between biological molecules allow the cell to regulate gene expression. Our experimental effort is focused largely on producing massive DNA sequence data sets that probe the inner workings of specific transcriptional regulatory sequences. To analyze these data, we use custom-written optimization software and high-performance computing. Some aspects of this analysis further touch on basic issues in machine learning and biophysics that have yet to be fully explored. So, in addition to our experimental and computational work, a portion of our effort is devoted to addressing basic but pertinent theoretical questions in biophysics and machine learning.

Programmable Inducibility in the *Escherichia coli* Transcriptional Regulatory Code

In collaboration with Rob Phillips (California Institute of Technology) and Curtis Callan (Princeton), my lab is pursuing experiments to investigate how the DNA sequence of promoters in the bacterium *E. coli* determines the quantitative aspects of transcriptional regulation. These experiments have, thus far, focused on the *E. coli lac* promoter. Decades of study have led to a detailed biochemical and structural understanding of how the *lac* promoter functions. The quantitative effects that the DNA sequence has on the *lac* promoter regulatory program, however, are not understood nearly as well. Indeed, it has proved exceedingly difficult to translate structural models into quantitative predictions about sequence-dependent activity. It seems likely that techniques that can directly probe the sequence dependence of protein–DNA and protein–protein interactions will

be needed to complement structural understanding before a complete description of the transcriptional regulatory code can be obtained. During my Ph.D. work, I helped to develop an experimental technique called "Sort-Seq" aimed at addressing this need. Sort-Seq uses ultra-high-throughput DNA sequencing and flow cytometry to provide precise quantitative information about how the biophysical mechanisms of a given transcriptional regulatory sequence are affected by DNA mutations (Kinney et al., *Proc Natl Acad Sci 107:* 9158 [2010]).

This assay was first tested on the *E. coli lac* promoter. Our initial analysis of the resulting data appeared to be consistent with the widely accepted model that the sequence of the RNA polymerase (RNAP)-binding site of the *lac* promoter encodes the energy with which RNAP binds DNA and that a protein–protein interaction of fixed energy between RNAP and the transcription factor CRP is the mechanism of transcriptional up-regulation. After repeating these experiments with Daniel Jones, a graduate student from Rob Phillips' lab, we noticed, however, that the energy of the CRP-RNAP interaction was not fixed, but rather, it exhibited substantial dependence on the sequence of the RNAP-binding site. Thus, by adjusting the sequence of the *lac* promoter RNAP-binding site, it is possible to independently vary two parameters: transcriptional activity in the absence of CRP and the fold-change by which CRP up-regulates transcription. We believe that this "programmable inducibility" may be a major and previously unappreciated component of the *E. coli* transcriptional regulatory code. Our current experimental work is now focused on deciphering this inducibility code, e.g., so that promoters with specified inducibility can be designed for applications in synthetic biology. We are also investigating the structural mechanism by which programmable inducibility is achieved.

Formal Diagrammatic Methods for Biochemical Systems

Analysis of Sort-Seq data requires fitting mathematical models that reflect hypothesized biophysical mechanisms for how a regulatory sequence of interest functions. But in doing so, my colleagues and I began to realize that, for all but the simplest systems, simply specifying and communicating models was problematic. This is because transcriptional regulatory systems, even if defined by just a small number of proteins, binding sites, and interaction rules, typically result in an exponentially large number of possible multiprotein–DNA complexes. Thermodynamic modeling of such systems requires that one compute the free energy of every possible complex, but this combinatorial explosion often makes listing the energy of each complex unfeasible.

One day, Phillips suggested to me that it might be possible to create formal diagrammatic methods for defining and analyzing biophysical models of transcriptional regulation, similar to how Feynman diagrams are used in quantum field theory. I was intrigued by this suggestion because such a diagrammatic method would also be applicable to a wide variety of other biochemical systems. And although Feynman diagrams are essentially just a bookkeeping device, it is difficult to overstate the importance of good bookkeeping when trying to figure out what a quantitative theory predicts.

I am currently finishing a proposal for one such diagrammatic technique. In this proposal, models of biochemical systems in thermal equilibrium are defined by diagrams representing elementary molecular components and interactions. Formal manipulations of these diagrams allow one to construct all possible multimolecule complexes. Even though the number of possible complexes can be very large, they can often be represented compactly by a small number of "compressed" diagrams. The grand partition function of the system can then be directly read from these compressed diagrams.

These diagrams, in fact, are literally the Feynman diagrams for a specific class of quantum field theories. These field theories, however, are unusual in a number of respects. In the simplest formulation, they have no dynamics and no space. The evaluation of individual Feynman diagrams is therefore trivial; the only challenge is counting them all. Use of these diagrams further allows one to solve the theory exactly, not just perturbatively. The connection to quantum field theory has important advantages. First, it provides a rigorous mathematical foundation for the stated diagrammatic methods. Moreover, these field theories closely resemble the Doi-Peliti formalism for the master equation, making it likely that these techniques can be extended in a natural way in order to treat nonequilibrium systems. Finally, this

connection suggests a straightforward manner in which computerized operator algebra might be used to study biochemical systems that are too large or complicated for analytical treatment.

Mutual Information as an Objective Function in Machine Learning

As is often the case in high-throughput biology, it is difficult to characterize and control noise in the Sort-Seq assay. Therefore, the fact that parametrized models can be fit to Sort-Seq data without having to model such noise is important. In my Ph.D. work, I helped show that maximizing the mutual information between a model's predictions and measurements provides a way of doing this. Specifically, in the large data limit, maximizing mutual information is mathematically equivalent to maximizing likelihood when the quantitative form of experimental noise is uncertain (Kinney et al., *Proc Natl Acad Sci 104:* 501 [2007]).

Alternatively, maximizing mutual information can be justified from the simple fact that it satisfies the data processing inequality (DPI), a fundamental relationship in information theory. However, there are other dependence measures that also satisfy DPI. This raises the question of whether maximizing a different DPI-satisfying measure will always lead to the same optimal model. In a preprint recently submitted to the arXiv (Kinney and Atwal 2012), Gurinder Atwal (CSHL) and I showed that in many cases, the answer to this question is "yes": When the correct model is in the space of models being considered, maximizing mutual information is provably equivalent to simultaneously maximizing all dependence measures that satisfy DPI. Thus, there exists a well-defined class of "DPI-optimal" models, all of which explain the data equally well according to every one of a large class of objective functions. Maximizing mutual information, however, often leaves certain model parameters unconstrained. These directions in parameter space were termed "diffeomorphic modes," because movement along them generates continuous invertible transformations of model predictions; such transformations affect neither mutual information nor any DPI-satisfying dependence measure. In our preprint, Atwal and I show that diffeomorphic modes result not from deficiencies of mutual information as an objective function, but rather from fundamental insensitivities of the type of experiments considered. Indeed, diffeomorphic modes are of a fundamentally different nature than other directions in parameter space; constraints on these modes do not depend on the number of data points and can only arise from separate calibration experiments.

In a separate arXiv preprint (Kinney and Atwal 2013), Atwal and I addressed the issue of "equitability" raised in a recent paper by Reshef et al. These authors argued that a measure of association between two stochastic variables should depend only on the relative signal-to-noise level, not on the underlying form of the relationship. Specifically, if $y = f(x) + noise$, then a dependence measure $D[x,y]$ should not depend on the specific functional form of f, but only on its amplitude relative to the amplitude of the noise. This proposal is sensible, but Reshef et al. come to the surprising conclusion that mutual information does not satisfy this definition of "equitability," and that a new statistical measure they propose, called the "maximal information coefficient" (MIC), does. In our preprint, Atwal and I show that this conclusion is incorrect. Specifically, we prove that no dependence measure $D[x,y]$ is able to satisfy the technical definition of equitability that Reshef et al. propose. However, the heuristic notion of equitability is still of value, and we show that it can instead be formalized in terms of a simple self-consistency condition closely related to DPI. We further prove that mutual information satisfies this revised definition of equitability while MIC does not. This finding was supported by a reexamination of the simulation evidence presented by Reshef et al.

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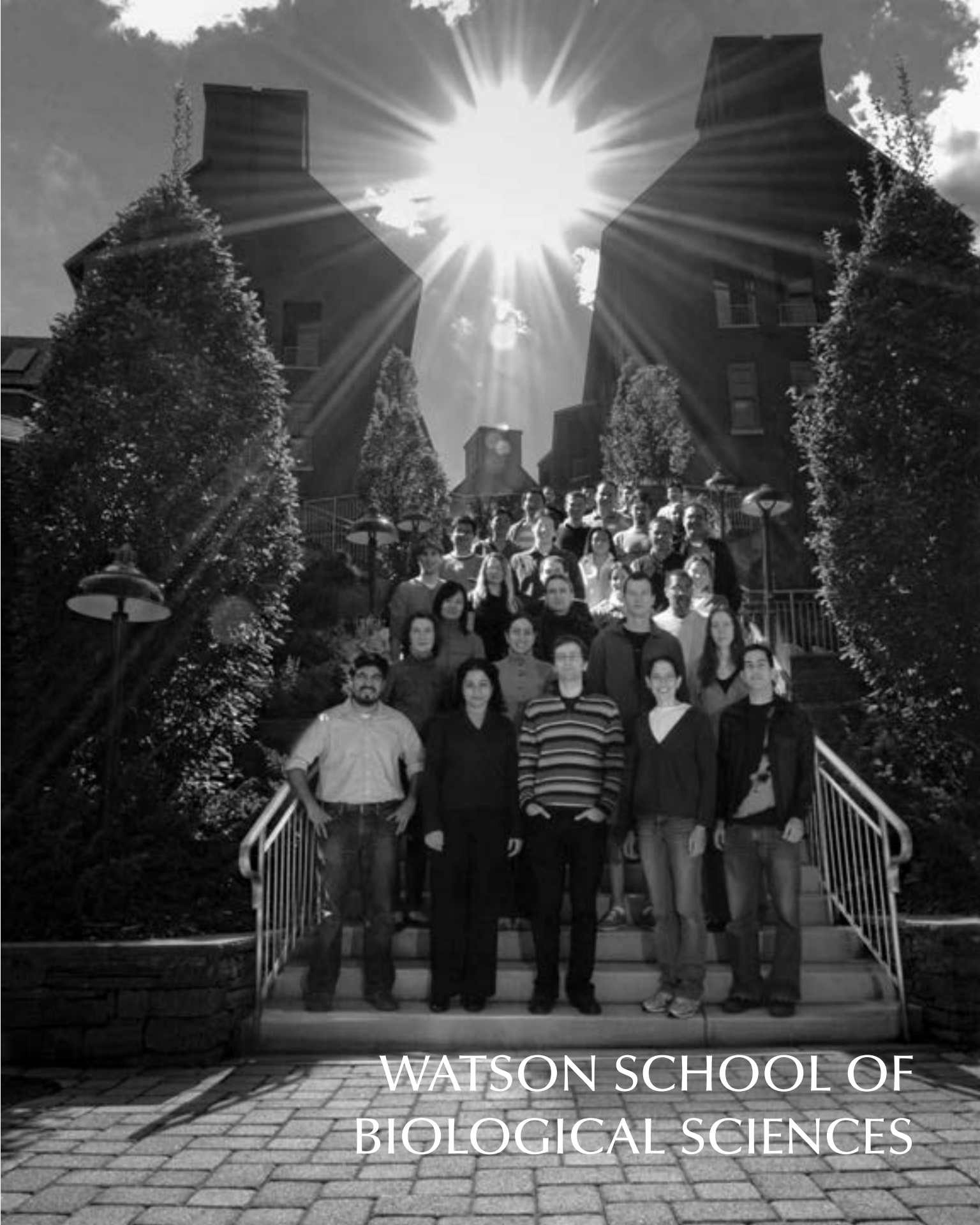
AUTHOR INDEX



WATSON SCHOOL OF BIOLOGICAL SCIENCES

ADMINISTRATION

Leemor Joshua-Tor, Ph.D., Professor and Dean (until August)
Adrian R. Krainer, Ph.D., Professor and Acting Dean (September–October)
Linda Van Aelst, Ph.D., Professor and Acting Dean (November–December)
Alyson Kass-Eisler, Ph.D., Postdoctoral Program Officer and Curriculum Director
Kimberley Geer, Administrative Assistant
Kimberly Creteur, M.Ed., M.S.Ed., Admissions Coordinator (from November)
Dawn Pologruto, B.A., Director, Admissions and Student Affairs (until September)
Keisha John, Ph.D., Associate Director, Recruitment and Undergraduate Research (until November)

EXECUTIVE COMMITTEE

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Leemor Joshua-Tor

Members
Mickey Atwal
Mikala Egeblad
Adrian R. Krainer
Linda Van Aelst
David Stewart
David L. Spector (Director of Research)

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Mitchell Bekritsky, WSBS
Deblina Chatterjee (until June)
Iona Rus (from June)

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Keisha John (until November)
Alyson Kass-Eisler (from November)

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WATSON SCHOOL OF BIOLOGICAL SCIENCES DEAN'S REPORT

Throughout a busy year at the Watson School of Biological Sciences (WSBS), the achievements of the graduate program continued to grow. The quality of the scientific publications produced by our students, even as they continue to graduate in times well below the national average, remains extremely impressive, as does the success of our alumni in getting excellent jobs. These measures reinforce the value of the program and its unique place in graduate education in the United States.

There have been a number of changes to the administrative staff at the WSBS this year. After completing her term of 5 years as Dean of the Watson School, Leemor Joshua-Tor, Ph.D., stepped down on July 31, 2012. She remains on the faculty of the WSBS as a Professor at Cold Spring Harbor Laboratory (CSHL) and Investigator of the Howard Hughes Medical Institute. As the third Dean of the WSBS, Dr. Joshua-Tor enhanced the curriculum in significant ways, adding courses in quantitative biology, physical biology, and imaging. Her outstanding leadership ensured that the School remained at the leading edge of graduate education and, by contributing to the national debate on graduate education, she further advanced the influence of the school in that arena as well. I was appointed the Lita Annenberg Hazen Dean of the WSBS as of January 1, 2013. In the interim—from August 1 to the end of 2012—two senior members of the WSBS Executive Committee, Adrian R. Krainer and Linda Van Aelst, stepped in to lead the WSBS temporarily as acting Deans. They both did an excellent job in this capacity, and I am immensely grateful to them.

In September 2012, Ms. Dawn Meehan Pologruto, who had been a major presence in the WSBS administration since 2003, most recently as the Director of Recruitment and Student Affairs, departed to raise her two young sons. In November, Keisha John, Associate Director for Recruitment and Undergraduate Research, also left the WSBS, taking a position as Assistant Dean at Florida State University. We were sorry to see them go, as both had contributed so much to the success of the school over the years.

On the other hand, we were very pleased to recruit Kimberly Creteur to the WSBS administration in November, where she immediately took on responsibility for the admissions process of both the graduate and URP programs. Kimberly received her Bachelor's degree and first Master's degree from Vanderbilt University, where she also worked in the admissions office. Subsequently, she worked in the Athletics departments of the University of Massachusetts and the University of Georgia doing marketing and promotion. She then received a second Master's degree in counseling from Hofstra University, where she again worked in the admissions office.

Dr. Alyson Kass-Eisler, who has been with the WSBS since 2003, and Ms. Kimberley Geer, who has been the WSBS Administrative Assistant since 2007, were marvelous in ensuring that the programs at the WSBS were seamlessly administered and managed through all these changes.

Faculty Changes

Six new faculty members joined the Watson School in 2012: Jesse Gillis, Molly Hammell, Ivan Iossifov, Dan Levy, Gholson Lyon, and David Tuveson.

Jesse Gillis, an Assistant Professor, studies gene networks under the principle of "guilt by association" (GBA), which states that genes with related functions tend to share properties. His work centers on identifying the limits of the GBA approach and making fundamental improvements to its operation, as well as applying those improvements to neuropsychiatric gene network data. Molly Hammell, an Assistant Professor and former CSHL Research Investigator, uses computational algorithms to integrate multiple types of genomic and transcriptomic profiling data into models of regulatory rewiring events in human disease. The ultimate goal is to understand how

human diseases such as cancer, take advantage of the cell's innate propensity for plasticity to rewire regulatory networks into programs that serve the needs of cancer cells. Ivan Iossifov, an Assistant Professor and former Quantitative Biology Fellow, focuses on understanding the genetics of complex phenotypes (i.e., human disease) and biomedical text mining. Dan Levy, an Assistant Professor and former CSHL Research Investigator, works on the synthesis of data from a range of mutational modalities into a coherent picture of autism genetics. He is also interested in determining the contribution of de novo and rare inherited mutations to the etiology of sporadic disease through mathematical modeling and computer simulation. Gholson Lyon, an Assistant Professor, focuses on analyzing human genetic variation and its role in severe neuropsychiatric disorders. A second focus of his laboratory is to elaborate the mechanistic basis of a new rare disease that he described in 2011, called Ogden Syndrome—the first-described human disease involving a defect in the amino-terminal acetylation of proteins. David Tuveson, Professor, investigates pancreatic cancer and melanoma by generating mouse models that mimic the human diseases closely and by participating in clinical trials with experimental therapeutics. The goals of the laboratory are to identify the essential components of malignant transformation of pancreatic cells and melanocytes in vivo and to translate this knowledge into effective tumor detection and treatment strategies.

These new faculty members have already participated in WSBS activities. Each of them has given a Research Topics talk to the first-year students. They are also serving as members of thesis committees and expert qualifying examiners and have lectured in WSBS core courses. We look forward to their growing participation as members of the faculty.

Two faculty members also departed the School this year. Yuri Lazebnik was one of the first instructors of the Scientific Exposition and Ethics Core Course and a guest lecturer in the Scientific Reasoning and Logic and Specialized Disciplines: Cellular Structure and Function Courses. He served as thesis committee member and was a thesis research mentor to a WSBS student. Yuri was also the Director of the Partners for the Future Program from 2001 to 2008. Robert Lucito was a guest lecturer in the Specialized Disciplines: The Genome Course. He was also a member of the Qualifying Exam and Admissions Committees and an academic mentor to three WSBS students. We will miss them both and wish them every success in their future endeavors.

The Ninth WSBS Graduation

On April 29, 2012, we celebrated the Watson School's ninth graduation ceremony. Five students were awarded the Ph.D. degree: Patrick Finigan and Fred Rollins from the Entering Class of 2006, Kyle Honegger and Zhenxun Wang from the Entering Class of 2007 and Elizabeth Nakasone from the Entering Class of 2008. Carrie Clendaniel from the Entering Class of 2008 was awarded a Masters degree, but did not attend the ceremony. An honorary degree was bestowed upon Sir Kenneth Murray, who also gave the commencement address. Dr. Murray, together with his late wife and scientific partner, Noreen Murray, was at the forefront of developments in recombinant DNA technology in the 1970s, and late in that decade, he was a cofounder of one of the first biotechnology companies, Biogen, where he was instrumental in the development of a vaccine for hepatitis B, the first genetically engineered vaccine approved for human use. With royalties from this breakthrough, Kenneth and Noreen Murray established the Darwin Trust of Edinburgh in 1983. During the last 30 years, this Trust has supported more than 300 graduate students and 50 undergraduates, as well as endowing faculty positions and supporting building projects.

As with each graduation, we extended a special welcome to the family members and friends of our students who attend the ceremony. Among these special guests were Beth's family members who traveled from Hawaii and brought beautiful and traditional handmade leis for this special event.

Teaching Award


Michael Schatz

At the graduation ceremony this year, Michael Schatz was presented with the seventh annual Winship Herr Faculty Teaching Award, named in honor of the School's founding dean. Michael, an instructor in the Specialized Disciplines Course in Quantitative Biology, was also the winner last year. He was chosen by the students for this award, based on his enthusiasm and creativity in teaching. The winner of this award is nominated and voted on by the students. Below are some of what they had to say about Michael:

> *"His lectures were challenging to some of the students but they were very successful because of Michael's great preparation."*
> *"He made sure we remembered the key concepts and came up with analogies that most of us will never forget."*

Admissions 2012

The School received 274 applications for the 2012/2013 academic year and is deeply indebted to the marvelous work of its Admissions Committee in reviewing, interviewing, and selecting candidates for our doctoral program. The Admissions Committee for the 2012 entering class comprised Gregory Hannon (Chair), Mickey Atwal, Anne Churchland, Leemor Joshua-Tor, Adrian R. Krainer, Zachary Lippman, W. Richard McCombie, Stephen Shea, Nicholas Tonks, and Linda Van Aelst.


2012 graduates: (*Left to right*) CSHL President Bruce Stillman, Fred Rollins, Kyle Honegger, Beth Nakasone, Patrick Finigan, Zhenxun Wang, WSBS Dean Leemor Joshua-Tor, Chancellor Emeritus Jim Watson

2012 WSBS DOCTORAL RECIPIENTS

Student	Thesis advisor	Academic mentor	Current position
Eyal Gruntman	Glenn Turner	Joshua Dubnau	Postdoctoral fellow, Janelia Farm; advisor is Michael Reiser
Kyle Honegger	Glenn Turner	John R. Inglis	Postdoctoral fellow with Dr. Glenn Turner, CSHL
Elizabeth Nakasone	Mikala Egeblad	Alea A. Mills	Medical student at the John A. Burns School of Medicine at the University of Hawaii
Maria Luisa Pineda	Raffaella Sordella	Adrian R. Krainer	CEO, Envisagenetics

2012 THESIS DISSERTATION DEFENSES

ENTERING CLASS OF 2006

Eyal Gruntman, June 14, 2012

Integration properties of Kenyon cells in the Drosophila melanogaster *mushroom bodies.*

Thesis Examining Committee

Chair:	**Stephen Shea**
Research Mentor:	**Glenn Turner**
Academic Mentor:	**Josh Dubnau**
Committee Member:	**Anthony Zador**
Committee Member:	**Yi Zhong**
External Examiner:	**Cori Bargmann,** *The Rockefeller University*

ENTERING CLASS OF 2007

Kyle Honneger, March 9, 2012

Neural coding in the Drosophila *mushroom body.*

Thesis Examining Committee

Chair:	**Adam Kepecs**
Research Mentor:	**Glenn Turner**
Academic Mentor:	**John R. Inglis**
Committee Member:	**Anthony Zador**
Committee Member:	**Josh Dubnau**
Committee Member:	**Pavel Osten**
External Examiner:	**Leslie Vosshall,** *The Rockefeller University*

Maria Pineda, June 22, 2012

Substrate specificity of receptor tyrosine kinases is critical for selective signaling.

Thesis Examining Committee

Chair:	**Gregory J. Hannon**
Research Mentor:	**Raffaella Sordella**
Academic Mentor:	**Adrian R. Krainer**
Committee Member:	**Scott Lowe**
Committee Member:	**Darryl Pappin**
External Examiner:	**Vivek Mital,** *Weill Cornell Medical College*

ENTERING CLASS OF 2008

Elizabeth Nakasone, February 24, 2012

A stromal CCL2/CCR2 signaling axis regulates chemotherapeutic response in a mouse model of breast cancer.

Thesis Examining Committee

Chair:	**Linda Van Aelst**
Research Mentor:	**Mikala Egeblad**
Academic Mentor:	**Alea A. Mills**
Committee Member:	**Scott Lowe**
Committee Member:	**David L. Spector**
External Examiner:	**Valerie M. Weaver,** *University of California, San Francisco*

DOCTORAL THESIS RESEARCH

Student	Academic mentor	Research mentor	Thesis research
ENTERING CLASS OF 2007			
Megan Bodnar *Starr Centennial Scholar*	Nicholas Tonks	David L. Spector	The nuclear choreography of chromatin dynamics, gene expression, and gene repression in embryonic stem cells.
Ralph Burgess *Starr Centennial Scholar*	Bruce Stillman	Gregory Hannon	Recombination hot spots: Characterizing fine-scale variation in the frequency of meiotic recombination across the mammalian genome.
Joseph Calarco *NSERC Scholar* *David H. Koch Fellow*	David Jackson	Robert Martienssen	Transposable element (TE) regulation in the flowering plant *Arabidopsis thaliana*.
Saya Ebbesen *NIH Predoctoral Trainee (ARRA)* *Starr Centennial Scholar*	David J. Stewart	Scott Lowe	In vivo identification and characterization of novel tumor suppressors relevant to human breast cancer.
Paloma Guzzardo *Leslie C. Quick, Jr. Fellow* *NIH Predoctoral Trainee* *William Randolph Hearst Student*	Adrian R. Krainer	Gregory Hannon	Dissecting the piRNA pathway in the *Drosophila* ovarian somatic sheet.
Marek Kudla *George A. and Marjorie H. Anderson Fellow*	David Jackson	Gregory Hannon	CLIP method as a way of direct readout of microRNA target sites.
Hassana Oyibo *Farish-Gerry Fellow* *William Randolph Hearst Student*	Hiro Furukawa	Anthony Zador	Reconstruction of connectivity of neurons by reading of oligonucleotide labels (ROC-N-ROL).
Michael Pautler *NSERC Scholar* *William R. Miller Fellow*	Robert Lucito	David Jackson	The RAMOSA pathway and inflorescence branching in maize.
Yevgeniy Plavskin *Alfred Hershey Fellow* *NIH Predoctoral Trainee*	Jan A. Witkowski	Marja Timmermans	The evolution of the miR390-dependent tasiRNA pathway and its function in bryophyte development.
Joshua Sanders *Edward and Martha Gerry Fellow*	Bruce Stillman	Adam Kepecs	*Trans*-regional coordination of activity in the mouse brain
Petr Znamenskiy *David and Fanny Luke Fellow*	Terri Grodzicker	Anthony Zador	Pathways for attention and action in the auditory system.
ENTERING CLASS OF 2008			
Philippe Batut *Florence Gould Fellow*	Zachary Lippman	Thomas Gingeras	Transposons and evolution of transcriptional regulation in the *Drosophila* clade.
Mitchell Bekritsky *Starr Centennial Scholar*	W. Richard McCombie	Michael Wigler	High-throughput microsatellite genotyping.
Dario Bressan *Robert and Teresa Lindsay Fellow*	Z. Josh Huang	Gregory Hannon	A genomic approach toward the elucidation of connectivity patterns at cellular resolution in complex neural networks.
Melanie Eckersley-Maslin *Genentech Foundation Fellow*	Gregory Hannon	David L. Spector	Making the choice: Mechanistic insights into random autosomal monoallelic expression in mammalian cells.
Sang Geol Koh *George A. and Marjorie H. Anderson Fellow*	Glenn Turner	Anthony Zador	Attention-dependent information routing in the mouse auditory cortex.
Katie Liberatore *Starr Centennial Scholar and National Science Foundation Predoctoral Fellow*	Adrian R. Krainer	Zachary Lippman	Inflorescence development and heterosis.
Ozlem Mert *George A. and Marjorie H. Anderson Fellow*	John R. Inglis	Scott Lowe	Characterization of the role of E2F7 in oncogene-induced senescence and tumorigenesis.
Zinaida Perova *Charles A. Dana Fellow*	Linda Van Aelst	Bo Li	The role of medial prefrontal cortex in behavioral depression.

DOCTORAL THESIS RESEARCH (*continued*)

Student	Academic mentor	Research mentor	Thesis research
Felix Schlesinger *Crick-Clay Fellow*	Gregory Hannon	Thomas Gingeras	Classification of novel transcription start sites.
Nilgun Tasdemir *Robert and Teresa Lindsay Fellow II*	Josh Dubnau	Scott Lowe	Investigating the molecular overlaps between epigenetic reprogramming and transformation.
Elvin Wagenblast *Starr Centennial Scholar* *Boehringer Ingelheim Fellow*	Jan A. Witkowski	Gregory Hannon	Role of stem/progenitor cells in mammary gland and breast tumors.
Susann Weissmueller *Annette Kade Fellow*	Raffaella Sordella	Scott Lowe	In vivo identification and characterization of tumor suppressor genes in hepatocellular carcinoma.
ENTERING CLASS OF 2009			
Stephane Castel *Cashin Fellow NSERC Scholar*	Lloyd Trotman	Robert Martienssen	RNAi-mediated heterochromatin in *S. pombe.*
Kristen Delevich *NIH Predoctoral Trainee*	Stephen Shea	Bo Li	Elucidating the role of Disrupted-in-Schizophrenia-1 in development of prefrontal cortical circuits.
Silvia Fenoglio *Elisabeth Sloan Livingston Fellow*	Linda Van Aelst	Gregory Hannon	RNAi screening to identify putative therapeutic targets for the treatment of pancreatic cancer.
Wee Siong Goh *A*STAR Fellow Delbrück Fellow*	Hiro Furukawa	Gregory Hannon	Determining piRNA primary biogenesis, and MIWI and late piRNA function in mice using *Caenorhabditis elegans* as a model system for genetic screening.
Ian Peikon *Dr. John and Consuelo Phelan Student*	Mickey Atwal	Anthony Zador	Reverse engineering the brain.
Kaja Wasik *George A. and Marjorie H. Anderson Fellow*	Jan A. Witkowski	Gregory Hannon	A screen for novel components of the piRNA pathway in *Drosophila melanogaster.*
Cinthya Zepeda Mendoza *Gonzalo Rio Arronte Fellow*	Thomas Gingeras	David L. Spector	Analysis of higher-order chromatin organization at the mouse syntenic region of human 1p36 upon genomic copy number changes.
ENTERING CLASS OF 2010			
Arkarup Bandyopadhyay *Goldberg Lindsay Fellow*	Zachary Lippman	Florin Albeanu	Identity and intensity encoding of odors in rodents.
Colleen Carlston *John and Amy Phelan Student*	Hiro Furukawa	Christopher Hammell	Identification and characterization of noise-suppressor genes that act via microRNAs in *Caenorhabditis elegans* larval development.
Matthew Koh *George A. and Marjorie Anderson Fellow*	Bo Li	Florin Albeanu	Roles of olfactory bulb inhibitory microcircuits in shaping the temporal response properties of mitral cells.
Lisa Krug *NIH Predoctoral Trainee*	Stephen Shea	Josh Dubnau	Mechanisms of transposon regulation in the central nervous system.
John Sheppard *Bristol Myers Squibb/NIH Predoctoral Trainee*	Josh Dubnau	Anne Churchland	Neural mechanisms of multisensory decision making.
Jack Walleshauser *Barbara McClintock/NIH Predoctoral Trainee*	Christopher Hammell	Leemor Joshua-Tor	Structural basis for TUT4 uridylation of pre-let-7/lin28 complex.
ENTERING CLASS OF 2011			
Robert Aboukhalil *NIH Predoctoral Trainee* *Starr Centennial Scholar* Proposal defense: May 2013	Josh Dubnau	Mickey Atwal Michael Wigler	Using single-cell RNA-Seq to investigate tumor heterogeneity and evolution.
Brittany Cazakoff *Edward and Martha Gerry Fellow* Proposal defense: January 2013	Christopher Hammell	Stephen Shea	Dynamic granule cell processing of odor information.
Joaquina Delas Vives *La Caixa Fellow* Proposal defense: January 2013	Nicholas Tonks	Gregory Hannon	Functional role of long noncoding RNAs in hematopoiesis.

DOCTORAL THESIS RESEARCH (*continued*)

Student	Academic mentor	Research mentor	Thesis research
Anja Hohmann *David H. Koch Fellow* Proposal defense: January 2013	John Inglis	Christopher Vakoc	Exploring the role of bromodomain-containing protein 9 (Brd9) in the maintenance of acute myeloid leukemia.
Justus Kebschull *David and Fanny Luke Fellow* *Genentech Foundation Fellow* Proposal defense: February 2013	Marja Timmermans	Anthony Zador	Grasping the brain.
Fred Marbach *Farish-Gerry Fellow* Proposal defense: April 2013	Josh Dubnau	Anthony Zador	A study of auditory corticostriatal cells in the behaving mouse.
Onyekachi Odoemene *NIH Predoctoral Trainee* *William Randolph Hearst Scholar* Proposal defense: March 2013	Stephen Shea	Anne Churchland	The role of neural inhibition in perceptual decision making.
Sophie Thomain *George A. and Marjorie H. Anderson Fellow* Proposal defense: February 2013	Josh Dubnau	Zachary Lippman	Characterization of a new meristem maintenance pathway in tomato and *Arabidopsis thaliana* and its relation to pollen tube growth.
Charles Underwood *William R. Miller Fellow* Proposal defense: February 2013	Michael Schatz	Robert Martienssen	Epigenetic inheritance through mitosis and meiosis in *Arabidopsis thaliana*.

Entering Class of 2012

On August 27, 2012, the Watson School welcomed its 14th class. This year, nine students joined the School: Nitin Singh Chouhan, William Donovan, Talitha Forcier, Tyler Garvin, Yu-Jui (Ray) Ho, Irene Liao, Paul Masset, Annabel Romero Hernandez, and Abram Santana.

Academic Mentoring

The Watson School takes pride in the level of mentoring afforded its students. One critical component of this is our two-tiered mentoring approach: Each student receives an academic as well as a research mentor. Because WSBS students select a research mentor only in June, right before their qualifying exam—some 10 months after they arrive—it is a particular responsibility of the academic mentor to monitor students during the intensive coursework of the first term, during their rotations, and to help them identify a suitable research mentor. Almost the first thing entering students do is select, by mutual agreement, a member of the research or nonresearch faculty to serve as their academic mentor. And even after the students choose their research mentors, the academic mentors remain important advisors and advocates and serve as members of the students' thesis committees. This program continues to receive much support from the faculty who volunteer to be academic mentors, and it has rightfully become a vital ingredient in our success. The Academic Mentors for the Entering Class of 2012 are:

STUDENT	MENTOR	STUDENT	MENTOR
Nitin Singh Chouhan	Florin Albeanu	Irene Liao	David Jackson
William Donovan	Christopher Hammell	Paul Masset	Jan A. Witkowski
Talitha Forcier	W. Richard McCombie	Annabel Romero Hernandez	Adrian R. Krainer
Tyler Garvin	Zachary Lippman	Abram Santana	Lloyd Trotman
Ray Ho	Michael Schatz		

ENTERING CLASS OF 2012

Nitin Singh Chouhan, Indian Institute of Science Education and Research (IISER); Pune M.S. Awarded: IISER, Pune (2011); Summer Undergraduate Research Program: Freie University, Berlin, Germany (2010); Ranked Top 1% IIT-JEE Exam (2006); IISER, Pune Fellowship (2006–2011)
Academic Mentor: Florin Albeanu

William Donovan, Brown University; Summer Undergraduate Teaching and Research Award: Brown University (2011)
Academic Mentor: Christopher Hammell

Talitha Forcier, Cornell University; Convair Alumni Association Endowment: Cornell University; Frank and Mary Ann Tataseo Scholarship: Cornell University; Jules and Joann Tanzer Scholarship: Cornell University; Tanner Dean's Scholarship: Cornell University
Academic Mentor: W. Richard McCombie

Tyler Garvin, University of Southern California; Dean's List Awarded: University of Southern California (2009); Summer Undergraduate Research: Sandia National Laboratories (2010)
Academic Mentor: Zachary Lippman

Yu-Jui Ho, National Taiwan University; M.S. Awarded: Cornell University (2010); Outstanding Achievement Scholarship, Taiwan Post Company (2006)
Academic Mentor: Michael Schatz

Irene Liao; University of California, Berkeley; Howard Hughes Medical Institute Biology Fellows Program, University of California, Berkeley (2009); Departmental Citation for Genetics and Plant Biology, University of California, Berkeley, Department of Plant and Microbial Biology (2009); Sponsored Projects for Undergraduate Research (SPUR), University of California, Berkeley, College of Natural Resources (2007 2007–2009); Phi Beta Kappa, University of California, Berkeley (2009); Research Training Program, Smithsonian Institution's National Museum of Natural History, Washington D.C. (2008); Golden Key International Honour Society, University of California, Berkeley (2007); College of Natural Resources Dean's List, University of California, Berkeley (2006–2009)
Academic Mentor: David Jackson

Paul Masset, University of Cambridge; M. Cog Sci Awarded: EHESS, ENS, Universite Paris Descartes (2012); B.A. and M. Eng. Awarded: University of Cambridge (2011); Cambridge iGEM Team: Finalist, "Best Wiki", and Gold Medal Awarded (2010); Cambridge European Trust Bursary; Trinity College Engineering Essay Prize; Honourable Mention: 37th International Physics Olympiads; 2nd place in Physics: Belgian Francophone Physics Olympiad (2006); 4th place in Chemistry: Belgian Francophone Physics Olympiad (2006)
Academic Mentor: Jan A. Witkowski

Annabel Romero Hernandez, Instituto Politecnico Nacional, Mexico; M.S. Awarded: Centro de Investigación y de Estudios Avanzados del IPN (2011); National Council on Science and Technology Scholarship (2009–2011); Lazaro Cardenas Medal: Instituto Politecnico Nacional, Mexico (2009); Awarded top student in class: Instituto Politecnico Nacional, Mexico (2005–2008); Fundacion TELMEX-IPN Scholarship (2005–2008)
Academic Mentor: Adrian R. Krainer

Abram Santana, University of California, Santa Cruz; Minority Access to Research Careers (MARC) Scholarship Award (2011–2012); Society for the Advancement of Chicanos and Native Americans in Science (SACNAS): Poster Award in Chemistry (2011); SACNAS National Conference Travel Award (2011); Initiative for Maximizing Student Diversity (IMSD) Summer Research Experience Award (2011); Frank and Sarah McKnight Prize in Chemistry Semi-Finalist: University of Texas Southwestern Medical Center; Deans Honors: University of California, Santa Cruz
Academic Mentor: Lloyd Trotman

2012 Entering Class: (*Front row from left to right*) Paul Masset, William Donovan, Tyler Garvin, Abram Santana, Nitin Chouhan, Ray Ho. (*Back row from left to right*) Annabel Romero Hernandez, Irene Liao, Talitha Forcier.

The Fall Term Curriculum

Our faculty continues to do an outstanding job developing and delivering the curriculum. We are extremely grateful for their considerable time and effort in maintaining the high-quality coursework that we strive to provide. The Curriculum Development and Integration Committee (CDIC)—Adrian Krainer (Chair), David Jackson, Leemor Joshua-Tor, Nicholas Tonks, and Glenn Turner—continues to carefully monitor and develop the curriculum. In addition to the outstanding course instructors and guest lecturers from within the Laboratory, our courses also continue to attract an impressive array of guest lecturers from other institutions.

Recruiting Efforts

As in years past, Dawn Pologruto organized most of the recruitment efforts, sending our faculty and students traveling the length and breadth of the country to talk about the WSBS graduate and undergraduate programs. Keisha John did most of the traveling again this year, making special recruitment visits aimed at enhancing diversity. The table below details recruitment fairs and conferences we attended, together with the names of faculty, students, and administrators who represented WSBS on these occasions. To further raise awareness of our programs, we also mailed more than 15,000 letters to colleges and universities in the United States and abroad. We are grateful to all the students and faculty members who enthusiastically go on recruitment trips on behalf of the WSBS.

2012 WATSON SCHOOL OF BIOLOGICAL SCIENCES RECRUITMENT SCHEDULE

Event	Location	Date	WSBS Attendees/Titles
Hunter College: MARC/RISE Program Visit and Information Session	Cold Spring Harbor Laboratory	January 19	Kristen Delevich, Graduate Student Chris Hammell, Assistant Professor Keisha John, Associate Director of Recruitment and Undergraduate Research Dawn Pologruto, Director of Admissions and Student Affairs
Vassar College: Genetics and Bioinformatics Course Visit and Information Session	Cold Spring Harbor Laboratory	February 23	Kristen Delevich Molly Hammell, Research Assistant Professor Dawn Pologruto Michael Schatz, Assistant Professor
SUNY, Farmingdale: Biology Club Visit	Cold Spring Harbor Laboratory	March 23	Hassana Oyibo, Graduate Student
The National Conference on Undergraduate Research (NCUR): Graduate School Fair	Weber State University	March 29–30	Keisha John
College of William and Mary: Visit and Information Session	Cold Spring Harbor Laboratory	March 30	Kristen Delevich Chris Hammell Molly Hammell Elizabeth Nakasone, Graduate Student Dawn Pologruto
American Association of Cancer Research: Annual Meeting	Chicago, Illinois	March 31–April 4	CSHL Meetings and Courses and Press personnel attended with WSBS information for distribution
Experimental Biology: Annual Meeting	San Diego, California	April 21–25	CSHL Meetings and Courses and Press personnel attended with WSBS information for distribution
American Society for Microbiology: Annual Meeting	San Francisco, California	June 16–19	CSHL Meetings and Courses and Press personnel attended with WSBS information for distribution

(*continued*)

2012 WATSON SCHOOL OF BIOLOGICAL SCIENCES RECRUITMENT SCHEDULE (*continued*)

Event	Location	Date	WSBS Attendees/Titles
Special Libraries Association: Annual Meeting	Chicago, Illinois	July 15–18	CSHL Meetings and Courses and Press personnel attended with WSBS information for distribution
American Society of Plant Biologists: Annual Meeting	Austin, Texas	July 20–25	CSHL Meetings and Courses and Press personnel attended with WSBS information for distribution
University of Medicine and Dentistry, New Jersey: Summer Internship Program Visit	Cold Spring Harbor Laboratory	July 30	Eugene Plavskin, Graduate Student
Brookhaven National Laboratory: Graduate School Fair and Undergraduate Research Program Symposium	Brookhaven National Laboratory	August 8	Keisha John
Hunter College: Minority Access for Research Careers (MARC) Program Research Talk and Information Session	Hunter College	September 12	Chris Hammell, Assistant Professor Anja Hohmann, Graduate Student
Massachusetts Institute of Technology: Career Fair	Massachusetts Institute of Technology	September 21	Stephane Castel
The Johns Hopkins University: Information Session	The Johns Hopkins University	September 25	Keisha John
Morgan State University MBRS-RISE Program: Graduate Career Workshop and Information Session	Morgan State University	September 26	Keisha John
Cornell University: Information Session	Cornell University	October 2	Hassana Oyibo, Graduate Student Eugene Plavskin
Cornell University: Graduate and Professional School Day	Cornell University	October 3	Hassana Oyibo Eugene Plavskin
Society for Advancement of Chicanos and Native Americans in Science (SACNAS)	Seattle, Washington	October 11–14	Keisha John
University of Cambridge: Information Session	University of Cambridge	October 11	Anja Hohmann
Society for Neuroscience: Annual Meeting; Graduate School Fair	New Orleans, Louisiana	October 13–17	Keisha John
California Institute of Technology: Career Fair	California Institute of Technology	October 16	Megan Bodnar, Graduate Student
Massachusetts Institute of Technology: Information Session	Massachusetts Institute of Technology	October 26	Keisha John
California Forum for Diversity in Graduate Education: Graduate School Fair	San Francisco State University	November 3	Keisha John
American Society for Human Genetics: Annual Meeting	San Francisco, California	November 6–10	CSHL Meetings and Courses and Press personnel attended with WSBS information for distribution
Annual Biomedical Research Conference for Minority Students (ABRCMS)	San Jose, California	November 7–11	Keisha John; Cinthya Zepeda Mendoza, Graduate Student
University of Puerto Rico, Rio Piedras: MARC Program Information Session	University of Puerto Rico, Rio Piedras	November 21	Alea Mills, Professor
American Society for Cell Biology: Annual Meeting	San Francisco, California	December 15–19	CSHL Meetings and Courses and Press personnel attended with WSBS information for distribution

Interinstitutional Academic Interactions

WSBS students account for approximately half of the total number of graduate students here at CSHL. The balance comprises visiting graduate students from other institutions who have decided to conduct some or all of their thesis research in the laboratories of CSHL faculty. The vast majority of these visitors are from Stony Brook University (SBU), via a program established between CSHL and SBU more than 30 years ago.

Over the years, we have also established relationships with other institutions around the world, thereby enabling their students to conduct research here at CSHL. Currently, we have visiting students from institutions in Germany, France, Mexico, and India, to name a few. The Watson School provides a contact person for these students and maintains relationships with administrations from their home institutions. These students are fully integrated into the CSHL community and receive all necessary assistance as they navigate the complexities of performing doctoral research away from their home institutions. Those students who joined us this year are listed in the table below.

STUDENT	CSHL RESEARCH MENTOR	AFFILIATION AND PROGRAM
Naishitha Anaparthy	James Hicks	Stony Brook, Molecular and Cellular Biology
Deanna Bahel	Christopher Hammell	Stony Brook, Molecular and Cellular Biology
Debjani Pal	Raffaella Sordella	Stony Brook, Molecular and Cellular Biology
Jayon Lihm	Seungtai Yoon	Stony Brook, Applied Mathematics and Statistics
Yiyang Wu	Gholson Lyon	Stony Brook, Genetics
Tomoki Nomakuchi	Adrian R. Krainer	Stony Brook, Medical Scientists Training Program
Robert Wysocki	Mikala Egeblad	Stony Brook, Medical Scientists Training Program
Nitin Sirole	Raffaella Sordella	Stony Brook, Genetics
Eric Szelenyi	Pavel Osten	Stony Brook, Neuroscience

Graduate Student Symposium

Each year, the students participate in three Graduate Student Symposia held in January, May, and October at the Laboratory's Genome Research Center in Woodbury. This year, each Symposium consisted of five sessions where senior students each gave 15-minute talks. Coffee breaks, lunch, and a wine and cheese reception at the end of the day rounded out the program and provided opportunities for more informal interactions. The Graduate Student Symposium is open to the entire Laboratory community, and, as in previous years, all in attendance found the Symposia to be very worthwhile. We are grateful to the two student chairs of the Symposium, Jia-Ray Yu (SBU) and Cinthya Zepeda-Mendoza (WSBS), and to Keisha John for providing administrative oversight.

Graduate Student and Postdoctoral Fellow Departures

With each year come not only new arrivals, but also departures. The following graduate students and postdoctoral fellows departed from the Laboratory during 2012:

Postdoctoral Fellows

Benjamin Boettner	Tomas Hromadka	Toma Marinov	Chunlao Tang
Peter Bommert	Mamoru Kato	Sylvain Mitelheiser	Hiroki Taniguchi
Santanu Chattopadhyay	Hironori Kawakami	Kate Revill	Emma Vernersson Lindahl
Stacy DeBlasio	Akiko Kobayashi	Ulla Schwertassek	Kazimierz Wrzeszczynski
Heike Demmer	Ying Lin	Puja Singh	Yao, Zhan
Xinglong Gu	Yilei Liu	FNU Sneh Lata	Zhang, Bin

Graduate Students

Ralph Burgess	Benjamin Czech	Elizabeth Nakasone	Yu-Ting Yang
Ying Cai	Zhen Gong	Maria Pineda	Taekyung Yun
Matthew Camiolo	Miao He	Vadim Pinskiy	
Deblina Chatterjee	Wanhe Li	Yi Su	
Michael Cressy	Yifan Mo	Matthew Titmus	

Executive Committee

The School's Executive Committee, which meets monthly, provides year-round direction for the School and its students through its invaluable policy recommendations. I wish to thank faculty members Mickey Atwal, Mikala Egeblad, Adrian Krainer, David Spector, David Stewart, and Linda Van Aelst, who served on the Executive Committee through 2012. In December, Mickey Atwal and Adrian Krainer were elected to a second term. SBU student representative Deblina Chatterjee successfully defended her thesis in June and was replaced by Ioana Rus. Mitchell Bekritsky was the WSBS student representative. We are thankful to Deblina, Ioana, and Mitch for their contributions to discussions and for providing useful suggestions and feedback.

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 designed specifically for the graduate students at the Laboratory. Dr. Dianne K. Newman, Professor of Geobiology at the California Institute of Technology and Investigator of the Howard Hughes Medical Institute, was this year's Gavin Borden Fellow. Her lecture, "Electron transfer in times of stress: New roles for redox active "'antibiotics,'" provided a unique look at how bacteria adapt to their environment. In addition, the students joined Dr. Newman during a roundtable lunch and discussion, where she shared insights into her personal scientific journey.

The Watson School Continues to Benefit from Generous Benefactors

We are extremely grateful for the generous donors, whose one-time gifts or continued support made our 2012 programs possible, including the Annenberg Foundation, Lita Annenberg Hazen Foundation, Bristol-Myers Squibb Corporation, Mr. and Mrs. Richard M. Cashin, Mr. and Mrs. Landon Clay, Lester Crown, the Dana Foundation, Henriette and Norris Darrell, the Samuel Freeman Charitable Trust, the William Stamps Farish Fund, the Genentech Foundation, Mr. and Mrs. Alan Goldberg, Florence Gould Foundation, William Randolph Hearst Foundation, Dr. Mark Hoffman, Annette Kade Charitable Trust, Estate of Gale Kavaliauskas, Mr. David H. Koch, Mr. and Mrs. Robert D. Lindsay and Family, Mr. and Mrs. David Luke III, Marjorie A. Matheson, Mr. and Mrs. William R. Miller, OSI Pharmaceuticals Foundation, Estate of Edward L. Palmer, Mr. and Mrs. John C. Phelan, the Quick Family, Estate of Elisabeth Sloan Livingston, the Starr Foundation, Universidad Nacional Autonoma de Mexico, and the Roy J. Zuckerberg Family Foundation.

We are also very fortunate to hold a prestigious National Research Service Award Predoctoral Training Grant from the National Institutes of Health, National Institute of General Medical Sciences. The School successfully competed for renewal of this award in 2012.

Student Achievements

The WSBS students continue to impress with their accomplishments. They publish their research findings in prestigious international journals—more than 220 to date. In addition, our current students and alumni have been successful in receiving prestigious awards and fellowships. In 2012,

- WSBS student Colleen Carlston was selected to join the National Science Foundation's 2012 East Asia and Pacific Summer Institutes for U.S. Program. She also received a National Science Foundation Graduate Research Fellowship.

- WSBS student Melanie Eckersley-Maslin was awarded a Keystone Symposium Traveling Fellowship. She was also awarded an American Society for Cell Biology travel fellowship to attend the annual conference.
- WSBS student John Sheppard was awarded a National Science Foundation Graduate Research Fellowship and received a National Defense Science & Engineering Graduate Fellowship from the Department of Defense.
- WSBS graduate Hiroshi Makino was awarded a fellowship from the Uehara Memorial Foundation (Japan). He also received a fellowship from the Japan Society for the Promotion of Science.
- WSBS graduate Elizabeth Murchison was the recipient of the Eppendorf award for Young European Investigators and the MRC Jewellery Heirloom Award.

2012 WSBS STUDENT (CURRENT OR PREVIOUS) PUBLICATIONS

Borges F, Gardner R, Lopes T, **Calarco JP**, Boavida LC, Slotkin RK, Martienssen RA, Becker JD. 2012. FACS-based purification of *Arabidopsis* microspores, sperm cells and vegetative nuclei. *Plant Methods* **8:** 44.

Calarco JP, Borges F, Donoghue MT, Van Ex F, Jullien PE, Lopes T, Gardner R, Berger F, Feijó JA, Becker JD, Martienssen RA. 2012. Reprogramming of DNA methylation in pollen guides epigenetic inheritance via small RNA. *Cell* **151:** 194–205.

Calarco JP, Martienssen RA. 2012. Imprinting: DNA methyltransferases illuminate reprogramming. *Curr Biol* **22:** R929–R931.

Djebali S, Lagarde J, Kapranov P, Lacroix V, Borel C, Mudge JM, Howald C, Foissac S, Ucla C, Chrast J, Ribeca P, Martin D, Murray RR, Yang X, Ghamsari L, Lin C, Bell I, Dumais E, Drenkow J, Tress ML, Gelpí JL, Orozco M, Valencia A, van Berkum NL, Lajoie BR, Vidal M, Stamatoyannopoulos J, **Batut P**, Dobin A, Harrow J, Hubbard T, Dekker J, Frankish A, Salehi-Ashtiani K, Reymond A, Antonarakis SE, Guigó R, Gingeras TR. 2012. Evidence for transcript networks composed of chimeric RNAs in human cells. *PLoS One* **7:** e28213.

Iossifov I, Ronemus M, Levy D, Wang Z, Hakker I, Rosenbaum J, Yamrom B, Lee Y, Narzisi G, Leotta A, Kendall J, Grabowska E, Ma B, Marks S, Rodgers L, Stepansky A, Troge J, Andrews P, **Bekritsky M**, Pradhan K, Ghiban E, Kramer M, Parla J, Demeter R, Fulton LL, Fulton RS, Magrini VJ, Ye K, Darnell JC, Darnell RB, Mardis ER, Wilson RK, Schatz MC, McCombie WR, Wigler M. 2012. De novo gene disruptions in children on the autistic spectrum. *Neuron* **74:** 285–299.

Dow LE, Premsrirut PK, Zuber J, Fellmann C, **McJunkin K**, Miething C, Park Y, Dickins RA, Hannon GJ, Lowe SW. 2012. A pipeline for the generation of shRNA transgenic mice. *Nat Protoc* **7:** 374–393.

Glaser SP, Lee EF, Trounson E, Bouillet P, Wei A, Fairlie WD, Izon DJ, Zuber J, **Rappaport AR**, Herold MJ, Alexander WS, Lowe SW, Robb L, Strasser A. 2012. Anti-apoptotic Mcl-1 is essential for the development and sustained growth of acute myeloid leukemia. *Genes Dev* **26:** 120–125.

Nakasone ES, Askautrud HA, Kees T, Park JH, Plaks V, Ewald AJ, Fein M, Rasch MG, Tan YX, Qiu J, Park J, Sinha P, Bissell MJ, Frengen E, Werb Z, Egeblad M. 2012. Imaging tumor-stroma interactions during chemotherapy reveals contributions of the microenvironment to resistance. *Cancer Cell* **21:** 488–503.

Preall JB, Czech B, **Guzzardo PM**, Muerdter F, Hannon GJ. 2012. Shutdown is a component of the *Drosophila* piRNA biogenesis machinery. *RNA* **18:** 1446–1457.

Raposo D, **Sheppard JP**, Schrater PR, Churchland AK. 2012. Multisensory decision-making in rats and humans. *J Neurosci* **32:** 3726–3735.

Sanders JI, Kepecs, A. 2012. Choice ball: A response interface for two-choice psychometric discrimination in head-fixed mice. *J Neurophysiol* **108:** 3416–3423.

Shi J, Wang E, Zuber J, **Rappaport A**, Taylor M, Johns C, Lowe SW, Vakoc CR. 2012. The Polycomb complex PRC2 supports aberrant self-renewal in a mouse model of MLL-AF9;Nras(G12D) acute myeloid leukemia. *Oncogene* **32:** 930–938.

(*continued*)

2012 WSBS STUDENT (CURRENT OR PREVIOUS) PUBLICATIONS (*continued*)

Simpson D, Muthuswamy S, Tansey WP. 2012. Impact of epithelial organization on Myc expression and activity: Response. *Cancer Res* **72:** 1036.

Wang Z, Chatterjee D, Jeon HY, Akerman M, Vander Heiden MG, Cantley LC, Krainer AR. 2011. Exon-centric regulation of pyruvate kinase M alternative splicing via mutually exclusive exons. *J Mol Cell Biol* **4:** 79–87.

Wang Z, Jeon HY, Rigo F, Bennett CF, Krainer AR. 2012. Manipulation of PK-M mutually exclusive alternative splicing by antisense oligonucleotides. *Open Biol* **2:** 120–133.

Zador AM, Dubnau J, **Oyibo HK**, Zhan H, Cao G, **Peikon I**. 2012. Sequencing the connectome. *PLoS Biol* **10:** e1001411. Review.

Watson School student

Alumni in Faculty and Senior Positions

To date, 54 students have received their Ph.D. degree from the WSBS. Of these graduates, 12 currently hold tenure-track faculty positions and one holds an independent research position. Twenty-eight of our former students are pursuing postdoctoral research and eight of our alumni completed their postdoctoral studies and have moved into positions in administration, education, industry, management consulting, and journal editing.

WSBS GRADUATES IN FACULTY AND SENIOR POSITIONS

Name	Current position
François Bolduc	Assistant Professor, University of Alberta, Canada
Darren Burgess	Assistant Editor, *Nature Reviews Cancer* and *Nature Reviews Genetics*, UK
Amy Caudy	Assistant Professor, University of Toronto, Canada
Michelle Cilia	Research Molecular Biologist, United States Department of Agriculture, The Boyce Thompson Institute and Adjunct Assistant Professor, Cornell University
Catherine Cormier	Scientific Liaison, Arizona State University
Yaniv Erlich	Whitehead Fellow, Whitehead Institute
Rebecca Ewald	Project Manager, Roche Diagnostics, Inc.
Elena Ezhkova	Assistant Professor, Mount Sinai School of Medicine
Ira Hall	Assistant Professor, University of Virginia School of Medicine
Christopher Harvey	Assistant Professor, Harvard University
Keisha John	Assistant Dean, Director—Office of Graduate Fellowships and Awards at The Graduate School, Florida State University
Zachary Lippman	Assistant Professor, Cold Spring Harbor Laboratory
Marco Mangone	Assistant Professor, Arizona State University
Patrick Paddison	Assistant Member, Fred Hutchinson Cancer Research Center
Emiliano Rial-Verde	Engagement Manager, McKinsey & Co., Inc., Geneva, Switzerland
Ji-Joon Song	Assistant Professor, Korea Advanced Institute of Science and Technology (KAIST), South Korea
Niraj Tolia	Assistant Professor, School of Medicine at Washington University, St. Louis
Wei Wei	Assistant Professor, University of Chicago

WSBS Family Events

Finally, we celebrated some personal occasions. Three students from the Entering Class of 2008 were married this year. On January 8, Mitchell Bekritsky married his fiancée Rikki Stern in Florida. On August 26, two students, Zina Perova and Jiahao Huang, were married in New York City. On November 17, Marek Kudla, from the Entering Class of 2007, and his wife Manuela, welcomed daughter Milena Trzebinska-Kudla to their family. Our congratulations and best wishes to all of them.

Alexander Gann
Professor and Dean

Zina and Jiahao with Dr. and Mrs. James Watson

Milena Trzebinska-Kudla

Rikki and Mitch

SPRING CURRICULUM

Topics in Biology

ARRANGED BY **Leemor Joshua-Tor, Alyson Kass-Eisler, and Jan A. Witkowski**

FUNDED IN PART BY **The Daniel E. Koshland, Jr. Visiting Lectureship; the David Pall Visiting Lectureship; the Fairchild Martindale Visiting Lectureship; the Lucy and Mark Ptashne Visiting Lectureship; the Michel David-Weill Visiting Lectureship**

Each year, one or a team of invited instructors offer 7-day courses at the Banbury Conference Center to explore specialized topics outside the expertise of the Cold Spring Harbor Laboratory faculty. These courses include morning or evening lectures as well as afternoon sessions during which students read assigned papers. These intensive courses are modeled on the Cold Spring Harbor Laboratory Lecture Courses held each summer at the Banbury Conference Center. In Spring 2012, one of our own faculty members, Florin Albeanu, developed a new Topics in Biology course on Optical Methods. The course included a large hands-on component, in which the students built their own microscopes, and for practical purposes, it was held on our main campus. The other Topics in Biology course in Spring 2012 was the Evolution course, taught once again by Nipam Patel and held at the Banbury Conference Center.

Optical Methods

Attended by the entering classes of 2008 and 2011

INSTRUCTORS **Florin Albeanu,** Cold Spring Harbor Laboratory
Adam Kampff, Champalimaud Neuroscience Programme, Portugal

VISITING LECTURERS **Florian Engert,** Harvard University
Tom Mrsic-Flogel, University College London

TEACHING FELLOWS **Francesca Anselmi,** Cold Spring Harbor Laboratory
Rob Campbell, Cold Spring Harbor Laboratory

Optical imaging techniques are widely used in all areas of modern biological research. Our aim for this course was to give students an introduction into widely used basic and advanced optical methods. Given the experimental nature of the topic, a central aim of our course was to offer students a practical hands-on experience. This included both the use of commercially available systems and, more importantly, a primer on custom building and adapting optical setups to address specific biological needs. The course began with an overview of basic optics (diffraction, refraction, reflection, resolution limits) and the components needed to build a microscope (lens combinations and photodetectors, such as photodiodes, PMTs, and CCD cameras). We introduced basic illumination techniques including Koehler, phase contrast, differential interference contrast (DIC), and dark field. One day was dedicated to discussing and measuring various sources of

noise (dark, read-out, shot noise). We further provided an overview of more advanced optical methods ranging from intrinsic optical imaging and bioluminescence to patterned illumination and superresolution. We continued by giving detailed theoretical and experimental description of wide-field (epifluorescence) and laser-scanning fluorescence microscopy (confocal and multiphoton). The course concluded with lectures from invited speakers on biological applications of multiphoton microscopy, bioluminescence, and digital holography. In the lab sessions, the students constructed optical rail systems using a combination of lenses, irises, and light sources to understand the principles of bright-field microscopy. They further adapted their custom-built setups for epifluorescence and added CCD cameras as detectors. They acquired pictures and real-time movies of samples ranging from tissue paper, pollen grains, fly wings, blood, and cheek cells to planaria and transgenic *Caenorhabditis elegans*. The students built a scanning fluorescence microscope and further modified it into a confocal system by introducing an aperture and descanning the beam. Different groups focused on different aspects of this project, from wiring up electronic circuits for the photodetector to writing scanning software in Matlab and Labview to actually assembling the optical components and operating the system. The course ran from Sunday, March 25, through Saturday, March 31.

Optical methods participants. (*From left to right*) Sang Geol-Koh, Melanie Eckersley-Maslin, Silvia Fenoglio, Mitchell Bekritsky, Zina Perova, Josh Sanders

Evolution

Attended by the entering classes of 2009 and 2010

INSTRUCTOR	**Nipam Patel**, University of California, Berkeley
VISITING LECTURERS	**Rob DeSalle,** American Museum of Natural History **David Jablonski,** University of Chicago **Mike Palopoli,** Bowdoin College **Dan Rokhsar,** University of California, Berkeley
TEACHING FELLOWS	**April Dinwiddie,** Yale University **Stephen Fairclough,** University of California, Berkeley

The field of evolutionary biology touches upon all other areas of the biological sciences, since every form of life and every biological process represents an ongoing evolutionary "experiment." Our aim in this course was to both discuss our understanding of the mechanisms of evolution and explore how evolutionary data can be used to further our understanding of various biological problems. The course began with a discussion of the diversity of organisms that currently exist, as well as methods for understanding the evolutionary relationships among these organisms. The students then went on to study how paleontological data are collected and used to understand the history of life on Earth and to examine how DNA sequence data can be used to understand the evolutionary history of organisms, genes, and genomes. Within this molecular and genetic framework, the focus shifted to the mechanisms of evolutionary change and how variation within

populations leads to the evolution of new species. Finally, 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, March 4, to Saturday, March 10, and included a class favorite field trip and curator tour at the American Museum of Natural History in New York City.

At the American Museum of Natural History: *Front* (*from left to right*): April Dinwiddie, Silvia Fenoglio, Colleen Carlston, Kaja Wasik, Kristen Delevich, Cinthya Zepeda-Mendoza, David Jablonski. *Rear* (*from left to right*): Arkarup Bandyopadhyay, Wee Siong Goh, Stephen Fairclough, Ian Peikon, Stephane Castel, Lisa Krug, Jack Walleshauser, John Sheppard, Matthew Koh.

Teaching Experience at the Dolan DNA Learning Center

DIRECTOR	**David A. Micklos**	
INSTRUCTORS	**Amanda McBrien (Lead)**	**Erin McKechnie**
	Jennifer Galasso	**Bruce Nash**
	Elna Gottlieb	**Jermel Watkins**

As science plays an increasing role in society, there is also an increasing need for biologists to educate nonscientists of all ages about biology. The Watson School of Biological Sciences doctoral program offers its students unique teaching experiences through the Laboratory's Dolan DNA Learning Center, where students teach laboratory courses to high school and middle school students. From these teaching experiences, they learn how to communicate with nonbiologists and to inspire and educate creative young minds.

The teaching module entailed pairs of students teaching one morning or afternoon a week for 12 weeks. In the initial weeks, the Dolan DNA Learning Center instructors taught the Watson School students the didactic process; it was not until the fifth week that the graduate students taught on their own. At the end of the 12 weeks, the students were very excited about their teaching experience.

Laboratory Rotations

The most important element of a doctoral education is learning to perform independent research that leads to a unique contribution to human knowledge. After the fall course term, students participate in laboratory rotations. These rotations provide students and faculty the opportunity to get to know each other and to explore possibilities for doctoral thesis research. At the end of each rotation, students make short presentations of their studies to the other students and their rotation advisors. These talks give students an opportunity to share their laboratory experiences and to learn how to give a scientific presentation. With this latter goal in mind, in addition to the research mentors, the instructors of the Scientific Exposition and Ethics Core Course and members of the School's Executive Committee attend the talks and give individual feedback to students on their presentations. This year, 20 WSBS faculty members served as rotation mentors, some mentoring more than one student.

ROTATION MENTORS	**Florin Albeanu**	**Gregory Hannon**	**Michael Schatz**
	Mickey Atwal	**Z. Josh Huang**	**Stephen Shea**
	Anne Churchland	**Adam Kepecs**	**David L. Spector**
	Mikala Egeblad	**Adrian R. Krainer**	**Christopher Vakoc**
	Hiro Furukawa	**Bo Li**	**Michael Wigler**
	Christopher Hammell	**Zachary Lippman**	**Anthony Zador**
	Molly Hammell	**Robert Martienssen**	

FALL CURRICULUM

CORE COURSES

The Leslie C. Quick, Jr. Core Course on Scientific Reasoning and Logic

INSTRUCTORS	**Gregory Hannon (Lead)**	**Leemor Joshua-Tor**
	Alexander Gann	**Bo Li**
	Christopher Hammell	**Nicholas Tonks**
GUEST LECTURERS	**Hiro Furukawa**	**Senthil Muthuswamy**
	Molly Hammell	**David Tuveson**
	Justin Kinney	**Linda Van Aelst**
	Robert Martienssen	

The SRL core course was revamped last year with regard to content and instructor participation. This year, only one module changed, with Christopher Hammell joining the team. Each module of the course has been designed to cover a different general theme, and proceeds with the goal of considering an open, still unanswered, scientific question. In addition, the first two modules of the course were combined into one longer module so that an integrated theme could be tackled using two different approaches: molecular biology and structural biology.

A fundamental aspect of earning the Ph.D. is training in the pursuit of knowledge. In this core course, which forms the heart of the curriculum, students (1) acquire a broad base of knowledge about the biological sciences, (2) learn the scientific method, and (3) learn how to think critically.

The beginning of the course is divided into four to five modules. For each module, students read an assigned set of research articles (generally four articles) and at the end of the module, provided written answers to a problem set that guided them through several of the articles. Twice weekly, students attended lectures related to the module's topic that included concepts and fundamental information as well as experimental methods. During each week, the students met among themselves to discuss the assigned papers not covered by the problem set. Each week, students spent an evening discussing the assigned articles with faculty.

In the final part of the course, students participated in a mock study-section in which real National Institutes of Health R01 grants were reviewed and critiqued. This allowed the students to evaluate the questions before the answers were known, evaluate routes toward discovery before knowing where they will end, and make critical judgments about how to proceed in the face of an uncertain outcome. The module topics for this course were as follows:

Topic	**Instructor(s)**
Mechanism and Structure of Gene Regulation	Alex Gann Leemor Joshua-Tor
Gene Regulatory Logic and the Construction of Multicellular Organisms: Insights from Flies, Plants, and Worms	Christopher Hammell
The Brain: Wiring, Plasticity, and Maladaptation	Bo Li
Signal Transduction and the Search for Novel Therapeutics	Nicholas Tonks
Study Section	Gregory Hannon

The Darrell Core Course on Scientific Exposition and Ethics

INSTRUCTORS	**David J. Stewart (Lead)** **Alea A. Mills** **Arne Stenlund**
TEACHING ASSISTANT	**Andrea Eveland**
GUEST LECTURERS	**Diane Esposito** **Jan A. Witkowski**
VISITING LECTURERS	**Olga Akselrod,** The Innocence Project **Keith Baggerly,** University of Texas, M.D. Anderson Cancer Center **Michael Beilski,** The Center for Biotechnology **Robert Charrow,** Greenberg Traurig, LLC **Avner Hershlag,** North Shore University Hospital **Amy Harmon,** *The New York Times* **Boyana Konforti,** Editor, *Nature Structural & Molecular Biology* **Tung-Tien (Henry) Sun,** New York University

The Scientific Exposition and Ethics (SEE) core course 2012 was taught by the same instructor team as last year, with David Stewart taking the lead role, joined by Alea Mills and Arne Stenlund. This year, one CSHL postdoctoral fellow, Andrea Eveland, participated in the course as a teaching assistant. As usual, the course hosted expert guest lecturers who covered topics including scientific presentations, the editorial review process, the ethical and legal responsibilities of scientists, DNA profiling and postconviction appeals, and reproductive genetics.

This core course offered instruction in the fundamental elements of scientific exposition—writing skills and public speaking—and ethics. The ability to communicate effectively and to appreciate the intricacies of ethical issues are essential skills for biologists; both subjects were taught in a series of example-based lectures and discussion groups. Writing skills included the fundamentals of modern scientific English and the organization and preparation of papers, research abstracts, and grant applications. Oral presentation skills were taught by instructors with different modes of presentation. Together with instructors, students critiqued formal seminar presentations at the Laboratory.

Instruction and discussions about ethics included the ethical implications of biological discovery for society as well as the nature and boundaries of ethical behavior of scientists and their rights and responsibilities. A primary objective of the course was that students consider exposition and ethics integral aspects of scientific research.

Research Topics

ORGANIZERS	**Kimberley Geer** **Alyson Kass-Eisler**

This core course provided students with an in-depth introduction to the fields of research that the Laboratory scientists investigate. Students and faculty attended a weekly Research Topics seminar, at which faculty members and CSHL fellows presented their current research topics and methods of investigation each Wednesday evening over dinner. The students learned how to approach important problems in biology. These seminars, together with the annual fall Laboratory In-House symposium, provided students with a basis for selecting laboratories in which to do rotations.

Specialized Disciplines Courses

The students in the Entering Class of 2012 took a total of four Specialized Disciplines courses this fall: *Cellular Structure and Function*, *Genetics and Genomics, Systems Neuroscience,* and *Quantitative Biology.*

Cellular Structure and Function

INSTRUCTORS	**Linda Van Aelst (Lead)** **Raffaella Sordella**
GUEST INSTRUCTORS	**Darryl Pappin** **David L. Spector** **Bruce Stillman**
VISITING LECTURERS	**Gregg Gundersen,** Columbia University **Aron Jaffe**, Novartis **Alexey Khodjakov**, Wadsworth Laboratory **Sandy Simon**, The Rockefeller University **Marc Symons,** The Feinstein Institute for Medical Research

With the complete genome sequence available for many organisms, there is now an increasing emphasis on understanding the function of the gene products. This understanding requires an increasing appreciation of the structure and function of the cell, as well as dynamic associations within the cell. This course provided a basic overview of the structural and functional organization of cells, with particular emphasis on cellular compartmentalization and communication. Topics of focus included the cytoskeleton, cell adhesion and signaling, membrane transport, gene expression, and nuclear organization. In addition, the course provided insight into the basic toolbox of the cell biologist of the 21st century.

Genetics and Genomics

INSTRUCTORS	**Thomas Gingeras (Lead)** **Zachary Lippman**
GUEST LECTURERS	**Josh Dubnau** **Marja Timmermans**

This course placed modern genetics and genomics into the context of classical genetics. History, technique, and perspective of genetic inference were described around four levels of analysis: forward genetics, natural genetic variants, gene interaction, and genomics. Emphasis was on integrating classical with modern questions of genetic analysis: How are genes mapped and "cloned"? How do gene mutations help to define biological processes? How are more complex traits genetically dissected into simpler (underlying) components? What concepts and techniques are used to organize genes into pathways and networks? What defines a gene and what gene variation exists in natural populations? What are the functional consequences of gene variation, and

how is it detected? How are genomes organized and coordinately regulated? How can genomic information be catalogued, organized, and mined? These questions and concepts were fleshed out using examples from the literature.

Quantitative Biology

INSTRUCTORS	**Mickey Atwal (Lead)** **Michael Schatz**
GUEST LECTURERS	**Ivan Iossifov** **Justin Kinney (CSHL Fellow)** **Alexander Krasnitz**

With the advent of high-throughput technologies in biology, it has become necessary for biological researchers to be able to analyze and interpret high-dimensional data and frame hypotheses mathematically. To this end, the aim of this course was to equip the students with basic training in modern statistical methods. By the end of the course, the students were able not only to answer many of the simple statistical questions that arise in data analyses, but also to become familiar with the more complex techniques used by fellow computational biologists.

Topics included error fluctuations, calculating the significance of an experimental result, Bayesian inference, information theory, power calculations, dimensional reduction, and DNA sequence analyses. In addition, this course introduced mathematical modeling, motivated by the classic examples in quantitative biology such as the Delbrück-Luria experiment, Hopfield's kinetic proofreading, and Kimura's neutral theory of population genetics.

For the first time, a 2-day Quantitative Biology boot camp, in which students were provided with the quantitative skills useful to their other coursework, was given at the very beginning of the fall semester.

Systems Neuroscience

INSTRUCTORS	**Adam Kepecs (Lead)** **Glenn Turner**
GUEST INSTRUCTORS	**Anne Churchland** **Alexei Koulakov** **Stephen Shea** **Anthony Zador**

This course introduced students to neuroscience, with a focus on learning and plasticity from its cellular basis, through development, to systems and behavior. Both experimental and theoretical viewpoints were explored. The course started with the basics of electrical signaling in neurons: ion channels, action potentials, and synaptic transmission. The cellular basis of learning, including Hebb's postulates and LTP (long-term synaptic potentiation), was discussed. The course explored the consequences of synaptic learning rules by examining how experience shapes the wiring of the nervous system during development and investigated how such building blocks translate into whole-organism behavior.

The course then examined classical conditioning and asked how changes in synaptic transmission could underlie such behavior. Associative learning computational models of the learning

process were discussed. From behaviors that focus on simple memories, the course turned to behaviors that require making perceptual decisions. This was accomplished by covering some basic concepts of perceptual neuroscience, such as neuronal "receptive fields," and we 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. It concluded with a discussion of the role of dopamine in reward and learning, the theory of reinforcement learning, and pathologies of reward-seeking behavior.

POSTDOCTORAL PROGRAM

PROGRAM DIRECTOR **Nicholas Tonks**

PROGRAM ADMINISTRATOR **Alyson Kass-Eisler**

Cold Spring Harbor Laboratory (CSHL) is proud of its rich tradition in postdoctoral education and has a deep commitment to the postdoctoral experience. CSHL has long been recognized as a place for nurturing young scientists, with postdoctoral researchers being an integral part of the discovery process. Current fields of research expertise include genetics; molecular, cellular, and structural biology; neuroscience; cancer; plant biology; genomics; and quantitative biology. The postdoctoral community at CSHL is diverse and international, arriving at the Laboratory with many different backgrounds and with needs that change during the course of the postdoctoral years. The Postdoctoral Program Office works closely with the fellows and the Laboratory's administration to coordinate and organize educational and career development activities. Alyson Kass-Eisler, the Postdoctoral Program Officer, and Nicholas Tonks, the Scientific Director of the Postdoctoral Program, provide the day-to-day support for the postdoctoral program, which is overseen by the Dean of the Watson School of Biological Sciences (WSBS).

In January 2011, Bruce Stillman held his annual Town Hall meeting with postdocs. One suggestion that came from the meeting was the establishment of a Postdoctoral Liaison Committee (PDLC)—an elected group of postdocs who would help share information and ideas between the administration and the postdoctoral community. In 2012, new members Jonathan Ipsaro and Benjamin Roche joined returning members Kate Creasey, Santiago Jaramillo, Dawid Nowak, and Kate Revill. The PDLC has been hard at work organizing two successful retreats this year aimed at fostering networking and collaboration. The PDLC also oversees and distributes funds to two career development groups as described below. These groups are primarily composed of postdoctoral fellows, but they also include graduate students.

Today's postdocs face a number of challenges, including a very difficult job market. CSHL works hard to give its postdocs the upper hand when it comes to competing for the scarce jobs that are available. More and more, it has become our responsibility to introduce a diversity of career opportunities that are available and to provide the tools to our postdocs to prepare for these positions. Thanks to the assistance of PDLC and these career development groups, 2012 was an extremely exciting year for us!

The Career Development Program (CDP) is interested in providing programming geared toward careers in academia. A six-part series was organized called "Conversations with Faculty," in which CSHL faculty and administration gave the postdocs career insights in an informal and interpersonal format. Sessions included The Postdoc Experience, with Christopher Hammell and Gregory Hannon; Going on the Job Market: Applicant Perspective, with Stephen Shea and postdoc Ted Karginov; Going on the Job Market: Search Committee Perspective, with Thomas Gingeras, Marja Timmermans, and Anthony Zador; Transitioning from Postdoc to PI: Negotiations, with Adrian Krainer, Nick Tonks, and David Spector; First Year as PI: A CSHL Perspective, with Anne Churchland and Michael Schatz; and First Year as PI: A Perspective from Outside CSHL, which featured CSHL alumni Elena Ezhkova (Assistant Professor, Mount Sinai School of Medicine) and Mikel Zaratiegui (Assistant Professor, Rutgers University).

The CDP also hosted another extremely well-received workshop on preparing for the Chalk Talk, an integral part of the academic job search. In this session, Martine Mirrione, a postdoc in Bo Li's laboratory, presented her future research plans in front of Alea Mills, Pavel Osten, Marja Timmermans, and Tony Zador, who served as the mock hiring panel.

Together, we have also been working on building connections with local colleges and universities to provide teaching experience opportunities for interested postdocs. The first of these connections has been made with Molloy College, and a CSHL postdoc has already started teaching as an Adjunct Professor. Finally, the CDP started a new series called "Getting to Know Your Faculty," where CSHL faculty members share stories of their journey throughout their career and highlight the person behind the scientific concepts and publications. The first guest was CSHL President Bruce Stillman.

The Bioscience Enterprise Club (BEC) is most interested in providing information for non-academic careers, and they have also been organizing a series of seminars and workshops. The areas of interest include biotechnology, intellectual property, law, regulatory affairs, and venture capitalism. This year's presentations included How to be Successful as a Scientist Working in a Start-up, with Melinda Thomas, Entrepreneur-in-Residence for the City of New York; Careers in Medical/Healthcare Communications, with Kakuri M. Omari, Ph.D., Scientific Director, and Julianne Dunphy, Ph.D., Vice President, Scientific Director at Publicis Healthcare Communications Group; Translating Your Talent into a Career: Decoding the Job Search Process, with Barbara Gebhardt, President of Opus Scientific; Entrepreneurial Science: From Sunless Tanning to Sickle Cell Disease, with Steven Isaacman, Ph.D., Founder and CEO of Nanometics LLC; Launching a Successful Venture Capital Backed Start-up, with Irvin Barash, President, Vencon Management, Inc.; and Startups vs. Biotech?, with Mark Fasciano, Ph.D., Managing Director, Canrock Ventures. Mark Lesko, Executive Director of Accelerate LI, a regional collaboration among the major academic and research organizations on Long Island to accelerate an innovation-based economy, also participated in the session. They were joined by a writer for *Newsday*, leading to a mention of the club in the paper! Finally, the BEC hosted Michael Bielski from the Fundamentals of the Bioscience Industry Program, a course for scientists interested in gaining entrepreneurial experience, located at Stony Brook University.

To celebrate National Postdoc Appreciation week, we planned a faculty/postdoc volleyball game, barbeque, and fair highlighting the career development initiatives. Unfortunately, the weather did not cooperate, forcing the cancellation of the volleyball game. We still enjoyed an indoor barbeque, and it was a great opportunity for the community to find out about the ongoing programs we have been running.

Through the Laboratory's Human Resources department, the postdocs have had access to free jobseeker webinars hosted by the Higher Education Research Consortium. The 2012 webinars included The Campus Visit, Insider Tips for Finding a Job Outside Academia, and Ask The Experts: Answers to Your Toughest Academic Job Search Questions.

Seven years ago, the WSBS initiated a prize for the best posters by a postdoctoral fellow and the best poster by a graduate student at the Laboratory's annual In-House Symposium. Each "prize" is given in the form of an educational grant of $500. In addition to providing a forum for the postdoctoral fellows to show off their research, and potentially win a prize, it gives the entire scientific community a chance to come together and share ideas beyond the walls of their individual laboratories. It has been a great success for both the presenters and the Laboratory community as a whole. This year, the postdoctoral prize, for the second year in a row, was won by Tim Kees from Mikala Egeblad's laboratory.

During the past couple of years, we have increasingly widened the role of postdoctoral fellows in the curricular activities of the School. Our fellows have been involved in preparing our students for the challenging fall term curriculum by working as one-on-one tutors during the summer. Throughout the fall term, postdoctoral fellows are used as tutors for all aspects of the curriculum, providing one-on-one tutoring, group tutoring, and participating in discussion sessions. The Scientific Exposition and Ethics (SEE) course takes advantage of the expertise of our postdoctoral community by hiring fellows as teaching assistants (TAs). These fellows serve

as an integral part of the course by providing their expertise in discussions, editing the students' written work, and critiquing oral presentations. In fall 2012, Andrea Eveland, a postdoctoral fellow from David Jackson's laboratory, was the TA for the SEE course.

A new program called "Demystifying Science," which started in 2011, enlists postdoctoral fellows who bring their expertise and teaching interests to the educationally diverse staff of the Laboratory. In the 2012 sessions, geared toward a general audience, postdocs have talked about Exploring the 3rd Dimension: The Neurobiology of Depth Perception, by Dennis Eckmeier; Seeing Scents: Watching Brain Cells Decipher the World, by Rob Campbell; The Clockwork Automaton: Machines in Biology, by Swagatam Mukhopadhyay; Mixed Messages in Human Disease, by Jackie Novatt; Reading and Writing Minds: How Neuroscience Can Revolutionize Communication, by Santiago Jaramillo; Evolution: What's New and Who Cares?, by graduate student Eugene Plavskin; and Turning Villains into Heros: Immune Cells in Cancer, by Tim Kees.

Since 2003, all postdoctoral fellows and graduate students at the Laboratory have been enrolled in a special initiative of the New York Academy of Science (NYAS), the Science Alliance. The Science Alliance for graduate students and postdoctoral fellows is a consortium of universities, teaching hospitals, and independent research facilities in the New York City metro area that have formed a partnership with NYAS. The aim of the Alliance is to provide career and professional development monitoring for postdoctoral fellows and graduate students in science and engineering, through a series of live events and a dedicated web portal. In addition, the Science Alliance gives graduate students and postdoctoral fellows the opportunity to network with their peers across institutions and with key leaders in academia and industry. This year, the Science Alliance meetings and workshops included Writing For Biomedical Publication; From Idea to IPO; Writing About Science for the Public; Introduction to Scientific Teaching; Leveraging the Scientific Mindset in the Entrepreneurial World; PepsiCo Journey through Science Day; From Scientist to CSO: Leadership & Management Development for Careers in Business and Industry; Making the Leap: A Non-Academic Career Planning & Job Search Boot Camp; and Communicating Science to the Public.

On December 6, the Office of Sponsored Programs, the Development Department, and the Postdoc Program Office held a very popular course on Grant Writing. The sessions included Introduction to Grants and External Funding; The Grant Application; Protecting Human Research Subjects/Use of Laboratory Animals in Research; The Grant Abstract/Public Summary; Peer Review Panel Discussion; Effective Writing Skills; Fellowships, Career Development Awards & Resources for Postdocs; and a Fellowship Discussion Panel featuring postdocs Olga Anczukow-Camarda, Jan Bergmann, and Leah Sabin, who all currently hold competitive, individual fellowships.

Finally, a most important measure of our postdoctoral program's success is the ability of postdoctoral fellows to secure positions at the end of their training. In 2012, the Laboratory's departing postdoctoral fellows went on to positions at Arnold & Porter, Bayer HealthCare Pharmaceuticals, *Cell Press*, Fraunhofer Institute for Cell Therapy and Immunology, H3 Biomedicine Inc., Kyushu University, Max-Planck Florida Institute, Memorial Sloan-Kettering Cancer Center, Roche Pharmaceuticals, Rutgers University, University of Montreal, and Vellore Institute of Technology, just to name a few.

UNDERGRADUATE RESEARCH PROGRAM

PROGRAM DIRECTORS **Anne Churchland**
Michael Schatz

PROGRAM ADMINISTRATOR **Keisha John**

Established more than 50 years ago, the CSHL Undergraduate Research Program (URP) plays a major role in providing and setting the standard for meaningful hands-on undergraduate research training in biology. The 10-week program begins the first week of June. During the first week, the students are oriented to the campus, receive a guided historical tour of campus, and a tour of all facilities and resources available to them, ensuring a smooth transition into the Laboratory community and research. The URPs work, live, eat, and play among CSHL scientists, and they have a very busy academic and social calendar throughout the remaining 9 weeks of the summer. The students receive training in Scientific Research, Science Communication, Career Preparation, and Bioinformatics and Computational Biology, all while interacting socially with fellow program participants and members of the CSHL community at large in formal and informal activities. Some of these activities in 2011 were a pizza party with Dr. and Mrs. Watson, dinner with Dr. and Mrs. Stillman, BBQ and pool parties, volleyball games, designing the URP T-shirt, competing in the annual Scavenger Hunt, and the ever-famous URP vs. PI volleyball match.

The students' scientific development is the most important component of the program; therefore, in the beginning of the summer, the URPs write an abstract and present a talk on their proposed research. The URP participants work alongside scientists and become increasingly independent throughout the summer. Concluding the program in August, the URPs prepare a final report and present their results in a 15-minute talk at the URP Symposium. As in previous years, the program directors and faculty mentors were highly impressed with the accomplishments of the URP students.

The URPs present their talks in the James Library.

The following 26 students, selected from 884 applicants, took part in the 2012 program:

Francesca Aloisio, University of Texas, Austin
Advisor: **Dr. Gregory Hannon**
Funding: Howard Hughes Medical Institute
Using RNA-FISH to characterize the localization of novel lincRNAs in the mouse hematopoietic system.

Sarah Anderson, University of North Carolina, Chapel Hill
Advisor: **Dr. Gregory Hannon**
Funding: Libby Fellowship
Characterizing the role of pachytene piRNAs in mice.

Marta Andres Terre, University of Pennsylvania, Universitat de Barcelona
Advisor: **Dr. Marja Timmermans**
Funding: 30th Anniversary URP Scholar
Defining the developmental profile of miRNA mobility.

Dhruba Banerjee, University of California, Berkeley
Advisor: **Dr. Florin Albeanu**
Funding: National Science Foundation Scholarship
Top-down control of invariant odor perception.

Alexandra Batchelor, University of Cambridge, United Kingdom
Advisor: **Dr. Adam Kepecs**
Funding: Clare College Watson Scholarship
How does cocaine affect optimal decision making?

Eleanor Batty, Brown University
Advisor: **Dr. Anne Churchland**
Funding: National Science Foundation Scholarship
Encoding of head movement in the posterior parietal cortex.

Eric Biggers, Macalester College
Advisor: **Dr. Michael Schatz**
Funding: National Science Foundation Scholarship
Assembling the pineapple genome.

Jeetayu Biwas, Brandeis University
Advisor: **Dr. Darryl Pappin**
Funding: Former URP Fund Scholarship
Human thymosin b-4: Searching for the mechanism behind the mystery.

Michael Bocek, University of Washington
Advisor: **Dr. Mikala Egeblad**
Funding: Robert H.P. Olney Fellowship
Extending the Brainbow system for live tumor imaging studies.

Rachel Charney, McMaster University, Canada
Advisor: **Dr. Pavel Osten**
Funding: Burroughs Wellcome Fellowship
The neurobiological effects of fever on wild-type mice and the 16p11.2 autistic mouse model.

Zachary Collins, George Washington University
Advisor: **Dr. Partha Mitra**
Funding: Former URP Fund Scholarship
Mapping GABAergic neuron subtypes in mouse models of autism spectrum disorders.

Karensa Crump, Binghamton University
Advisor: **Dr. Stephen Shea**
Funding: Alfred L. Goldberg Fellowship
Granule cell activity in the main olfactory bulb of awake mice.

David Ding, University of Oxford, United Kingdom
Advisor: **Dr. Lloyd Trotman**
Funding: Dorcus Cummings Scholarship
In vitro studies of IL-6 signaling in prostatic cancers and metastases.

Emily Glassberg, Dartmouth College
Advisor: **Dr. Alexander Krasnitz**
Funding: National Science Foundation Scholarship
A novel computational strategy to determine nucleosome positioning in *S. cerevisiae.*

Servan Gruninger, University of Zurich, Switzerland
Advisor: **Dr. Josh Dubnau**
Funding: William Shakespeare Fellowship
Tracking transposition events of the *gypsy* retrotransposon in neural cells of *Drosophila melanogaster.*

Julian Homburger, Cornell University
Advisor: **Dr. Mickey Atwal**
Funding: National Science Foundation Scholarship
Associations between rare variants and complex disease.

Nathan Huey, Kenyon College
Advisor: **Dr. W. Richard McCombie**
Funding: Steamboat Foundation Scholarship
Identifying mutational burden within the DISC1 interactome in a case-control study for psychiatric disorders.

Scott Johnson, University of Maryland, Baltimore County
Advisor: **Dr. Robert Martienssen**
Funding: William Townsend Porter Foundation Scholarship
Uncovering the role of the centromere in the *Arabidopsis* male germline development.

Marissa LaMoure, University of Texas, Austin
Advisor: **Dr. Bruce Stillman**
Funding: James D. Watson Undergraduate Scholarship
Elucidating the Orc2–BubR1 interaction.

Ryan Lee, California State University, San Bernardi
Advisor: **Dr. Yi Zhong**
Funding: Garfield Fellowship
Mapping a complete neural value circuit.

Rebecca Marton, University of Notre Dame
Advisor: **Dr. Christopher Hammell**
Funding: University of Notre Dame URP Scholarship
Development of a high-throughput RNAi screen to identify modulators of heterochronic miRNA activity.

James Morton, Miami University
Advisor: **Dr. Thomas Gingeras**
Funding: National Science Foundation Scholarship
A computational analysis of allele-specific expression.

Helen Mueller, Columbia University
Advisor: **Dr. Alea Mills**
Funding: National Science Foundation Scholarship
Chd5 expression in fetal stem cells.

Amanda Raimer, Widener University
Advisor: **Dr. Adrian R. Krainer**
Funding: National Science Foundation Scholarship
Splicing variability of spinal muscular atrophy.

Ian Stephens, Trinity College, Dublin, Ireland
Advisor: **Dr. Z. Josh Huang**
Funding: Trinity College
Genetic targeting of cortical pyramidal neuron subtypes using mouse engineering.

Edward Twomey, Seton Hall University
Advisor: **Dr. Leemor Joshua-Tor**
Funding: Joan Redmond Read Fellowship
Characterization of Gtsf1 involvement in the piRNA pathway.

PARTNERS FOR THE FUTURE

PROGRAM DIRECTOR **David Jackson**

PROGRAM ADMINISTRATOR **Michele Borriello**

The Partners for the Future Program, established by Dr. James Watson in 1990, provides an opportunity for talented Long Island high school students to have hands-on experience in biomedical research at Cold Spring Harbor Laboratory. This highly competitive program is open to Long Island high school students in their junior year. Each high school science chairperson may nominate up to three students. The top candidates are interviewed by CSHL scientists. Students selected for the program are paired with a scientist mentor and spend a minimum of 10 hours per week, September through March of their senior year, conducting original research. At the conclusion, the students present their projects to an enthusiastic audience of the students, scientific mentors and colleagues, CSHL administrators, parents, and teachers. Although the students learn a great deal about modern biology and state-of-the-art research techniques, the main advantage of the program is that they are exposed to day-to-day life in a lab. Interacting with scientists and support staff, the students learn to define and pursue a research goal while solving problems that may occur along the way. The 2012–2013 Partners for the Future were chosen from among 49 nominations. They are listed below.

Partner	High School	Laboratory	CSHL Mentor
Zachary Graziano	Earl L. Vandermeulen High School	Z. Josh Huang	Josh Huang
Meghan Joline	Miller Place High School	Florin Albeanu	Gonzalo Hugo
Mallory Rutigliano	St. Anthony's High School	Christopher Hammell	Cristina Chen
Rebecca Molinsky	The Wheatley School	Alexander Krasnitz	Guoli Sun
Lina Kim	Mineola High School	Doreen Ware	Lifang Zhang
Meher Walia	Syosset High School	Adrian R. Krainer	Mads Jensen
Ariana Cyrstal Farahani	Friends Academy	David Jackson	Yael Galon-Wolfenson
Morgan Lange	Harborfields High School	Molly Hammell	Molly Hammell
Razvan Tohanean	Mineola High School	Partha Mitra	Swagatam Mukhopadhyay
Emaad Khwaja	Huntington High School	Gregory Hannon	Gregory Hannon
Elias Blinkoff	Glen Cove High School	Adam Kepecs	Sachin Ranade
Michelle Long	Syosset High School	Mikala Egeblad	Juwon Park
Alain Sherman	Roslyn High School	Alea A. Mills	Guy Horev
Steven Trezza	West Hempstead High School	Robert Martienssen	Evan Ernst

Partners for the Future participants

MEETINGS AND COURSES

ACADEMIC AFFAIRS

The Cold Spring Harbor Laboratory Meetings and Courses Program attracts scientists from all over the world to communicate, learn, and critique the latest ideas and approaches in the biological sciences. The program consists of advanced laboratory and lecture courses as well as large meetings and biotechnology conferences that are held almost year-round. The Meetings and Courses Program at the Laboratory attracted strong attendance, with more than 7100 Meeting participants and almost 1300 course participants (trainees, teaching, and support faculty). The Cold Spring Harbor Asia (CSHA) program, including 18 conferences and one summer school, has to date attracted more than 3000 participants, bringing the anticipated year-end total for both United States– and China-based programs to almost 11,400.

This year, the Laboratory held 27 academic meetings that brought together scientists from around the world to discuss their latest research. The Symposium this year focused on plant biology, highlighting the tremendous research progress achieved in recent years, and was intended to provide a broad synthesis of the current state of the field, setting the stage for future discoveries and application. The first Symposium in this historic series that focused exclusively on the botanical sciences attracted more than 300 participants, including notable plant biologists such as David Baulcombe, Philip Benfey, Joanne Chory, John Doebley, Joseph Ecker, Mark Estelle, Sarah Hake, Ottoline Leyser, Marjori Matzke, and Craig Pikaard.

Cold Spring Harbor Laboratory meetings are unique in assembling a program with very few invited speakers. Instead, meeting organizers are encouraged to select talks from abstracts that are submitted on the basis of scientific merit, ensuring that the latest findings will be presented and that young scientists will have the chance to describe their work. The introduction of several successful new meetings occurred in 2012 with Regulatory and Noncoding RNAs, Epigenetics and Chromatin, and the merger of the Pharmacogenomics and Personal Genomes meetings. The annual meetings on The Biology of Genomes and Retroviruses were again oversubscribed, and many others attracted strong or record attendances. In the immediate aftermath of Hurricane Sandy, the Laboratory played host to a diminished but resilient meeting on Nuclear Receptors and Disease under the slogan "Science beats Sandy." Many of these meetings have become essential for those in the field and are held on a biannual basis. Partial support for individual meetings is provided by grants from the National Institutes of Health (NIH), the National Science Foundation (NSF), other foundations, and companies. Core support for the meetings program is provided by the Laboratory's Corporate Sponsor Program.

The Courses program covers a diverse array of topics in molecular biology, neurobiology, structural studies, and bioinformatics. The primary aim of the courses remains to teach advanced 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.

The opening in June 2012 of the new Howard Hughes Medical Institute (HHMI)–funded Hershey Laboratory provided valuable additional space for cell biology and computational courses and is proving to be very popular with instructors and course participants alike. This expansion also allowed for the hosting of a new 2-week laboratory course on Single Cells, due to be held annually, and a discussion course on Cognitive Aging, anticipated to repeat on a biennial basis.

Instructors, course assistants, and course lecturers come from universities, medical schools, research institutes, and companies around the world to teach at CSHL. Their excellence and dedication make the course program work well indeed. We would especially like to thank Drs. Joaquin Espinosa, Kat Hadjantonakis, Jason Mitchell, Mark Reimers, Jaime Rivera-Perez, David Skuse, and Jeff Smith all of whom retired after many years of service.

Grants from a variety of sources support the courses. The core support provided through the recently renewed grant from the HHMI remains critical to our course program. The courses are further supported by multiple awards from the NIH and the NSF, and additional support for individual courses is provided by various foundations and other sources. The courses also depend on equipment and reagents that are loaned or donated by a large number of companies, partnerships that are invaluable in ensuring that the courses offer training in the latest technologies.

Now in its third year of operation, the CSHA program, under the direction of Dr. Maoyen Chi, is headquartered at the Suzhou Dushu Lake Conference Center, a purpose-built academic conference center on the outskirts of old Suzhou, within a high-technology suburb (SIP). CSHA's scientific program includes large symposia and meetings, training workshops, and Banbury-style discussion meetings. CSHA is a wholly owned subsidiary of CSHL and is not beholden to outside partners in terms of our scientific programming. In 2012, 18 scientific conferences and one summer school were held in Suzhou, predominantly attracting scientists from the Asia/Pacific region. Attendance by junior Asian scientists continues to be one of the challenges faced by the program, but the 50% growth in attendance between the first and third year of operations bodes well for the future. This program is described in more detail in a separate Annual Report.

Alumni Notes: The 2012 Nobel Prize in Physiology or Medicine was awarded jointly to Dr. Shinya Yamanaka, who participated in the 1994 course on immunocytochemistry and frequently attended our Translational Control and Mouse Molecular Genetics meetings, and Dr. John Gurdon, a frequent lecturer in the *Xenopus* course and a speaker at several CSHL Symposia, most recently at the 2012 Organ Development meeting.

The meetings and courses program staff is comprised of a lean team of talented professionals, many who wear multiple "hats." They handle the complexities of database design, programming, web and multimedia design, educational grants management, marketing and recruitment, conference and course administration, audiovisual services, and other activities.

Terri Grodzicker
Dean of Academic Affairs

David Stewart
Executive Director,
Meetings and Courses Program/
President, Cold Spring Harbor Asia

77TH COLD SPRING HARBOR LABORATORY SYMPOSIUM ON QUANTITATIVE BIOLOGY

The Biology of Plants

May 30–June 4 319 Participants

ARRANGED BY **Terri Grodzicker, Rob Martienssen, David Stewart, and Bruce Stillman**
Cold Spring Harbor Laboratory

R. Martienssen, S. Hake

The Cold Spring Harbor Symposia on Quantitative Biology bring together scientists from all over the world to present and evaluate new data and ideas in rapidly moving areas of biological research. Each year, a topic is chosen that appears to be at a stage where general and intensive scrutiny and review are needed. Criteria for selection of topics are numerous, but they include the rate of progress in a given field, how recent research is highlighting connections between fundamental biological mechanisms, and the potential applications of the new discoveries to human health and society. Cold Spring Harbor Laboratory selected the theme of The Biology of Plants for the historic 77th Symposium in the series.

Plants are integral to human well-being, and many species have been domesticated for more than 10,000 years. Evidence of plant scientific investigation and classification can be found in ancient texts from cultures around the world (Chinese, Indian, Greco-Roman, Muslim, etc.), whereas early modern botany can be traced to the late 15th and early 16th centuries in Europe. During the past several decades, plant biology has been revolutionized first by molecular biology and then by the genomic era. The model organism *Arabidopsis thaliana* has proved to be an invaluable tool for investigation into fundamental processes in plant biology, many of which share commonalities with animal biology. Plant-specific processes from reproduction to immunity and second messengers have also yielded to extensive investigation. With the genomes of more than 30 plant species now available and many more planned in the near future, the impact on our understanding of plant evolution and biology continues to grow. Our increased ability to engineer plant species to a variety of ends may provide novel solutions to ensure adequate and reliable food production and renewable energy even as climate change impacts our environment.

The decision to focus the 2012 Symposium on plant science reflected the enormous research progress achieved in recent years, and it was intended to provide a broad synthesis of the current state of the field, setting the stage for future discoveries and application. This is the first Symposium in this historic series that focused exclusively on the botanical sciences. The Symposium spanned a broad range of areas of investigation including genetics, biochemistry, molecular and cell biology, developmental biology, physiology, and population/evolution studies at levels ranging from the single cell to the entire organism and from single genes to genomes; plant-specific processes and pathways featured broadly throughout the meeting. Effort was made to balance fundamental biological discoveries with applications relevant to societal well-being including improved crops, fuel, and habitat.

In arranging this Symposium, the organizers were dependent on the guidance of a broad cadre of advisors including Drs. Phil Benfey, Joanne Chory, Nam-Hai Chua, Jeff Dangl, Joe Ecker, and Chris Somerville. Opening night speakers included Sarah Hake, Craig Pikaard, Joanne Chory,

and Jeff Dangl. Rob Martienssen delivered a compelling Dorcas Cummings Lecture on "Send in the Clones" to Laboratory friends, neighbors, and Symposium participants in advance of the annual dinner parties.

This Symposium was attended by almost 320 scientists from more than 20 countries, and the program included 65 invited presentations and 169 poster presentations. To disseminate the latest results and discussion of the Symposium to a wider audience, attendees were able to share many of the Symposium talks with their colleagues who were unable to attend using the Leading Strand video archive, and interviews by Gemma Bilsborough, Inês Chen, Kate Creasey, Emilie Marcus, Richard Sever, and Jan Witkowski with leading experts in the field were arranged during the Symposium and distributed as free video from the Cold Spring Harbor Symposium interviews website.

Funds to support this meeting were obtained from the National Science Foundation, the Gordon & Betty Moore Foundation, and the Gatsby Charitable Foundation. Financial support from the corporate sponsors of our meetings program is essential for these Symposia to remain a success, and we are most grateful for their continued support.

PROGRAM

Introduction
B. Stillman, *Cold Spring Harbor Laboratory*

Germline
Chairperson: X. Chen, *University of California, Riverside*

Signaling
Chairperson: J. Ecker, *The Salk Institute for Biological Studies, La Jolla, California*

Epigenetics
Chairperson: D. Baulcombe, *University of Cambridge, United Kingdom*

Plant Pathogen Responses
Chairperson: S. Harmer, *University of California, Davis*

Domestication and Evolution
Chairperson: P. Benfey, *Duke University, Durham, North Carolina*

Timing
Chairperson: O. Leyser, *University of Cambridge, United Kingdom*

Photosynthesis, Metabolism, and Biofuels
Chairperson: S. Jacobsen, *University of California, Los Angeles*

Small RNAs
Chairperson: U. Grossniklaus, *University of Zürich, Switzerland*

Dorcas Cummings Lecture: Send in the Clones
R. Martienssen, *Cold Spring Harbor Laboratory*

Stem Cells, Polarity, and Nuclear Organization
Chairperson: D. Shippen, *Texas A&M University, College Station*

Development and Abiotic Stress
Chairperson: D. Bergmann, *Stanford University, California*

Summary
V. Chandler, *Gordon & Betty Moore Foundation, Palo Alto, California*

Z. Lippman, D. Zamir, D. Ware

Y. Plavskin, J. Ecker

MEETINGS

PTEN Pathways and Targets

March 13–16 109 Participants

ARRANGED BY **Suzanne Baker,** St. Jude Children's Research Hospital
Lewis Cantley, Beth Israel Deaconess Medical Center/Harvard Medical School
Pier Paolo Pandolfi, Beth Israel Deaconess Medical Center/Harvard Medical School
Ramon Parsons, Columbia University

S. Baker, H. Dbouk

This fourth conference was a dynamic discussion forum, bringing together top scientists studying the PTEN/PI3 kinase pathway using biochemical and molecular approaches as well as in vivo model systems. An exciting series of talks and poster presentations addressed the complex regulation of this signal transduction cascade and its critical role in normal development, cancer, aging and metabolism, and other pathological disease states. The conference provided the opportunity for researchers of all levels to get together to discuss their latest research findings and technical approaches toward the analysis of the PTEN pathway. Particular emphasis was placed on pathway regulation and cross-talk, emerging complex mechanisms of PTEN regulation, human cancer pathogenesis, disease models, and therapy.

A total of 76 investigators presented in the nine scientific sessions, with 45 platform and 31 poster presentations. The seven platform and two poster sessions were marked by dynamic and enthusiastic exchanges of new results. In the scientific sessions, many audience members participated in the question and answer sessions, and the poster sessions were well attended.

The sessions are listed below in the Program. Essential funding for the meeting was provided by the National Cancer Institute, a branch of the National Institutes of Health, and by Novartis.

PROGRAM

PI3 K and Upstream Regulation of PTEN Signaling
Chairperson: P. Vogt, *The Scripps Research Institute, La Jolla, California*

Downstream Targets of PTEN
Chairperson: A. Toker, *Beth Israel Deaconess Medical Center, Boston, Massachusetts*

mTORC1 and Intersecting Pathways
Chairperson: B. Manning, *Harvard School of Public Health, Boston, Massachusetts*

PI3 K/PTEN Signaling In Development and Disease
Chairperson: S. Baker, *St. Jude Children's Research Hospital, Memphis, Tennessee*

R. Parsons, B. Hopkins

Complex Mechanisms of PTEN Regulation
Chairperson: P.-P. Pandolfi, *Beth Israel Deaconess Medical Center/Harvard Medical School, Boston, Massachusetts*

Modeling PI3 K/PTEN Signaling in Vivo
Chairperson: J. Zhao, *Harvard Medical School, Boston, Massachusetts*

Therapeutic Inhibitors of PI3 K Signaling
Chairperson: J. Engelman, *Massachusetts General Hospital, Boston*

G. Mills, N. Sonenberg

J. Den Hertog

L. Trotman, S. Muthuswamy

Systems Biology: Global Regulation of Gene Expression

March 20–24 273 Participants

ARRANGED BY **Martha Bulyk,** Brigham & Women's Hospital and Harvard Medical School
Marian Walhout, University of Massachusetts Medical School
Brad Bernstein, Massachusetts General Hospital/Harvard Medical School/Broad Institute
Timothy Hughes, University of Toronto, Canada

Systems Biology aims to understand the emergent properties of molecular networks in cells by using systematic and global approaches. One of the most actively researched areas of systems biology in recent years has been global regulation of gene expression, which coordinates complex metabolic and developmental programs in organisms. This eighth conference, like the ones in previous years, captured the continuing rapid progress and many new discoveries in this young and exciting field. This meeting featured 46 talks and 109 poster presentations covering a broad range of topics. The speakers, poster presenters, and other conference attendees were composed of a mix of students, postdocs, and PIs at all levels. Two keynote speeches were exciting highlights of the meeting. The first was presented by Dr. Michael Levine, who has been a leader in the area of transcriptional regulation for many years. Dr. Levine described the identification and analysis of gene regulatory sequences in *Drosophila* and presented new ideas on the significance of paused RNA polymerase at promoters. The second keynote was presented by Dr. Robert Kingston, a leader in the field with a long history of the development of new approaches to the study of chromatin and gene regulation. Dr. Kingston presented a new technique for identifying the genomic loci associated with noncoding RNAs, as well as a recently published structure of a nucleosome-associated BAF domain. This year's meeting featured new techniques utilizing second-generation sequencing; for example, several presentations included systematic dissection of regulatory sequences by assaying thousands of synthesized variants. Many of the presentations also described new approaches to the analysis of data from established techniques, such as ChIP-Seq. Relative to previous years, the analyses generally involved much larger data sets due to ongoing increases in sequencing volume, systematic efforts such as ENCODE and modENCODE, and ever-expanding resources on the binding specificities of transcription factors. Many presentations focused on the difficult problems of relating transcription factor sequence preferences to their in-vivo-binding sites and of relating the in-vivo-binding sites of transcription factors and chromatin proteins to gene expression outputs. The three-dimensional architecture of chromosomes also featured prominently. Representation of DNA-binding and RNA-binding activities and the role of long ncRNAs continued to be topics of interest. It was clear that much remains to be done in understanding virtually all levels of global gene regulation; however, many of the presented studies featured tight integration of experimental and computational work, so clearly, the mission of this conference in bringing together experimental and computational scientists is succeeding.

This meeting was funded in part by the National Cancer Institute, a branch of the National Institutes of Health; the National Science Foundation; and Roche.

PROGRAM

Keynote Speaker
M. Levine, *University of California, Berkeley*

Developmental Networks
Chairperson: S. Teichmann, *MRC Laboratory of Molecular Biology, Cambridge, United Kingdom*

Transcriptional Regulatory Networks
Chairperson: S. Churchman, *Harvard Medical School, Boston, Massachusetts*

Keynote Speaker
R. Kingston, *Massachusetts General Hospital, Boston*

Chromatin
Chairperson: J. Dekker, *University of Massachusetts Medical School, Worcester*

Epigenomics
Chairperson: T. Hughes, *University of Toronto, Canada*

***Cis*-Regulatory Logic**
Chairperson: H. Bussemaker, *Columbia University, New York*

Cellular Responses
Chairperson: B. Andrews, *University of Toronto, Canada*

Posttranscriptional Regulation
Chairperson: M. Walhout, *University of Massachusetts Medical School, Worcester*

Variation and Evolution
Chairperson: A. Stark, *Research Institute of Molecular Pathology, Vienna, Austria*

P. Freddolino, G. Fundenberg

Discussion during poster session

F. Schlesinger, T. Gingeras

O. Bahcall

Neuronal Circuits

March 28–31 194 Participants

ARRANGED BY **Cori Bargmann,** The Rockefeller University
Dmitri Chklovskii, Janelia Farm Research Campus
Carl Petersen, Ecole Polytechnique Federale de Lausanne

D. Chklovskii, C. Petersen, C. Bargmann

In the quest to understand the brain, neuronal circuits represent a central level of description. Establishing connectivity in neuronal circuits seems as essential for solving the brain as having a geographic map for planning one's travel. Although such realization has motivated Cajal to describe a variety of neuronal circuits using Golgi stains, his contributions were technologically limited. With the recent appearance of novel molecular genetic, imaging, and computational techniques, a comprehensive description of the wiring diagram, an old dream of neuroscientists, is about to become a reality. Because technological advances have been made in different organisms and systems, we wanted to create a forum that brings together researchers working on different topics, yet focusing on neuronal circuits. The 2008 meeting on "Neuronal Circuits: From Structure to Function" included six broad slide sessions covering the olfactory system, the visual system, methods, behavior learning and memory, motor systems and the cortex, and a very interactive poster session.

For the second meeting of this kind, the response of the field was very enthusiastic. The meeting brought together 194 participants from all over the world, most of whom made either oral or poster presentations. Invited talks were given by Cori Bargmann, The Rockefeller University; Tobias Bonhoeffer, Max-Planck Institute of Neurobiology; Karl Deisseroth, Stanford University; Winfried Denk, Max-Planck Institute for Medical Research; Jeff Lichtman, Harvard University; Eve Marder, Brandeis University; Edvard Moser, Center for the Biology of Memory; Sacha Nelson, Brandeis University; Clay Reid, Harvard Medical School; Massimo Scanziani, University of California, San Diego; Kristin Scott; University of California, Berkeley; Stephen Smith, Stanford Medical School; Charles Stevens, The Salk Institute; Karel Svoboda, Janelia Farm Research Campus, Howard Hughes Medical Institute; Gabor Tamas, University of Szeged, Hungary/HHMI; Rachel Wilson, Harvard Medical School; and Rafael Yuste, Columbia University/HHMI. The meeting provided an important clearinghouse for ideas and approaches and helped scientists in the field get the most up-to-date information, as well as enabling them to meet, to network, and to establish collaborations. Based on the uniformly enthusiastic comments of the participants, the intensity of the oral and poster sessions, and the large crowds that stayed up late every night at the bar to discuss science, the meeting was a great success.

A highlight of the meeting program was the inaugural Larry Katz Memorial Lecture; thanks to the generous donation of many colleagues in the field, 25 excellent nominations were received all over the world. A committee composed of the three organizers and Cori Bargmann selected two speakers: Andrea Hasenstaub of Salk Institute for Biological Studies, and Feng Zhang of Stanford University. Both gave wonderful talks about their graduate studies.

This meeting was funded in part by the National Institute of Neurological Disorders and Stroke, a branch of the National Institutes of Health.

PROGRAM

Information Processing
Chairperson: D. Chklovskii, *HHMI/Janelia Farm Research Campus, Ashburn, Virginia*

Chemosensation and Decision Making
Chairperson: C. Bargmann, *HHMI/The Rockefeller University, New York*

Vision/Hearing
Chairperson: A. Konnerth, *Technical University Munich, Germany*

Innate and Learned Behaviors
Chairperson: M. Feller, *University of California, Berkeley*

Locomotion and Technology
Chairperson: R. Friedrich, *Friedrich-Miescher-Institute, Basel, Switzerland*

Development and Plasticity
Chairperson: Y. Dan, *University of California, Berkeley*

Larry Katz Lecture
W. Hong, *Stanford University, California*

H. Oyibo

A. Reid, T. Hromadka

I. Pikon, J. Bargmann

Evolution of *Caenorhabditis* and Other Nematodes

April 3–6 129 Participants

ARRANGED BY **Dee Denver,** Oregon State University
Mark Blaxter, University of Edinburgh

D. Denver, M. Blaxter

This meeting was well attended by researchers representing 11 countries. The keynote speaker was Marie-Anne Félix, who discussed diverse aspects of key advances that her lab and collaborators have made in our understanding of nematode evolution, ranging from the discovery of many critical new species in the model genus *Caenorhabditis* to the mutational and selective forces shaping natural variation in nematode developmental processes. Scientific sessions included eight presentations, and the poster session included 26 additional presentations of the topics listed in the program below as well. The conference was preceded by an NSF-supported workshop of the NemaSym Research Coordination Network, focusing on symbiotic nematode–bacteria interactions, which included several presentations on diverse nematode-related symbioses, as well as discussions on how to move forward in understanding these interorganismal relationships. During a meeting planning session, extensive enthusiasm was expressed about the 2012 meeting at Cold Spring Harbor, new organizers were recruited, and it was agreed to meet again at the Wellcome Genome Campus in Cambridge, England (as in 2010) and then hopefully return to Cold Spring Harbor in 2016.

This meeting was funded in part by the National Human Genome Research Institute, a branch of the National Institutes of Health; the National Science Foundation; and the Nathan A. Cobb Nematology Foundation. The Nathan A. Cobb Nematology Foundation provides all nematologists and friends the opportunity to build self-sustaining endowments for the advancement of nematology. The endowments provide grants to students for study, travel, and participation in annual meetings of nematological and related societies. For more information about the Foundation and its endowments, visit http://nematologists.org/na_cobb_foundation.php.

PROGRAM

Biodiversity and Phylogenetics
Chairpersons: H. Bik, *University of California, Davis;* T. Moens, *Ghent University, Belgium*

Keynote Speaker
M.-A. Félix, *Ecole Normale Supérieure, Paris, France*

Genomes and Transcriptomes
Chairpersons: J. Spieth, *Washington University, St. Louis, Missouri;* M. Berriman, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*

Discussion Session: Multi-*Caenorhabditis* genomes analysis

Population and Evolutionary Genomics I
Chairperson: A. Cutter, *University of Toronto, Canada*

Panel Discussion: Future Directions for Our Research Community

Sex and Recombination
Chairpersons: J. Anderson, *University of Oregon, Eugene;* M. Rockman, *New York University, New York*

Parasitism, Pathogenesis, and Symbiosis
Chairpersons: H. Schulenburg, *University of Kiel, Germany;* D. Bird, *North Carolina State University, Raleigh*

Evolution of Molecular, Cellular, and Developmental Pathways
Chairperson: M. Mitreva, *Washington University, St. Louis, Missouri*

Population and Evolutionary Genomics II
Chairperson: A. Cutter, *University of Toronto, Canada*

From Genotype to Phenotype
Chairpersons: J. Kammenga, *Wageningen University, The Netherlands;* M. Viney, *University of Bristol, United Kingdom*

Automated Imaging and High-Throughput Phenotyping

April 10–14 104 Participants

ARRANGED BY **Philip Benfey,** Duke University
Anne Carpenter, Broad Institute of Harvard and Massachusetts Institute of Technology
Robert Waterston, University of Washington
Uwe Ohler, Duke University

This second conference proved to be as successful as the first. A total of 41 investigators presented in the seven scientific sessions, with many of the other 104 registered attendees presenting in the poster session. The one poster and seven platform sessions were marked by dynamic and enthusiastic exchanges of new results. In the scientific sessions, many audience members participated in the question and answer sessions, and the poster session was well attended. The meeting Sessions are listed below in the program.

An additional session engaged the community in discussion of the future directions for this field. During this session, there was a high level of enthusiasm to hold another meeting at Cold Spring Harbor on the same topic.

This meeting was partially supported by LemnaTec GmbH and Molecular Devices.

P. Benfey, B. Peshlov

Poster Session in Bush Auditorium

PROGRAM

Microscopy and Technology
Chairperson: S. Fraser, *California Institute of Technology, Pasadena*

Single Cell and Subcellular Phenotyping I
Chairperson: A. Carpenter, *Broad Institute of Harvard and Massachusetts Institute of Technology, Cambridge*

Models for Development and Behavior
Chairperson: C. Godin, *University of Chicago, Illinois*

Developmental Phenotyping
Chairperson: R. Waterston, *University of Washington, Seattle*

Discussion Session: Current Challenges/Outlook/ Planning for Next Meeting
P. Benfey, *Duke University, Durham, North Carolina*
A. Carpenter, *Broad Institute of Harvard and MIT, Cambridge*
R. Waterston, *University of Washington, Seattle*
U. Ohler, *Duke University, Durham, North Carolina*

Cellular Phenotyping: Differentiation and Migration
Chairperson: J. Kovacevic, *Carnegie Mellon University, Pittsburgh, Pennsylvania*

Data Mining, Modeling, Management, and Visualization
Chairperson: U. Ohler, *Duke University, Durham, North Carolina*

Organ(Ismal)/Behavioral Phenotyping
Chairperson: P. Benfey, *Duke University, Durham, North Carolina*

A. Assadi, A. Sangari

T. Chen

Molecular Pathways in Organ Development and Disease

April 17–21 125 Participants

ARRANGED BY **Sharon Dent**, University of Texas M.D. Anderson Cancer Center
Jonathan Epstein, University of Pennsylvania School of Medicine
Guillermo Oliver, St. Jude Children's Research Hospital

J. Epstein, S. Dent, G. Oliver

Recent advances in stem cell biology are impressive and have strengthened our collective optimism that gene and stem-cell-based therapies may eventually become realistic approaches in the treatment of many diseases, particularly those that have a significant pathological effect on organs and specific organ systems. Central to that goal is a clear understanding of the genes and mechanisms controlling vertebrate organogenesis in health and disease. Accordingly, the specific goal of this new conference was to bring together a diverse group of scientists working in various animal models and interested in studying different molecular, cellular, and genetic aspects of vertebrate organogenesis in basic and translational settings.

This meeting provided a forum in which researchers working in various animal models (mice, zebrafish, chicken) in diverse aspects of organ development presented their latest findings and an overview of the field. In this setting, the conference provided the opportunity for an important exchange of information and ideas and to help to establish fruitful collaborations. Six major topics were chosen for detailed discussion, each including basic and disease-oriented presentations as a reflection of the most advanced and interesting areas of study in the field of vertebrate organogenesis. These topics were all ultimately united by the use of approaches that pursue answers to key biological questions in the intact organism rather than in simplified cell culture and ex vivo studies.

PROGRAM

Keynote Speakers
L.B. Buck, *Fred Hutchinson Cancer Research Center, Seattle, Washington:* Deconstructing Smell.
J.B. Gurdon, *Wellcome Trust/CR UK, Cambridge, United Kingdom:* The Stabilization of Cell Differentiation.

Signaling Pathways/Morphogenesis
Chairperson: O. Pourquie, *IGBMC, Illkirch, France*

Regulation of Gene Expression
Chairperson: J. Epstein, *University of Pennsylvania School of Medicine, Philadelphia*

Evolution
Chairperson: C. Tabin, *Harvard Medical School, Boston, Massachusetts*

Gene Regulatory Networks/Computational Biology
Chairperson: S. Fraser, *California Institute of Technology, Pasadena*

Y. Serinagaoglu, R. Deckelbaum

Stem Cells in Development
Chairperson: Y. Sasai, *RIKEN Center for Developmental Biology, Kobe, Japan*

Epigenetics
Chairperson: S. Dent, *University of Texas M.D. Anderson Cancer Center, Houston*

Cancer and Disease
Chairperson: S. Piccolo, *University of Padua, Italy*

E. Fuchs

M.-I. Chung

Wine and cheese reception

Gene Expression and Signaling in the Immune System

April 24–28 351 Participants

ARRANGED BY **Doreen Cantrell,** University of Dundee
Sankar Ghosh, Columbia University College of Physicians & Surgeons
Dan Littman, HHMI/New York University School of Medicine
Mark Schlissel, University of California, Berkeley


M. Schlissel

This meeting was held for the sixth time in 2012, in the bright sunshine and mild temperatures of springtime. More than 350 registrants, including ~30% from outside of the United States and many first-time attendees, participated in a highly engaging and interactive 4-day meeting.

Although there are many immunology meetings, the unique aspect of this Cold Spring Harbor meeting is its focus on molecular and biochemical aspects of the development and function of the immune system. In addition, this meeting attracts a broad range of scientists who use the immune system as a model to study basic principles of biological regulation.

Talks were presented by a mix of invited speakers and investigators selected from a group of more than 250 submitted abstracts. There were a significant number of women and junior investigators who were asked to give talks. Most speakers focused almost exclusively on their unpublished work. A number of exciting talks, such as the presentation by Alexander (Sasha) Rudensky (Memorial Sloan-Kettering Cancer Center), explained a long-standing biological phenomenon, namely, the ability of a mother to carry a fetus that is not immunologically identical during pregnancy. His work showed that induced T-regulatory cells are critical for the suppression of the maternal immune response. Steve Smale presented exciting studies that showed by using the technology of RNA-Seq that the composition of the inflammatory gene expression program in macrophages is determined by not just transcription, but also the process of splicing of transcripts and their export to the cytosol. Such talks highlighted the relevance of the data discussed at this meeting not just to immunology, but to the broader scientific community.

Oral presentations were supplemented by three afternoon poster sessions that were extremely well attended and provided a forum for all meeting participants (including many graduate students and postdocs) to share their most recent data. In addition, attendees took advantage of walking tours of the CSHL campus, an engaging piano recital, and an evening performance by the Yale-based music group "The Cellmates" led by Richard Flavell.

Support for this meeting was obtained from the NIH/NIAID as well as from corporate sponsors.

PROGRAM

SESSION 1: Developmental Decisions
Chairperson: I. Weissman, *Stanford University School of Medicine, California*

SESSION 2: Regulation of Immune Cell Development
Chairperson: S. Smale, *University of California School of Medicine, Los Angeles*

SESSION 3: Poster Session I

SESSION 4: Chromatin Structure and Epigenetic Regulation
Chairperson: S. Ghosh, *Columbia University, New York*

SESSION 5: Antigen Receptor Gene Assembly and Modification
Chairperson: M. Schlissel, *Brown University, Providence, Rhode Island*

SESSION 6: Poster Session II

SESSION 7: Signal Transduction in Immune Cells
Chairperson: D. Cantrell, *College of Life Sciences, Dundee, United Kingdom*

SESSION 8: Regulation of Lymphocyte Function I
Chairperson: R. Germain, *National Institute of Allergy and Infectious Diseases/NIH, Bethesda, Maryland*

SESSION 9: Regulation of Lymphocyte Function II
Chairperson: D. Littman, *HHMI/New York University School of Medicine, New York*

SESSION 10: Innate Immunity
Chairperson: J. Hoffmann, *IBMC, Strasbourg, France*

D. Cantrell, R. Sever

F. Alt, D. Baltimore

R. Flavell fronting the Cellmates

Caricaturist in action

Molecular Chaperones and Stress Responses

May 1–5 220 Participants

ARRANGED BY **Andrew Dillin,** HHMI/University of California, Berkeley
F. Ulrich Hartl, Max-Planck Institute for Biochemistry, Germany
Jonathan Weissman, HHMI/University of California, San Francisco

A. Dillin, J. Weissman

This meeting continued the rich tradition of a forum where cutting-edge analysis of molecular mechanisms of protein folding is presented in the context of its biological importance and health relevance. The high attendance, large number of posters (131 posters over three sessions), and lively audience participation in the sessions and in the informal activities that followed are testament to the success of the meeting and to the successful balance of the sessions. The field of chaperone research is evolving and this meeting is evolving with it and is proving to be the cornerstone meeting for this rich and exciting field.

In addition to well-established biochemical and biophysical methods for studying chaperone function, this meeting featured presentations using single-molecule methods and advanced computational approaches to the study of signaling networks involved in control of chaperone gene expression. This year's meeting featured two new sessions: one on Therapeutics of Protein Misfolding, chaired by Dr. Jeff Kelly, and the other on Chaperones and Proteolysis, chaired by Dr. Dan Finley. The former provided a forum to discuss new approaches to entering the clinic using the knowledge base found within the chaperone field and the latter for work of special relevance to the prospects of linking chaperone function to emerging fields of protein degradation. Among the cutting-edge themes explored in this meeting were presentations of single-molecule studies of protein interactions and folding in vitro and new approaches to attempt in vivo; talks on protein trafficking in the cell that emphasized the fluidity of subcellular compartmentalization, when it comes to intracellular protein turnover; and connections between ribosomes and chaperones and novel signaling pathways involved in protein-folding homeostasis. An entire section was devoted to aging, illustrating how this field is beginning to impact the thinking within the gerontology community.

This meeting was funded in part by the National Institute on Aging, a branch of the National Institutes of Health, and Enzo Life Sciences.

Poster session in Bush Auditorium

PROGRAM

Protein Folding and Chaperone Biochemistry
Chairperson: S. Marqusee, *University of California, Berkeley*

Quality Control and Protein Trafficking
Chairperson: K. Nagata, *Kyoto Sangyo University, Japan*

Therapeutics of Protein Misfolding
Chairperson: J. Kelly, *Scripps Research Institute, La Jolla, California*

Protein Folding and Disaggregation Machines
Chairperson: S. Marqusee, *University of California, Berkeley*

Stress Signaling and Aging
Chairperson: L. Sistonen, *Åbo Akademi University, Turku, Finland*

Chaperone Networks in Development and Disease
Chairperson: A. Bertolotti, *MRC Laboratory of Molecular Biology, Cambridge, United Kingdom*

Chaperones and Proteolysis
Chairperson: D. Finley, *Harvard Medical School, Boston, Massachusetts*

R. Morimoto, T. Gidalevitz, A. Ben-Zvi

Wine and cheese reception

The Biology of Genomes

May 8–12 541 Participants

ARRANGED BY **Carlos Bustamante,** Stanford University
Susan Celniker, Lawrence Berkeley National Laboratory
Joel Hirschhorn, Broad Institute of Harvard and Massachusetts Institute of Technology
Christopher Ponting, University of Oxford

This meeting marked the 25th annual gathering of genome scientists at the Cold Spring Harbor Laboratory. Just over 540 people from around the world attended the meeting, with more than 360 abstracts presented describing a broad array of topics relating to the production, analysis, and interpretation of genomes from diverse organisms. The meeting built upon the remarkable progress in the sequencing, functional annotation, and analysis of genomes from many human individuals, "model organisms," and other animals including new genomic studies of pigeons, plants, microbiomes, and prokaryotes. Talks from the ENCODE and modENCODE consortium described their efforts to determine the function of every base in the human, worm, and fly genomes. Many investigators reported on their application of the most recent generation of DNA-sequencing technologies to determine sequence variants within populations that convey differences in molecular function and organismal phenotype and in susceptibility to disease, including cancer and complex disease. The new data are also providing important insights into functional genomic elements and population structure for humans and other species. Many groups are generating transcriptome, transcription-factor-binding site, and epigenomic maps to illuminate transcriptional complexity and regulation. Whole-genome single-cell sequencing and single-cell transcriptome analyses were also announced.

Session topics are listed below in the Program. There were numerous reports on progress in using next-generation sequencing technologies, with mounting expectation for further advances as these continue to be applied widely. Susan Wessler and Debbie Nickerson gave the Keynote Presentations on transposable elements and on human genetic variation, respectively.

The ELSI (Ethical, Legal, and Social Implications) panel was moderated by Vence Bonham. The area of discussion was Genomic Literacy for the Public and the panelists included Wayne Grody, Kimberly Kaphingst, and Jon Miller.

This meeting was funded in part by the National Human Genome Research Institute, a branch of the National Institutes of Health; Complete Genomics; Illumina; Omicia; and OpGen. The *Genome Research* poster prize was sponsored by Oxford Nanopore Technologies Ltd.

PROGRAM

High-Throughput Genomics and Genetics
Chairpersons: B. Cohen, *Washington University School of Medicine, St. Louis, Missouri;* S. Quake, *HHMI/Stanford University, California*

Genetics of Complex Traits
Chairpersons: B. Voight, *University of Pennsylvania Perelman School of Medicine, Philadelphia;* E. Dermitzakis, *University of Geneva Medical School, Switzerland*

Functional and Cancer Genomics
Chairpersons: E. Ostrander, *National Human Genome Research Institute/NIH Bethesda, Maryland;* P. Spellman, *Oregon Health & Science University, Portland*

J. Watson, E. Birney

Computational Genomics
Chairpersons: S. Myers, *Oxford University, United Kingdom;* L. Pachter, *University of California, Berkeley*

ELSI Panel and Discussion: Genomic Literacy for the Public
Moderator: V.L. Bonham, *National Human Genome Research Institute/NIH*, Bethesda, Maryland, *Panelists:* W.W. Grody, *University of California School of Medicine, Los Angeles;* K. Kaphingst, *Washington University School of Medicine, St. Louis, Missouri;* J.D. Miller, *University of Michigan, Ann Arbor*

Evolutionary Genomics
Chairpersons: D. Odom, *University of Cambridge, United Kingdom;* P. Wittkopp, *University of Michigan, Ann Arbor*

Genetics and Genomics of Nonhuman Species
Chairpersons: A. Stathopoulos, *California Institute of Technology, Pasadena;* L. Herrera Estrella, *National Polytechnic Institute, Irapuato, Mexico*

Guest Speakers
S.R. Wessler, *University of California, Riverside:* The Success Strategies of Transposable Elements that Rapidly Diversify Genomes.
D. Nickerson, *University of Washington, Seattle:* Human Genetic Variation: A Walk on the Rare Side.

Population Genomic Variation
Chairpersons: D. Bachtrog, *University of California, Berkeley;* J. Novembre, *University of California, Los Angeles*


Y. Hu, X. Ni


W. Kent, A. Hinrichs


D. Spector and A. Krainer pose for M. Macaluso

The Cell Cycle

May 15–19 256 Participants

ARRANGED BY **Sue Biggins,** Fred Hutchinson Cancer Research Center
Jacqueline Lees, Massachusetts Institute of Technology Center for Cancer Research
David Toczyski, University of California, San Francisco

S. Biggins, J. Lees, D. Toczyski

This 11th biannual meeting is internationally recognized for its ability to bring together scientists who study cell cycle regulation in eukaryotes ranging from yeast to humans. As usual, the meeting began with a keynote talk from a leader in the field. We were fortunate to have the Director of Cold Spring Harbor Laboratories, Dr. Bruce Stillman, tell us about the initial discovery of the origin recognition complex and the regulation of origin firing that ensures accurate duplication of chromosomes. He also talked about subsequent and unexpected roles for the origin recognition complex in the cell cycle. After the keynote talk, the evening session continued with five talks focused on the cross-talk between nuclear and cytoplasmic events. The remainder of the meeting was organized around seven lecture sessions and two poster sessions, which focused on the major stages of the cell division cycle, with an emphasis in each session on the molecular mechanisms that govern cell cycle progression. Many of these sessions emphasized the long-standing problems in cell cycle control, including the transcriptional mechanisms controlling entry in the cell cycle, the mechanisms that initiate and control chromosome duplication, the events that trigger the complex events of chromosome segregation in mitosis, and the numerous checkpoint systems that ensure that cell cycle events occur in the correct order. Cutting-edge techniques to address problems as well as talks that emphasized computational modeling and quantitative approaches were represented. The meeting ended with a new session on cell cycle decisions that brought together talks from yeast to human cancer. There were also a number of talks and posters about quiescence and other specialized states of the cell cycle. The meeting also connected cell cycle regulation with cancer biology with reports about the functions of oncogenes and tumor suppressors in cell cycle control. As always, major model systems for cell cycle analysis were represented, and the striking phylogenetic conservation of cell cycle regulatory mechanisms was readily evident. It was another landmark meeting for the cell cycle field, and the participants continue to look forward to equally exciting meetings in future years.

PROGRAM

Nucleocytoplasmic Cross-Talk
Chairperson: S. Bell, *HHMI/Massachusetts Institute of Technology, Cambridge*

Keynote Address: The Origin Recognition Complex: Chromosome Duplication and Separation
B. Stillman, *Cold Spring Harbor Laboratory*

G_0-G_1 Control
Chairpersons: N. Dyson, *Massachusetts General Hospital Cancer Center, Charlestown;* B. Andrews, *University of Toronto, Canada*

S Phase
Chairpersons: P. Jallepalli, *Memorial Sloan-Kettering Cancer Center, New York;* H. Ulrich, *Cancer Research UK London Research Institute, United Kingdom*

Kinetochores and the Spindle Checkpoint
Chairpersons: E. Nigg, *University of Basel, Switzerland;* F. Uhlmann, *Cancer Research UK London Research Institute, United Kingdom*

DNA Damage Response
Chairpersons: T. Paull, *University of Texas, Austin;* M. Jasin, *Memorial Sloan-Kettering Cancer Center, New York*

Cell Cycle Regulation by the Ubiquitin Pathway
Chairpersons: J. Pines, *University of Cambridge, United Kingdom;* M. Pagano, *New York University Medical Center, New York*

Mitosis and Cytokinesis
Chairpersons: A. Amon, *HHMI/Massachusetts Institute of Technology, Cambridge*; B. Earnshaw, *University of Edinburgh, United Kingdom*

Cell Cycle Decisions
Chairpersons: M. Fuller, *Stanford University School of Medicine, California;* G. Evan, *University of Cambridge, United Kingdom*

M. Mechali, S. Prasanth

F. Uhlman

T. Maresca, T. Fakagasa

S. Bell

Retroviruses

May 21–26 420 Participants

ARRANGED BY **Paula Cannon,** University of Southern California
Vineet KewalRamani, National Cancer Institute

V. KewalRamani, P. Cannon

Drs. Paula Cannon and Vineet KewalRamani are long-time attendees who in many ways have "grown-up" at this meeting and within its distinct community of scientists. Indeed, it is a characteristic feature of the Retroviruses meeting that a high number of repeat attendees come each year, where friendships and collaborations are both instigated and renewed.

In keeping with this theme, the organizers chose to recognize with keynote talks the contributions of one of the strongest supporters of the meeting, Dr. Jonathan Stoye. Jonathan took us on a tour of his many contributions to retrovirology by posing the question "Germ-line retroviruses–good or bad?" in a generous and entertaining talk that garnered many laughs along the way. His work has encompassed mouse retroviruses, pig viruses and xenotransplantation, and the ongoing standoff between retroviruses and their hosts.

A second keynote speaker was Dr. Maxine Linial, who has made many important contributions to our understanding of retrovirology with more recent research on molecular aspects of foamy retrovirus replication. Like Dr. Stoye, Maxine is a former organizer of the Retroviruses meeting. Maxine's talk was all the more extraordinary because she delivered it despite a life-altering event that resulted in her losing her sight. The power of her words, accompanied by a simple easel of paper, reminded the audience that you do not need a polished PowerPoint presentation to convey important insights about scientific discoveries. The final keynote speaker was Dr. John Mellors, an AIDS clinician at the interface of patient care and basic research on anti-HIV drugs. John's talk was a thought-provoking opinion piece that put into perspective both his own insights and the broader community of clinicians who deal with the consequence of retrovirology's most notorious family member. John's talk ran over the allotted time as audience members kept him on the stage with questions about drug treatments for HIV and the possibility of a "cure."

As is usual, the meeting was organized into 13 sessions (10 oral, 3 poster) with the emphasis on unpublished, basic research. The sessions consisted of traditional areas that focused on specific aspects of the retrovirus life cycle, but they were also mixed up a little to reflect new themes that

A. Andrew, C. Valmas

E. Campbell, P. Green

are emerging in the study of retroviruses and to showcase, for example, the contribution of retrovirology to studies of evolution and innate immunity. A significant number of abstracts focused on a newly discovered host-cell factor that can limit HIV replication, the SAMHD1 restriction factor. The oral session included a presentation by Nadine Laguette, who was the first author on one of the two original papers describing this factor. Nadine presented her work as the recipient of the sixth annual Andy Kaplan prize in Retrovirology. Other highlights of the submitted research presentations included the identification of a new retroelement "Steamer" in steamer clams; the finding that the host restriction factor, BST2/tetherin, which is targeted by the HIV-1 Vpu protein, also activates NF-kB; the description of a new antiviral restriction factor, Schlaffen 11, which disrupts HIV protein translation by interfering with tRNA synthesis; several presentations that used state-of-the-art techniques of imaging to provide new insights, including a talk from Walter Mothes' lab that used intravital imaging to investigate mouse retrovirus infections in vivo; a presentation by Alan Frankel that described reconstitution in vitro of the HIV nuclear export complex and its visualization by electron microscopy; advances in our structure/function understanding of TRIM5 restriction of HIV-1 by several groups; recognition that CPSF6 and TNPO3 interact in regulating early steps of HIV replication; and the identification of CD169/Siglec-1 as a *trans*-receptor for HIV-1 replication that regulates virus interactions with dendritic cells. As always, the Retroviruses meeting provided both descriptions of new discoveries in the field and education about new techniques of scientific investigation that could be applied to other work.

Y. Lazebnik, G. Melikyan

J. Witkowski, J. Stoye

This meeting was funded in part by the National Institute of Allergy and Infectious Diseases, a branch of the National Institutes of Health, and Gilead Sciences, Inc.

Evolution
Chairpersons: H. Fan, *University of California, Irvine;* R. Gifford, *Aaron Diamond AIDS Research Institute, New York*

Uta Von Schwedler Prize For Retrovirology
Awarded by: V. Planelles, *University of Utah, Salt Lake City*
To: O. Cingöz, *Tufts University, Boston, Massachusetts*

Entry, Reverse Transcription, and Uncoating
Chairpersons: S. Hughes, *National Cancer Institute/NIH, Frederick, Maryland;* S. Sarafianos, *University of Missouri School of Medicine, Columbia*

Cytoplasmic Trafficking, Nuclear Entry, and Integration Site Selection
Chairpersons: L. James, *Medical Research Council, Cambridge, United Kingdom;* M. Yamashita, *Aaron Diamond AIDS Research Center, New York*

Keynote Address: 40 Years in Retrovirology, A Reminiscence
M. Linial, *Fred Hutchinson Cancer Research Center, Seattle, Washington.*

Assembly and Maturation
Chairpersons: O. Pornillos, *University of Virginia, Charlottesville*; V. Vogt, *Cornell University, Ithaca, New York*

Keynote Address: Germ-line Retroviruses—Good or Bad?
J. Stoye, *MRC National Institute for Medical Research, London, United Kingdom*

TRIM5α and Innate Antiviral Factors
Chairpersons: E. Campbell, *Loyola University Chicago, Maywood, Illinois;* J. Young, *Salk Institute for Biological Studies, La Jolla, California*

Tetherin, VPU, and NEF
Chairpersons: E. Barker, *Rush University Medical Center, Chicago, Illinois;* L. Wu, *Ohio State University, Columbus*

RNA Trafficking, Incorporation, and Interacting Factors
Chairpersons: J. Lingappa, *University of Washington, Seattle,* A. Telesnitsky, *University of Michigan Medical School, Ann Arbor*

Keynote Address: Can We Eradicate HIV from the Individual and from the World?
J. Mellors, *University of Pittsburgh, Pennsylvania*

SAMHD1
Chairpersons: R. Koenig, *Paul-Ehrlich-Institut, Langen, Germany;* J. Skowronski, *Case Western Reserve University School of Medicine, Cleveland, Ohio*

Sixth Annual Andy Kaplan Prize
Awarded by: N. Landau, *New York University School of Medicine*
To: N. Laguette, *CNRS, Montpellier, France*

Integration and Latency
Chairpersons: V. Pathak, *National Cancer Institute/ NIH, Frederick, Maryland;* B.M. Peterlin, *University of California, San Francisco*

Second Annual Daniel Wolf Prize
Awarded by: S. Goff, *Columbia University*, *New York*,
To: Jury-selected poster presenter at the 2012 Retroviruses meeting

APOBEC3 Proteins, VIF, and Cell Biology
Chairpersons: P. Bates, *University of Pennsylvania, Philadelphia;* K. Bishop, *National Institute for Medical Research, London, United Kingdom*

It's been a long day!

I. Rouzina, A. Hulme

Glia in Health and Disease

July 19–23 188 Participants

ARRANGED BY **Dwight Bergles,** Johns Hopkins University
William Talbot, Stanford University

D. Bergles, W. Talbot

Glial cells constitute the majority of cells in the human nervous system, but their roles in the healthy and diseased brain are still poorly understood. In this summer's meeting, students and scientists from across the world gathered to discuss recent progress in the field. The talks focused on exciting, unpublished results. There was lively discussion in the oral sessions, poster sessions, and informal settings. Sessions focused on the development of glia, genetic analysis of glial function, myelination, axon–glial interactions, microglia function, astrocyte function at synapses and blood vessels, and roles of glia in CNS disease. Vertebrate and invertebrate systems were well represented, and the meeting featured excellent presentations using diverse approaches, ranging from genomics to in vivo imaging in mammals, to central questions in glial biology. One of the highlights of the meeting was the keynote address from Prof. Klaus Nave, who presented exciting new information on signaling and metabolic transactions between axons and myelinating oligodendrocytes. As in the previous meetings in 2006, 2008, and 2010, there was again a sense of excitement that our understanding of the function of glia is quickly moving forward, with glia having central roles in normal brain development, function, and disease.

This meeting was funded in part by the National Institute of Neurological Disorders and Stroke, a branch of the National Institutes of Health.

PROGRAM

Glial Development
Chairpersons: R. Lu, *University of Texas Southwestern Medical Center, Dallas;* A. Brand, *University of Cambridge, United Kingdom*

Myelinating Cells
Chairpersons: D. Lyons, *University of Edinburgh, United Kingdom;* L. Feltri, *State University of New York, Buffalo*

Genetic Analysis of Glial Function
Chairpersons: M Götz, LMU Munich, Germany; M. Freeman, *HHMI/University of Massachusetts Medical School, Worcester*

Glial Function at Synapses
Chairpersons: B. Barres, *Stanford University School of Medicine, California;* A. Volterra, *Université de Lausanne, Switzerland*

K. Nave, R. Armstrong

Gliovascular Interactions
Chairpersons: S. Charpak, *INSERM, Paris, France;* B. MacVicar, *University of British Columbia, Vancouver, Canada*

Keynote Speaker
K.-A. Nave, *Max-Planck Institute of Experimental Medicine*

Axon–Glial Interactions
Chairpersons: M. Rasband, *Baylor College of Medicine, Houston, Texas;* M. Bhat, *University of North Carolina, Chapel Hill*

Microglia Function in Health and Disease
Chairpersons: B. Stevens, *Children's Hospital, Harvard Medical School, Boston, Massachusetts;* R. Ransohoff, *Cleveland Clinic, Ohio*

Role of Glia in CNS Injury and Disease
Chairperson: R. Armstrong, *Uniformed Services University of the Health Sciences, Bethesda, Maryland*

D. Parkinson, H. Colognato

A. Brand, E. Peco

M. Katz, Q. Aftab

Mechanisms and Models of Cancer

August 14–18 464 Participants

ARRANGED BY **Dafna Bar-Sagi,** New York University Medical Center
Karen Vousden, Beatson Institute for Cancer Research
William Weiss, University of California, San Francisco
Eileen White, Rutgers University/The Cancer Institute of New Jersey

Molecular alterations affecting tumor suppressor genes and oncogenes continue as a central focus driving human cancers. The last few years have witnessed an explosion in molecular cataloging of mutation, expression analyses, and epigenetic regulation that illuminates distinct human cancer types. This allows us to understand both commonalities and differences among and within cancer types, as well as presenting opportunities to test targeted agents against pathways not previously realized to be active in a specific subset of cancers. In addition, the cancer community continues to focus significant effort on understanding interactions between tumor cells and other cell types within the tissue microenvironment, and on abnormalities that alter tumor cell metabolism, both of which influence malignant progression, invasion, and metastasis. Since our last meeting, there has been increased appreciation of the role for epigenetic abnormalities in cancer and further comparisons between neoplastic transformation and cellular reprogramming. This fourth meeting convened an international group of investigators whose collective work focused on these themes. Oral and poster presentations provided numerous examples of how new insights have been gained from application of interdisciplinary approaches that utilize genetics, developmental biology, genomics and proteomics, and model organisms (principally sophisticated mouse models) to advance the development of rational therapeutics. A keynote address by Jackie Lees identified fascinating new roles for the RB pathway in cancer, whereas a keynote address by Chuck Sherr provided an educational and entertaining overview of a distinguished career in science. Eight oral sessions and two poster sessions focused on the topics listed below in the Program.

This conference was funded in part by the National Cancer Institute, a branch of the National Institutes of Health. The meeting will be held again in the summer of 2014.

SESSION 1: Tumor Suppressor Networks
Chairpersons: C. Prives, *Columbia University, New York;* J. Lees, *MIT Center for Cancer Research, Cambridge, Massachusetts*

Keynote Speaker
J. Lees, *MIT Center for Cancer Research, Cambridge, Massachusetts*

W.-X. Zong, E. White

N. Chandiramani, M. Blank, R. Navab

SESSION 2: Signaling Mechanisms
Chairpersons: K. Cichowski, *Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts; R. Marais, Paterson Institute of Cancer Research, Manchester, United Kingdom*

SESSION 3: Poster Session I

SESSION 4: Genomics/Epigenomics
Chairpersons: L. Chin, *University of Texas M.D. Anderson Cancer Center, Houston;* A. Melnick, *Weill Cornell Medical College, New York*

SESSION 5: Cancer Development and Metabolism
Chairpersons: S. Lowe, *Memorial Sloan-Kettering Cancer Center, New York;* J. Brugge, *Harvard Medical School, Boston, Massachusetts*

SESSION 6: Poster Session II

SESSION 7: Therapeutics I
Chairpersons: J. Maris, *Children's Hospital of Philadelphia, Pennsylvania;* F. DeSauvage, *Genentech, South San Francisco, California*

SESSION 8: Tumorigenesis
Chairpersons: M. Vander Heiden, *Massachusetts Institute of Technology, Cambridge;* T. Jacks, *HHMI/Massachusetts Institute of Technology, Cambridge*

SESSION 9: Therapeutics II
Chairpersons: B. Neel, *Ontario Cancer Institute, Toronto, Canada;* C. Sherr, *HHMI/St. Jude Children's Research Hospital, Memphis, Tennessee*

Keynote Address: Notes from the Fossil Record
C. Sherr, *HHMI/St. Jude Children's Research Hospital, Memphis, Tennessee*

SESSION 10: Tumor Microenvironment/Metastasis
Chairpersons: G. Bergers, *University of California, San Francisco;* M. Karin, *University of California, San Diego*

S. Lowe, E. Manchado

S. Muthuswamy, U. Guha

H. Varmus, D. Tuveson

L. Siskind, L. Obeid

Bacteria, Archaea, and Phages

August 21–25 196 Participants

ARRANGED BY **Petra Levin,** Washington University
Patricia Kiley, University of Wisconsin, Madison
Mark Goulian, University of Pennsylvania

P. Kiley, P. Levin, M. Goulian

This meeting featured 79 oral presentations and 85 poster presentations. It reinforced the power of basic research in bacteria and phages to provide critical insights into areas of biology ranging from ecology and evolution, to signal transduction and genetic regulatory mechanisms, to pathogenesis. The 2012 meeting featured presentations covering all of these topics and continued the historical strength of phage meetings in combining long-term themes of molecular microbiology with cutting-edge approaches and exciting new research areas. The keynote lecture by Jonathan Beckwith, a pioneer in molecular biology, focused on the contribution of a technology he pioneered, *lac* fusions, to our understanding of diverse areas of bacterial physiology including transcription, translation, secretion, and cell division. Another highlight was the Sternberg Lecture by Elie Diner, which focused on his Ph.D. work on specialized contact-dependent inhibition systems.

The opening session focused on transcriptional and translational regulation. Session chair Ann Hochschild focused on new work from her laboratory employing *E. coli* as a tool to study protein–protein interactions involved in the formation of protein aggregates similar to those of prion proteins. Recent National Academy inductee Gisela Storz chaired the session on stress response and global regulation; she spoke about her work on the role of very small proteins in different

S. Maloy, J. Watson, W. Szybalski, B. Stillman at the Milestones in Microbiology Awards

aspects of bacterial physiology. William Dowhan presented recent work investigating the role of lipids as determinants of membrane protein topology. The session on bacterial pathogenesis and host–microbe interactions was chaired by Gautam Dantas, who discussed the role of environmental microbes as reservoirs for antibiotic resistance. Lyle Simmons presented recent findings from his laboratory on DNA replication fidelity and mismatch repair. National Academy member Rich Lenksi gave a somewhat retrospective talk on his pioneering work in experimental evolution. Another celebration was for the long, continuous participation of Wisconsin's Waclaw Szybalski, whose generous support of the CSHL library was warmly recognized. Valéry de Crécy-Lagard, who chaired the session on genomics and genome organization, discussed comparative genomics as an effective tool for identifying gene function. The final session of the meeting, Physiology, was chaired by John Kirby, who spoke about his laboratory's work on a developmentally regulated signal transduction system in *Myxococcus xanthus.* Session talks and posters were presented by a mix of PIs, postdoctoral fellows, and graduate students whose high-quality presentations are a unique aspect of this meeting.

This meeting was funded in part by the National Institute of Allergy and Infectious Diseases, a branch of the National Institutes of Health; MedImmune; *Nature Reviews Microbiology*; and Promega.

PROGRAM

The Nat L. Sternberg Thesis Prize Award Announcement

Transcription and Transcriptional Regulation
Chairperson: A. Hochschild, *Harvard Medical School, Boston, Massachusetts*

Stress Response and Global Regulation
Chairperson: G. Storz, *National Institute of Child Health and Human Development/NIH Bethesda, Maryland*

Cell Surfaces and Cell Cycle Control
Chairperson: W. Dowhan, *University of Texas-Houston Medical School*

Bacterial Pathogenesis and Host–Microbe Interactions
Chairperson: G. Dantas, *Washington University School of Medicine, St. Louis, Missouri*

DNA Structure, Repair, and Dynamics
Chairperson: L. Simmons, *University of Michigan, Ann Arbor*

Evolution, Ecology and Environmental Biology
Chairperson: R. Lenski, *Michigan State University, Lansing*

Genomics and Genome Organization
Chairperson: V. de Crécy-Lagard, *University of Florida, Gainesville*

Keynote Address: From Lac Fusions 1964 to Lac Fusions 2012: Is This Progress?
J. Beckwith, *Harvard Medical School, Boston, Massachusetts*

Physiology
Chairperson: J. Kirby, *University of Iowa, Iowa City*

R. Washburn, J. Beckwith

A. Krainer, K. Tilly

J. Roberts, A. Hochschild

Regulatory and Noncoding RNAs

August 28–September 1 277 Participants

ARRANGED BY **Gregory Hannon,** Cold Spring Harbor Laboratory
Elisa Izaurralde, Max-Planck Institute for Developmental Biology, Germany
Michael Terns, University of Georgia

RNAs have a central role in gene expression. In addition to their function as messengers, RNAs perform many diverse and multifaceted roles across a spectrum of biological processes required for cell viability and function. Moreover, prokaryotic and eukaryotic genomes are pervasively transcribed to generate many additional RNAs of unknown function. In this summer's meeting, students and scientists from across the world engaged in interdisciplinary discussion of the roles of noncoding RNAs with the aim of enhancing our understanding of gene regulation and function. There was lively discussion in the oral sessions, the poster sessions, and in informal settings. Sessions focused on recent and unpublished discoveries in the fields of long and short noncoding RNAs. The meeting featured excellent presentations on the role of noncoding RNAs in diverse organisms ranging from prokaryotes to humans and using diverse experimental approaches including genomics, genetics, and cellular, molecular, and structural biology. There was a sense of excitement that our understanding of the function of noncoding RNAs is quickly moving forward and that this meeting provided an excellent framework for bolstering discussions in this emerging and exciting field of noncoding RNAs.

PROGRAM

Keynote Speakers

J. Doudna, *HHMI/University of California, Berkeley:* Programmable RNA-Guided Genome Defense in Bacteria.

H. Chang, *Stanford University, California:* Genome Regulation by Long Noncoding RNAs.

N. Djodji, S. Marquez

J. Witkowski, M. Gierszewska

J. Bergmann

Noncoding RNAs in Prokaryotes and RNA Modification
Chairpersons: J. van der Oost, *Wageningen University, The Netherlands;* A. Ferre D'Amare, *National Heart, Lung, and Blood Institute/NIH, Bethesda, Maryland;* J. Vogel, *University of Würzburg, Germany*

Transcriptional Gene Silencing and Genome Integrity
Chairperson: S. Jacobsen, *University of California, Los Angeles*

Mechanisms of miRNA-Mediated Gene Silencing
Chairpersons: D. Bartel, *HHMI. Whitehead Institute, Massachusetts Institute of Technology, Cambridge;* A. Pasquinelli, *University of California, San Diego*

Biological Functions of miRNAs
Chairpersons: V. Ambros, *University of Massachusetts Medical School, Worcester;* J. Mendell, *Johns Hopkins University School of Medicine, Baltimore, Maryland*

siRNAs and piRNAs
Chairpersons: M. Siomi, *Keio University School of Medicine, Tokyo, Japan;* J. Brennecke, *IMBA-Institute of Molecular Biotechnology, Vienna, Austria*

Long Noncoding RNAs
Chairperson: T. Gingeras, *Cold Spring Harbor Laboratory*

Transcriptional Gene Silencing
Chairpersons: D. Moazed, *Harvard Medical School, Boston, Massachusetts;* S. Grewal, National Cancer Institute/NIH, Bethesda, Maryland

A. Martinez-Sanchez

M. Hafner

C. Ronda

G. Hayes, R. Batey

Translational Control

September 4–8 389 Participants

ARRANGED BY **Thomas Dever,** National Institutes of Health
Ian Mohr, New York University
Tatyana Pestova, SUNY Downstate Medical Center

T. Dever, T. Pestova, I. Mohr

This meeting attracted participants from around the world, and included Richard Jackson, Samie Jaffrey, and David Sabitini as invited speakers, eight platform sessions, and three poster sessions that covered 313 abstracts. Of special note, the meeting marked the 25th anniversary of the first Cold Spring Harbor Meeting on Translational Control held in September 1987. This anniversary was marked by Richard Jackson's keynote talk highlighting the progress over the last 25 years as well as interesting questions for the future. Novel findings on the mechanism of translation included high-resolution cryo-EM structures of eukaryotic 80S ribosomes that revealed substantial rRNA expansions as well as an unexpected rolling of the 40S ribosomal subunit during translation elongation. Complementary biochemical and structural studies revealed how the ATP-binding protein EttA binds in the ribosomal E site to control translation in response to cellular energy status. In addition, studies examining the regulation of ribosomal activity by antibiotics and regulatory proteins were also presented. Studies on translation elongation revealed the important role of the factor EF-P in synthesizing polyproline, whereas genome-wide ribosomal profiling studies highlighted the use of alternative translation initiation sites on mRNAs, globally assessed pause sites during translation elongation, and highlighted the importance of Dom34 in ribosome recycling. Several breakthroughs were reported regarding the mechanism of translation initiation, including novel activities of initiation factors. The guanine nucleotide exchange factor eIF2B was reported to catalyze the dissociation of the eIF2-GDP • eIF5 complex, and yeast eIF4B was reported to bind directly to 40S ribosomal subunits to promote the recruitment of mRNAs. Evidence was presented that the m^7G cap-binding protein eIF4E, besides tethering eIF4G to the cap, strongly stimulates the RNA helicase activity of eIF4A in the presence of eIF4G and eIF4B, explaining how elevated levels of eIF4E promote the translation of oncogenic mRNAs with highly structured 5′-UTRs. In addition, a novel mechanism was described for generating peptides for presentation by the HMC that involves cryptic initiation with Leu-tRNA at CUG, not AUG, codons.

The prominent role of the protein kinase mTOR in regulating protein synthesis was documented in several presentations. This protein is a nexus, connecting inputs such as nutrient supply and hormone signaling to outputs that control the translation of particular mRNAs and overall protein synthesis rates in the cell. Discoveries showed how the synthesis of proteins central to plant growth is controlled and revealed the participation of mTOR in the synthesis (on cytosolic ribosomes) of mitochondrial proteins. Other themes included the functions of stress granules and initiation factors, and of modifications of the ribosome itself, in translational control. Studies of the protein kinase PKR, and its inhibitors made by viruses, illuminated the evolutionary contest waged between cells and infectious agents. Finally, fresh vistas were opened up by an investigation revealing the potential for additional layers of complexity in regulating viral gene expression. Several talks highlighted the important role of translational control in disease. Interestingly,

loss of the phosphatase-targeting subunit GADD34 was found to ameliorate some of the defects observed in a mouse model of vanishing white matter disease caused by mutations in eIF2B. Similarly, deletion of the translational regulator CEBP rescued phenotypic defects in a mouse model of fragile-X syndrome. Translational control in learning and memory, prion disease, and schizophrenia were also discussed. Moreover, overexpression of eIF4G or eIF4B was shown to promote cancer cell growth, while deleting one of the eIF4E genes in mice specifically impaired oncogenic transformation. Finally, the metabolic enzyme GAPDH was reported to protect ribosomal protein L13a in its extraribosomal role to regulate translation of inflammation-related proteins.

New insights into the mechanism of miRNA-mediated translational repression were also reported. Poly(A)-binding protein (PABP) was found to facilitate the recruitment of RISC to miRNA-binding sites in target transcripts. Upon binding, GW182 facilitates the release of PABP to repress translation prior to deadenylation. In studies using IRES-containing mRNA reporters, the eIF4AII helicase was found to be required for miRNA-induced translational repression. In support of this finding, mRNAs with conserved miRNA target sites were found to have longer 5′-untranslated regions and higher GC content than mRNAs without miRNA target sites. Additional talks provided insights into both global strategies used by cells to regulate translation and more specific novel mechanisms at work to control protein synthesis. An example of the former was a presentation on efforts to catalog the entire mRNA regulatory element "interactome" with *trans*-acting proteins in yeast; data gleaned from these studies are sure to seed new hypotheses and avenues of experimentation in the coming years. In another more targeted study, a new role for Dicer-2 in polyadenylation of cytoplasmic mRNAs was reported. Finally, a series of talks illustrated the ability of internal ribosome entry sites (IRESs) to use protein binding, specific cell types, and RNA structural changes to control translation initiation via mechanisms that still remain largely mysterious.

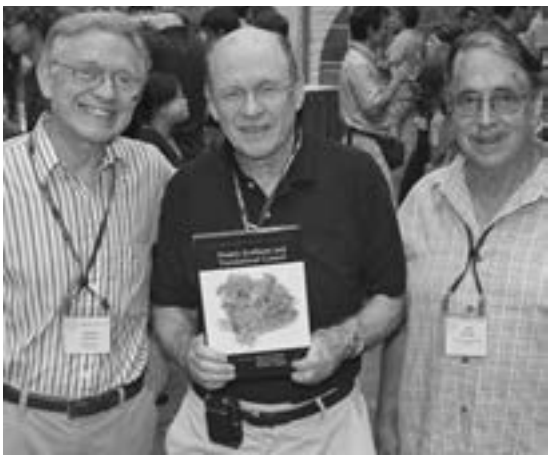
PROGRAM

Ribosome
Chairperson: R. Beckmann, *University of Munich, Germany*

Cell Signaling and Viruses
Chairperson: M. Mathews, *University of Medicine and Dentistry of New Jersey, Newark*

Initiation
Chairperson: J. Hershey, *University of California, Davis*

Cancer and Disease
Chairperson: N. Sonenberg, *McGill University, Montréal, Canada*



M. Mathews, N. Sonenberg, N. Ashourian

D. Ruggero, C. Calkhoven

mRNA Turnover and miRNAs
Chairperson: P. Anderson, *Brigham and Women's Hospital, Harvard University, Boston, Massachusetts*

Development and CNS
Chairperson: N. Gray, *University of Edinburgh, United Kingdom*

Regulatory Elements
Chairperson: J. Kieft, *University of Colorado Denver School of Medicine, Aurora*

Elongation and Termination
Chairperson: M. Sachs, *Texas A&M University, College Station*

I. Aznarez Da Silva, G. Singh

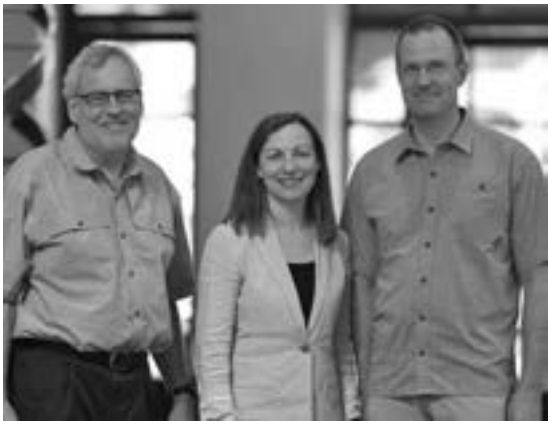
Posing for the caricaturist

Epigenetics and Chromatin

September 11–15 389 Participants

ARRANGED BY **Shelley Berger,** University of Pennsylvania
Robert Kingston, Massachusetts General Hospital and Harvard Medical School
Juerg Mueller, Max-Planck Institute of Biochemistry, Germany

This inaugural meeting was a great launch, with lively discussion of the roles for chromatin structure in regulation of regulatory processes. The focus was on the role for chromatin in the epigenetic mechanisms that create regulatory phenotypes that are more diverse than can be generated solely by genotype.



B. Kingston, S. Berger, J. Mueller

The high attendance, large numbers of posters (232), and sustained involvement of the participants throughout the meeting were testaments to the success of the meeting and the successful balance of the sessions. Scientists from 25 countries attended, and for over half of these scientists, it was their first visit to Cold Spring Harbor. This is a complex field with continually emerging mechanistic themes, which fostered sustained interest by the large and diverse audience as the topics shifted throughout the eight sessions.

These sessions covered covalent modification of DNA and of histones (two sessions) and the histone variants that can be used during regulation of chromatin function (one session). Mechanisms that allow repression of chromatin and the long-range effects of chromatin structure on regulatory processes were each discussed in a session, with the latter topic providing reports on the emerging technologies used to map three-dimensional interactions along and between chromosomes. The remaining three sessions covered enzymatic functions on chromatin, including those involved in remodeling nucleosomes, the mechanisms involved in the regulation of development, and the chromatin-based processes involved in DNA repair and recombination.

Among the highlights of the conference were studies directed toward development of therapeutics based on targeting proteins that bind chromatin modifications, the emerging roles for long noncoding RNAs in regulation, the dynamic nature of nucleosomes including the exchange of histone variants, and new studies concerning derivatives of methylated DNA.

S. Elgin, S. Heinkoff

C. Wang, J. Platt, A. Harwood

PROGRAM

Assembly, Repair, and Metabolism
Chairperson: G. Almouzni, *Institute Curie, Paris, France*

Enzymatic Function on Chromatin
Chairpersons: J. Workman, *Stowers Institute for Medical Research, Kansas City, Missouri;* T. Jenuwein, *Max-Planck Institute of Immunobiology, Freiburg, Germany*

DNA Methylation and Derivatives
Chairperson: C. Peterson, *University of Massachusetts Medical Center, Worcester*

Keynote Speaker
A. Bird, *University of Edinburgh, United Kingdom*

Repression
Chairpersons: D. Duboule, *University of Geneva and EPFL, Switzerland;* A. Shilatifard, *Stowers Institute for Medical Research, Kansas City, Missouri*

Histone Variants and Regulation
Chairperson: K. Luger, *Howard Hughes Medical Institute, Colorado State University, Fort Collins*

Keynote Speaker
C. Wu, *National Cancer Institute/NIH, Bethesda, Maryland*

D. Duboule, N. Loufat

Development and Inheritance of State
Chairpersons: B. Meyer, *HHMI/University of California, Berkeley;* R. Martienssen, *Cold Spring Harbor Laboratory*

Long-Range Effects
Chairperson: C. Dean, *John Innes Centre, Norwich, United Kingdom*

Covalent Modification and Regulation
Chairpersons: E. Heard, *Institut Curie, Paris, France;* S. Grewal, *National Cancer Institute/NIH, Bethesda, Maryland*

K. Sanbonmatsu

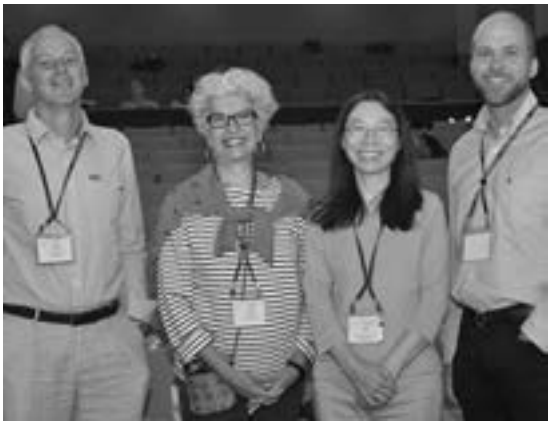
J. Van der Knaap, P. Verrigner

T. Jenuwein, G. Bloble

Axon Guidance, Synapse Formation, and Regeneration

September 18–22 296 Participants

ARRANGED BY **Alain Chedotal,** Vision Institute, INSERM, Paris
Graeme Davis, University of California, San Francisco
Yishi Jin, University of California, San Diego
Carol Mason, Columbia University



A. Chedotal, C. Mason, Y. Jin, G. Davis

The human brain has billions of nerve cells (neurons) and each neuron is typically connected to hundreds of other neurons via synapses in a highly precise fashion. This complex neural wiring underlies the ability of humans, and other animals, to interact with the outside world, to learn, and to perform complex behaviors. Defects in the development of neural connections are being increasingly linked to the cause of neurological disease. One of the major challenges for the field of neuroscience is to understand how nerve connections are made accurately and reliably. In the past decade, our understanding of the mechanisms that control axon growth and guidance, synaptogenesis, and the remodeling of neural circuits during development has progressed rapidly from phenomenology to the identification of specific molecular control mechanisms.

This eighth meeting in the series focused on key issues in axon guidance, circuit formation, synaptogenesis, and axon regeneration and included sessions devoted to particular problems in the assembly, plasticity, and repair of the nervous system, with speakers chosen from among the participants submitting abstracts by session chairs who are leaders in the field. The response of the field to the 2012 conference was one of overwhelming enthusiasm—with more than 296 participants, 240 of whom submitted abstracts, despite several Gordon Conferences held late in the summer on similar topics and the concurrent Japanese Neuroscience meeting. A total of 49 abstracts were selected for talks, in seven sessions, the remaining abstracts being presented as posters. Senior researchers, starting assistant professors, postdoctoral fellows, and graduate students were well-represented as speakers and participants. Session chairs at the meeting were well-balanced between men and women, and the meeting had a clear international presence with participants from Europe and Asia. Many of the outstanding talks this year were by graduate students. All of the major areas of research in the field were covered, as were all of the major approaches (cellular, physiological, anatomical, molecular, dynamic imaging, biochemical, and genetic). In addition, there were three keynote addresses. The first, by a speaker outside of the field of neural development, was given by Professor Charles Zuker and reviewed his work on taste sensory perception. The second and third keynote lectures were a pairing of speakers whose early work had major roles in moving the field of axon guidance and synapse formation forward. Jeff Lichtman focused on his recent work that combines ultrastructural reconstruction of brain anatomy with computation and functional dissection to decipher the logic of the mammalian brain. Barry Dickson spoke on his recent work that has led to the elucidation of the complete neural map underlying *Drosophila* sex behavior.

Also for the first time, this meeting held an informal event on professional development. Topics included how to search for mentors (postdoc or faculty), how to prepare for job interviews (academic or industry), how to write grants, managing teaching and research, how to balance life and work, and how to handle paper reviews. Two special discussion sessions were dedicated to

publishing by the attending journal editors and to careers as a physician-scientist by investigators with an M.D. or M.D.-Ph.D. degree and clinical experience. About half of the senior investigators participated in the event. The students and postdocs expressed high appreciation for the close interaction and valuable advice they gained through this event.

Overall, this meeting provided an important forum for ideas and approaches and helped scientists in the field get the most up-to-date information, as well as enabling them to meet, to network, and to establish collaborations. As at the previous meeting, a need was identified for an additional poster session because the number and quality of abstracts submitted for posters was so high. The three poster sessions were extremely well-attended. In addition, fewer talks were scheduled in the evening, and a talk session after the evening keynote was eliminated to allow time for discussion and attendee interactions. Based on the enthusiastic comments of the participants, the intensity of the oral and poster sessions, and the large crowds that stayed up late every night at the bar to discuss science, the meeting was a great success.

This meeting was funded in part by National Institute of Neurological Disorders and Stroke, a branch of the National Institutes of Health, and Merck.

PROGRAM

Axon to Synapse I
Chairpersons: G. Bashaw, *University of Pennsylvania, Philadelphia;* L. Erskine, *University of Aberdeen, United Kingdom*

Regeneration and Disease I
Chairpersons: J. Goldberg, *University of Miami, Florida;* A. Bonni, *Harvard Medical School, Boston, Massachusetts*

Special Lecture: Common Sense about Taste: From the Tongue to the Brain
C. Zuker, *Columbia University, New York*

Synapse to Circuit I
Chairpersons: H. Baier, *Max-Planck Institute of Neurobiology, Martinsried, Germany;* P. Arlotta, *Massachusetts General Hospital, Boston*

Axon to Synapse II
Chairpersons: R. Murphy, *Florida Atlantic University, Boca Raton,* V. Castellani, *CNRS, University of Lyon 1, France*

Synapse to Circuit II
Chairpersons: K. Shen, *Stanford University, California;* M. Goulding, *The Salk Institute, La Jolla, California*

Regeneration and Disease II
Chairpersons: E. Engle, *Children's Hospital, HHMI/Harvard Medical School, Boston, Massachusetts;* G. Rouleau, *University of Montréal, Canada*

Special Lectures
J. Lichtman, *Harvard University, Cambridge, Massachusetts:* Seeking Circuit Motifs with Connectomics.
B. Dickson, *Research Institute of Molecular Pathology, Vienna, Austria:* Wired for Sex; The Neurobiology of *Drosophila* Mating Decisions.

Axon to Synapse III
Chairpersons: D. Schmucker, *Vesalius Research Center, University Leuven, Belgium;* C. Hoogenraad, *Utrecht University, the Netherlands*


L. Goodrich, C. Mason


A. Erturk, A. Yaron


A. Davy, A. Kania

Dynamic Organization of Nuclear Function

September 27–October 1 222 Participants

ARRANGED BY **Edith Heard,** Curie Institute, France
Martin Hetzer, The Salk Institute for Biological Studies
David Spector, Cold Spring Harbor Laboratory

D. Spector, E. Heard, M. Hetzer

This meeting focused on the relationships between nuclear structure and function. The opening session highlighted current studies on Nuclear Function in Development and Disease, with the session chair (Jane Skok) giving a very nice introduction with an overview of nuclear and chromosome dynamics and raising several of the critical questions that would be touched on during the session. She also presented her own work on the dynamic choreography of higher-order looping and nuclear dynamics of antigen receptor loci during their recombination in mouse lymphocytes. Carolyn Larabell presented a talk on the powerful technique of soft X-ray tomography for the exploration of changes in euchromatin and heterochromatin organization, at high resolution, during development in olfactory neurons. The Friday morning session was dedicated to chromosome conformation capture approaches, which have come into the limelight in recent years and have provided major insights into the short- and long-range organization of the genome, at the molecular level. The session was one of the highlights of the meeting, with a significant amount of new data presented, including a talk by Peter Fraser on single-cell analysis of X-chromosome topology using novel 3C technology, as well as several presentations describing new levels of chromosome organization, such as topological domains, and discussions about the technical issues related to such techniques. After a very active poster session and discussions at the wine-and-cheese get-together, John Lis chaired the Transcriptional Regulation session. His introduction beautifully illustrated the refined picture that is emerging of how RNA polymerase navigates through different genes during transcription. The session covered several aspects of transcription, in particular features of genes that can greatly influence elongation rates, such as exon number (J. Lis), as well as epigenetic marks and nuclear proteins that can lead to transcriptional priming, and perpetuate the memory of such priming, for example, Nup98 (J. Brickner). A recurring theme in this meeting was single-molecule resolution where nascent mRNA quantification was undertaken.

The Nucleocytoplasmic Transport session on Saturday morning was chaired by Karsten Weis, who gave a broad introduction to the nuclear pore complex and transport mechanisms and presented new data on the role of Dbp5 in mRNA export including single-molecule imaging in yeast. The session included an update on the role of nuclear pores in *Tetrahymena* differentiation, the misregulation of mRNA export in human disease, links between signaling and mRNA export, and the description of a Ran-independent import pathway for Hsp70. The final talk addressed the ubiquitylation of nuclear pore proteins, a poorly understood but exciting new area.

The afternoon session on Epigenetics and Nuclear Organization was ably chaired by Wendy Bickmore. This was another animated session touching on chromatin and chromosome structure, as well as nuclear organization. Several talks focused on the long-range interactions in the nuclear genome. W. Bickmore described her work on Hox cluster genes using imaging and 3C techniques and raised some important technical considerations for HiC methods and data analysis. The

M. Ekersley, K. Sengupta

P.E. Gleizes, M. Ruault

session also provided mechanistic insights into the role of JMJD3 in transcriptional induction (M. Huebner) as well as the distribution of polycomb proteins on mitotic chromosomes (N.J. Francis). In addition, topics as diverse as asymmetric sister chromatid segregation and condensin-mediated chromosome compaction were presented.

On Sunday morning, M. Carmo-Fonseca chaired an exciting session on the Biology of Nuclear RNAs. She presented impressive new data using a system in which two RNA tags located in different introns within a transgene could be used to visualize RNA splicing in living cells. A host of new long and short noncoding RNAs were also discussed in this session. The nuclear RNA universe is clearly still expanding, and in addition to their more classical roles, RNAs are emerging as central actors for gene regulation, chromatin structure, and nuclear architecture.

The Sunday afternoon session on the nuclear pore complex and nuclear lamina was chaired by Yixian Zheng, who gave an exciting talk about the role of Nup153 in EGF signaling during germ cell differentiation. Three talks discussed aspects of the intranuclear life of nucleoporins, nuclear pore assembly, and the longevity of NPC proteins. Other exciting new findings provided insights into the targeting and structure of inner nuclear membrane proteins, nuclear membrane formation, and NPC assembly. The role of lamins in Hutchinson-Gilford progeria and chromatin organization was also discussed in great detail.

The meeting ended with a session on Cell Cycle and DNA Repair chaired by Julian Blow, who presented exciting data on replication complex assembly and the regulation of DNA replication. David Gilbert's lab presented data to demonstrate a relationship between the establishment and developmental regulation of replication timing to the topological boundaries of structural chromosome domains and how these properties can be uncoupled. In addition, several talks addressed the problem of DNA repair including its relation to cell cycle phase (R. Ileng Kumaran), the dynamics of chromosomal translocations (V. Roukos), and the effect of nuclear architecture on the efficiency of repair (E. Fabre).

The enthusiasm of the meeting participants was overwhelming, and it is remarkable how with each successive meeting the interest has grown and strengthened. The slightly smaller number of participants compared to previous meetings was partly due to the fact that the last meeting was the Symposium and that a new meeting (Epigenetics) with overlapping topics had actually taken place just a few weeks earlier. All of the feedback on the scientific interest and quality of the meeting was extremely positive and it is anticipated that the CSHL 2014 meeting will be an even greater success!

PROGRAM

Nuclear Function in Development and Disease
Chairperson: J. Skok, *New York University School of Medicine, New York*

Chromosome Conformation
Chairperson: P. Fraser, *Babraham Institute, Cambridge, United Kingdom*

Transcriptional Regulation
Chairperson: J. Lis, *Cornell University, Ithaca, New York*

Nucleocytoplasmic Transport
Chairperson: K. Weis**,** *University of California, Berkeley*

Epigenetics and Nuclear Organization
Chairperson: W. Bickmore, *MRC Human Genetics Unit, Edinburgh, United Kingdom*

Biology of Nuclear RNAs
Chairperson: M. Carmo-Fonseca, *University of Lisbon, Portugal*

The Nuclear Pore Complex and Nuclear Lamina
Chairperson: Y. Zheng, *Carnegie Institution, Baltimore, Maryland*

Cell Cycle and DNA Repair
Chairperson: J. Blow, *University of Dundee, United Kingdom*

S. Parker

Break time

M. Mechali, M. Prioleau

Germ Cells

October 2–6 205 Participants

ARRANGED BY **Laurinda Jaffe,** University of Connecticut Health Center
Erika Matunis, Johns Hopkins University School of Medicine
Tim Schedl, Washington University School of Medicine

L. Jaffe, T. Schedl, E. Matunis

This meeting began with five speakers in Session 1 focusing on issues of epigenetics, including Azim Surani as keynote speaker. As with all of the sessions, lively discussion followed each of the talks and continued after the conclusion of the sessions. Likewise the diversity in the model systems used for study, including mammals, fish, plants, and a wide range of invertebrates, highlighted deeply conserved emerging mechanisms.

Session 2 focused on germ cell specification and migration, a theme revisited with different emphasis throughout the meeting, particularly in Session 4, which focused on germline stem cells and their niches, and Session 5 concerned with environmental, hormonal, and somatic influences on germ cells. Evolving concepts about the nature of the germ cell niche and the molecules driving stem cell dynamics provoked considerable discussion. Talks included presentations of how Royalactin induces germ cell development during queen differentiation in honeybees, and how the bacterium *Wolbachia* influences mosquito oogenesis and the implications of this for disease control.

Poster Sessions (Sessions 3 and 6) presented studies on virtually all aspects of germ cell development in diverse model species. This meeting, compared to previous meetings, included a number of posters on how metabolic signaling (e.g., TOR) and environmental inputs control germ cell development and function, particularly in stem cells and during gametogenesis. Sessions 7 through 9 reported advances in our understanding of the regulation of meiosis, posttranscriptional control, and the cell biology of germ cells. Highlights included remarkable imaging of chromosome pairing during meiosis, and the discovery of a protein that mediates nuclear fusion during early development. The meeting concluded with Session 10 on sex determination, including new insights on sex chromosome evolution. There has been remarkable progress since the last Germ Cells meeting (in 2010). Recent findings in two areas stimulated much discussion. The first concerns the two major processes that occur during the formation of gametes, the meiotic events that lead to recombination, and appropriate chromosome segregation and the differentiation events that form oocytes or sperm. A surprising report from the Page and Eppig laboratories indicates that the entire process of gametogenesis to form fertilizable oocytes can occur completely independently of the meiosis. The second concerns the recent controversial proposal that the adult female ovary in mammals contains a population of stem cells, contrary to the long-held belief that the full endowment of oocytes in the mammalian ovary is present prior to birth. In a very elegant lineage study in the mouse system from the Spradling laboratory, no evidence was found that adult stem cells produce oocytes; instead, oocytes all arise from germ cells set aside before birth. An international cast, including speakers from Argentina, Australia, Canada, China, Czech Republic, France, Germany, India, Israel, Japan, Mexico, Netherlands, Norway, Poland, Portugal, Singapore, Spain, and the United Kingdom, participated in this meeting. There was general excitement about continued advances that will affect broad basic and clinical fields of development, reproduction, and

clinical sciences. Once again, the ease of interactions between established and new participants and the strong representation of unpublished, cutting-edge work were highly valued aspects of this meeting. The next Germ Cells meeting will be held at Cold Spring Harbor October 7–11 of 2014 and will be organized by Mary Ann Handel, Jane Hubbard, and Steve DiNardo.

This meeting was funded in part by the National Institute of Child Health and Human Development, a branch of the National Institutes of Health, and the Lalor Foundation.

PROGRAM

Epigenetics
Chairperson: S. DiNardo, *University of Pennsylvania School of Medicine, Philadelphia*

Keynote Address: Principles and Programming of the Mammalian Germ Line
A. Surani, *University of Cambridge, Cambridge, United Kingdom*

Germ Cell Specification and Migration
Chairperson: S. Strome, *University of California, Santa Cruz*

Germline Stem Cells and Their Niches
Chairperson: D. Zarkower, *University of Minnesota, Minneapolis*

Environmental, Hormonal and Somatic Influences on Germ Cells
Chairperson: M. Fuller, *Stanford University School of Medicine, California*

Meiosis
Chairperson: E. Gavis, *Princeton University, New Jersey*

Posttranscriptional Control
Chairperson: P. Newmark, *HHMI/University of Illinois at Urbana-Champaign*

Cell Biology of Germ Cells
Chairperson: P. Hunt, *Washington State University, Pullman*

Sex Determination
Chairperson: M. Matzuk, *Baylor College of Medicine, Houston, Texas*

A. Surani, F.J. Berger

C. Silva

Molecular Genetics of Aging

October 9–13 245 Participants

ARRANGED BY **Steven Austad,** University of Texas Health Science Center, San Antonio
Judith Campisi, Buck Institute for Age Research/Lawrence Berkeley National Laboratory
David Sinclair, Harvard University Medical School

D. Sinclair, J. Campisi, S. Austad

Aging is the largest single risk factor for developing a panoply of diseases, ranging from neurodegeneration to cancer. In recent years, enormous progress has been made in understanding how genetics and the environment influence the pace of aging and disease. Among the remarkable findings is the identification of molecular pathways that control aging in diverse species, from yeast to humans. This conference provided an intense forum for the latest results and emerging ideas in aging research at the molecular and organismal levels. The conference opened with a session on genetic variation—an exploration into why species, and particularly individuals within a species, age at different rates. A related session discussed genetic and epigenetic stability, which focused on the mechanisms that maintain the DNA sequence and its packaging (chromatin), how these entities change during aging, and how such changes affect organismal health and fitness. A subsequent session highlighted the role of mitochondria and energy metabolism in preserving organismal health and longevity, and the molecules that are crucial for optimal energy production and utilization. One session was devoted to cellular stress responses, and the intimate relationship between stress resistance and longevity. The conference then featured a session on stem cells, which emphasized recent findings on how these important sources of tissue repair and regeneration change with age, and how aging alters the tissue environment in which stem cells must function. Another session discussed protein homeostasis, with a focus on why proteins aggregate and how they may be prevented or eliminated to prevent aging and disease. A session then focused on the power of genetic manipulations of conserved pathways to alter the rates and outcome of aging. The final session highlighted the latest findings on the role of nutrition and nutrient sensing in

S. Helfand, S. Ahmed

H. Bussemaker, J. Tollervey

aging and discussed interventional strategies that are currently being developed. The conference brought together scientists from diverse fields, mirroring the expanding multidisciplinarity of the field, and reflected the excitement and growing understanding of the processes that drive aging and the possibilities for developing interventions that could slow the aging process and treat common diseases of aging.

This meeting was funded in part by the National Institute on Aging, a branch of the National Institutes of Health.

PROGRAM

Genetic Variation
Chairpersons: Y. Suh, *Albert Einstein College of Medicine, Bronx, New York;* C. Franceschi, *University of Bologna, Italy*

Epigenetic Stability
Chairpersons: A. Brunet, *Stanford University, California;* J. Vijg, *Albert Einstein College of Medicine, Bronx, New York*

Metabolism and Energetics
Chairpersons: D. Wallace, *Children's Hospital of Philadelphia/ University of Pennsylvania, Philadelphia;* M. Hansen, *Sanford-Burnham Medical Research Institute, La Jolla, California*

Genetics Stability and Cell Senescence
Chairpersons: J. Van Deursen, *Mayo Clinic, Rochester, Minnesota;* P. Hasty, *University of Texas Health Science Center, San Antonio*

Stem Cells
Chairpersons: I. Conboy, *University of California, Berkeley;* H. Jasper, *University of Rochester, New York*

Protein Homeostasis
Chairpersons: D. Sinclair, *Harvard Medical School, Boston, Massachusetts;* D. Green, *St. Jude Children's Research Hospital, Memphis, Tennessee*

Genetic Manipulation
Chairpersons: P. Kapahi, *Buck Institute, Novato, California;* S.-i. Imai, *Washington University School of Medicine, St. Louis, Missouri*

Nutrient Sensing and Interventions
Chairpersons: M. Tatar, *Brown University, Providence, Rhode Island;* R. Miller, *University of Michigan, Ann Arbor*

J. Wright, B. Schneider

J. Wilkowski, D. Wallace

O. Pougovkin

Nuclear Receptors and Disease

October 30–November 3 188 Participants

ARRANGED BY **Keith Yamamoto,** University of California, San Francisco
Ron Evans, Salk Institute Biological Studies
Susanne Mandrup, University of Southern Denmark

S. Mandrup, K. Yamamoto

This meeting has been held biennially since 2006 and is the fourth of its kind. Nuclear receptors constitute a unique group of transcription factors that regulate transcription in response to small lipophilic biomolecules that bind to the receptors. These proteins have very important roles as regulators of cellular growth, differentiation, and metabolism, and dysregulation can lead to metabolic diseases and different types of cancer. Accordingly, the meeting focused on basic mechanisms of nuclear receptor function as well as the role of nuclear receptors in metabolism and cancer.

As a result of Hurricane Sandy, the beginning of meeting had to be rescheduled to November 1. Furthermore, since many speakers and participants could not make it, the meeting was restructured to a workshop format and many younger scientists were given the opportunity to give talks. This allowed for excellent discussions between talks and also an evening discussion session. In addition, many participants gave short talks.

This meeting was funded in part by the National Institute of Diabetes and Digestive and Kidney Diseases, a branch of the National Institutes of Health, and Novo Nordisk.

PROGRAM BEFORE SANDY

Keynote Speakers
C. Sawyers, *HHMI/Memorial Sloan-Kettering Cancer Center, New York;* G.L. Hager*, National Cancer Institute/NIH, Bethesda, Maryland*

Chromatin and Transcription
Chairperson: G. Hager, *National Cancer Institute, NIH, Bethesda, Maryland*

Welcome notice

Science beats Sandy!

Cofactors
Chairperson: R.M. Evans, *HHMI/Salk Institute for Biological Studies, La Jolla, California*

Metabolism and Metabolic Disorders I
Chairperson: S. Mandrup, *University of Southern Denmark, Odense*

Metabolism and Metabolic Disorders II
Chairperson: C. Glass, *University of California, San Diego*

Cancer
Chairperson: A. Kralli, *Scripps Research Institute, La Jolla, California*

Development and Aging
Chairperson: A. Maggi, *University of Milan, Italy*

Structure and Ligands
Chairperson: D. Moore, *Baylor College of Medicine, Houston, Texas*

DURING SANDY

Keynote Speaker
G.L. Hager, *National Cancer Institute/NIH, Bethesda, Maryland*

Chromatin and Cofactors
Chairperson: G. Hager, *National Cancer Institute/NIH, Bethesda, Maryland*

Metabolism and Metabolic Disorders
Chairperson: S. Mandrup, *University of Southern Denmark, Odense*

Cancer
Chairperson: A. Kralli, *Scripps Research Institute, La Jolla, California*

M. Malewicz, G. Hager, D. Kelly

Personal Genomes and Medical Genomics

November 14–17 228 Participants

ARRANGED BY **Evan Eichler,** University of Washington
Deanna Kroetz, University of California, San Francisco
Richard Lifton, Yale University School of Medicine
James R. Lupski, Baylor College of Medicine
Richard Weinshilboum, Mayo Clinic

For the first time, this fifth meeting on Personal Genomes combined both the content of the Personal Genomes Meeting and that of the Pharmacogenomics Meeting to bring together scientific communities interested in gleaning medically actionable information from individual personal genomes. The meeting was entitled "Personal Genomes and Medical Genomics" and was a step toward precision medicine, which utilizes genomic information to guide differential diagnoses and potentially to optimize and direct therapeutic interventions. The meeting was initiated with an outstanding presentation from Retta Beery, whose fraternal twin children underwent personal genome sequencing to identify variants causative for a dopa-responsive dystonia. A specific gene mutation that was identified helped optimize therapy for the children. Mrs. Beery presented a very moving talk on dealing with this medical odyssey and how it affected their family for many years. The keynote speaker was followed by a series of talks in a session on "Genomics to guide differential diagnosis and therapy" in which it became obvious that personal genomes are being used around the world at the leading medical institutions by thoughtful leaders in genomics who are trying to leverage the clinical utility of information that can be gleaned from personal genomes. The second session dealt with somatic cell genomics and cancer pharmacogenomics, and the third with germline genomics emphasizing Mendelizing traits and locus heterogeneity. Three other sessions included genomic variation and common traits/disease, clinical integration of genomic variation, and evolving genomic technologies. A lively and informative ethics panel was presented in which challenges of clinical information on genomic variation were discussed in detail. Furthermore, the President's commission on Bio-Ethics presented the first public report of their findings related to human genome sequence information and its utility. Their deliberations stressed the desire for safeguarding and maintaining privacy of genomic information. Finally, a keynote address, given by Maynard Olson, enthusiastically embraced the rapid movement of genomic sequence information into the clinical arena; it also enlightened us by "cautionary notes" as we move into a molecular era

J. Lupski welcomes group

D. Kroetz

E. Eichler, *center*

Ethics panelists (*left to right*): G. Lyon, A. Beaudet, J. Beckmann, A. Allen

in which precision medicine is delivered by virtue of molecular knowledge of the disease process in each individual as elucidated through the information embodied in their personal genome.

This meeting was funded in part by the National Institute of General Medical Sciences, a branch of the National Institutes of Health, and Illumina.

PROGRAM

Genomics to Guide Differential Diagnosis and Therapy
Chairperson: J. Lupski, *Baylor College of Medicine, Houston, Texas*

Keynote Address: The Beery Family Journey: A Sequencing Success
R. Beery, *Encinitas, California*

Somatic Cell Genomics and Cancer Pharmacogenomics
Chairperson: D. Kroetz, *University of California, San Francisco*

Germline Genomics, Mendelizing Traits and Locus Heterogeneity
Chairperson: R. Lifton, *Yale University School of Medicine, New Haven, Connecticut*

Genomic Variation in Common Traits/Disease
Chairperson: E. Eichler, *University of Washington, Seattle*

Clinical Integration of Genomic Variation
Chairperson: R. Weinshilboum, *Mayo Clinic, Rochester, Minnesota*

Ethics Panel: Challenges of Clinical Implementation of Genomic Variation
Moderator: S. Plon, *Baylor College of Medicine, Houston, Texas*

Evolving Genomic Technologies
Chairperson: M. Hurles, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*

Keynote Address: Three Cautions about Personalized Medicine
M.V. Olson, *University of Washington, Seattle, Washington*

C. Aquadro

F. De La Vega

R. Beery, J. Watson

Neurodegenerative Diseases: Biology and Therapeutics

November 28–December 1 122 Participants

ARRANGED BY **Karen Duff,** Columbia University Medical Center
Jeffrey Rothstein, Johns Hopkins University School of Medicine
John Trojanowski, University of Pennsylvania School of Medicine

J. Rothstein, K. Duff, J. Trojanowski

Up to one-half of those aged 85 years or older will develop debilitating degenerative disease of the central nervous system. These various diseases include Alzheimer's disease (AD), Parkinson's disease (PD), frontal temporal lobe degeneration (FTD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS). To reflect some of the newest and most relevant research while providing diverse base topics, the 2012 meeting included prion-like spread of diseases including AD, PD, and tauopathies; new thoughts about biomarkers and comorbidities; the role of RNA in the pathogenesis of neurodegenerative diseases; the role of glia, especially their potential as targets for therapy; and a number of therapeutic targets and approaches including immunomodulation, small molecules, and gene inactivation strategies.

Although most forms of neurodegenerative disease occur in the absence of obvious heritability or identifiable genetic mutations, it has been possible during the past 20 years to discover uncommon genetic mutations as well as risk-modifying DNA changes in some examples and predictable causative changes in others. For some of the neurodegenerative diseases, common genetic abnormalities lead to a spectrum of disease phenotypes, which is clearly seen for FTD and ALS. New insight into disease mechanisms and possible overlapping therapeutic approaches are implicated. From these findings, transgenic technology has rapidly led to the development of mouse, fruit fly, and nematode model systems that partially recapitulate the clinical abnormalities of the human diseases as well as some of the hallmark molecular and morphological pathology of the conditions. Of particular relevance in this respect is the emergence of new ideas about the spread of disease entities within the brain and from the periphery, similar to that seen in the prion diseases, which has become a dominant topic in AD, FTD, and PD research. This new and exciting field of research was well-represented at the 2012 meeting.

The biannual meeting series started in 2000, and as in previous years, the explicit goal of the meeting focuses on identifying disease pathways and facilitating the translation of "breakthrough" science into effective medicines. At this year's meeting, numerous academic labs and commercial drug discovery organizations presented data on novel compounds, clinical trial results, or new druggable pathways for AD, PD, FTD, HD, ALS, spinal muscular atrophy, spinocerebellar ataxia, and prion diseases. In recent years, various molecular, biochemical, and cell-based screens have led to the development of small-molecule, peptide, and oligonucleotide compounds that show promise in neurodegenerative disease models. Importantly, the emergence of gene inactivation strategies using stabilized oligonucleotides has shown particular promise both as a tool for studying pathogenesis and, more importantly, as a clinically relevant therapeutic agent that could be used to knock down pathological proteins or pathways affected in several diseases.

For this meeting, platform sessions were organized around common technological themes. Chairpersons, invited speakers, and speakers selected from submitted abstracts were drawn from

the academic and pharma sectors, representing 20+ countries. The discussion of new, unpublished data was emphasized and adequate time was left for discussion of each presentation. Poster presentations also covered a wide range of neurodegenerative disease pathways, new animal and insect models, and novel therapeutic insights. Posters were displayed for an extended period during the meeting, and poster viewing was especially encouraged during the cocktail hours before the evening meals. In general, the meeting was most notable for its breadth of coverage of different neurodegenerative diseases, insightful and novel presentations and discussions, and the insight gleaned from lively interaction between the diverse participants.

PROGRAM

Prion-Like Neurodegeneration: Initiation and Disease Spread
Chairperson: V. Lee, *University of Pennsylvania School of Medicine, Philadelphia*

Risk Factors and Comorbidities: Aging, Biomarkers, and Genetics
Chairperson: G. Schellenberg, *University of Pennsylvania School of Medicine, Philadelphia*

RNA Metabolism in Neurodegenerative Disease
Chairperson: D. Cleveland, *University of California, San Diego*

Submitted Abstracts
Chairperson: P. Brundin, *Van Andel Research Institute, Grand Rapids, Michigan, and Lund University, Sweden*

Glial Biology: New Targets for Therapy in Neurodegeneration
Chairperson: J. Rothstein, *Johns Hopkins University School of Medicine, Baltimore, Maryland*

Immune and Small-Molecule Therapy for AD and PD
Chairperson: J. Trojanowski, *University of Pennsylvania School of Medicine, Philadelphia*

Gene Inactivation Strategies
Chairperson: A. Krainer, *Cold Spring Harbor Laboratory*

D. Cleveland

N. Kfoury

L. McConlogue, M. Diamond

Blood Brain Barrier

December 5–8 124 Participants

ARRANGED BY **Richard Daneman,** University of California, San Francisco
Britta Engelhardt, University of Bern, Switzerland
Ryan Watts, Genentech, Inc.

R. Daneman, B. Engelhardt, R. Watts

This conference focused on molecular mechanisms controlling blood brain barrier (BBB) development and function. The conference encouraged new conceptual approaches and novel methods to our understanding of the neuroprotective physiologies of BBB structures, pairing genetics of model organisms and associated biological methods with recent insights into vertebrate BBB physiology and development. The conference fostered cross-disciplinary exchange of ideas and expertise between developmental vascular and BBB biologists and disease-oriented industry scientists interested in targeting drugs across the BBB. The keynote address was given by N. Joan Abbott on the evolution of the BBB. Topics addressed by the conference are listed below in the Program. As is traditional at Cold Spring Harbor meetings, selection of material for oral and poster presentation was made by the organizers and individual session chairs on the basis of scientific merit.

PROGRAM

Molecular Control of BBB Development and Function
Chairpersons: S. Liebner, *Goethe University Clinic, Frankfurt am Main, Germany;* A. Prat, *CHUM-Université de Montréal, Canada*

Cellular and Acellular Elements
Chairpersons: C. Betsholtz, *Karolinska Institute, Stockholm, Sweden;* L. Sorokin, *University of Münster, Germany*

Model Organisms
Chairpersons: B. Anand-Apte, *Cleveland Clinic Lerner College of Medicine, Ohio;* R. Bainton, *University of California, San Francisco*

Retinal Vasculature and Other Tissue Barriers
Chairpersons: C.Y. Cheng, *The Rockefeller University, Population Council, New York;* H. Gerhardt, *London Research Institute-Cancer Research, United Kingdom*

S. Wang, C. Chen

BBB in Inflammation and Disease
Chairpersons: E. Tournier-Lasserve, *Université Paris Diderot - Paris 7, France;* E. de Vries, *MS Center, Amsterdam, The Netherlands*

Keynote Address: Evolution of the Blood Brain Barrier and Neurovascular Unit
N.J. Abbott, *King's College London, United Kingdom*

Crossing the BBB
Chairpersons: M. Bynoe, *Cornell University Veterinary Medical College, Ithaca, New York;* M. Wood, *University of Oxford, United Kingdom*


U. Frevert


D. Wilhelms


C. Klämbt

POSTGRADUATE COURSES

The Postgraduate Courses program at Cold Spring Harbor Laboratory is aimed at meeting the special need for training in interdisciplinary subjects that are either so new or so specialized that universities do not adequately teach them. Our aim is to provide intensive study in the most recent developments and techniques in these subjects and to prepare students to enter directly into research in a particular area. To ensure up-to-date coverage of current research work, we bring together course faculty from many laboratories around the world and supplement this staff with a series of seminar speakers.

Cold Spring Harbor Laboratory Workshop on Leadership in Biosciences

February 24–27

INSTRUCTORS **C.M. Cohen,** Science Management Associates, Newton, Massachusetts
D. Kennedy, Massachusetts School of Professional Psychology, Newton, Massachusetts

In this highly interactive workshop, participants learned and developed the skills to lead and interact effectively with others in both one-on-one and group settings. The workshop focused on techniques, situations, and challenges that relate specifically to leading and managing in the scientific workplace. It emphasized learning by doing and involved role playing, giving and receiving feedback, and group problem solving. The workshop's goal was to help participants identify areas where they needed guidance and growth, as well as how to capitalize on areas of strength. Participants had the opportunity to share their experiences and challenges with others and to receive feedback and guidance from others with experience in leading scientists in a variety of settings. Key focus areas of the workshop included recognizing and understanding leadership in a science setting; using negotiation as a tool in scientific discussions and problem solving; identifying and resolving conflicts in the lab; dealing with difficult people and situations; communicating ideas and plans in a way that engages others; leading effective and productive meetings; and leveraging scientific skills in the community and in public settings.

This course was supported with funds provided by American Express Philanthropy.

PARTICIPANTS

Azad, N., Ph.D., Hampton University, Hampton, Virginia
Chen, K., Ph.D., Mount Sinai School of Medicine, New York
Cohen, D., Ph.D., HHMI/Harvard University, Cambridge, Massachusetts
Dean, H., Ph.D., New York University, New York
Dorer, M., Ph.D., Fred Hutchinson Cancer Research Center, Seattle, Washington
Eriksson, C., Ph.D., Uppsala University, Sweden
Fehrenbacher, N., Ph.D., New York University, New York
Ganesan, R., Ph.D., MedImmune, Gaithersburg, Maryland
Goto, D., Ph.D., Hokkaido University, Japan
Gutierrez-Nava, M. de la Luz, Ph.D., DuPont Agricultural Biotechnology, Wilmington, Delaware
Iyer, A., Ph.D., Hampton University, Seattle
Lucs, A., Ph.D., Feinstein Institute for Medical Research, New York
Lyssenko, N., Ph.D., University of Pennsylvania, Philadelphia

Malone, C., Ph.D., New York University Medical School, New York
Mar, J., Ph.D., Albert Einstein College of Medicine, Bronx, New York
Miller, J., Ph.D., Mayo Clinic and Foundation, Rochester, Minnesota
Mishra, R., Ph.D., University of Illinois, Chicago
Murray, D., Ph.D., Royal College of Surgeons in Ireland, Dublin
Nieminen, J., B.S., Laval University, Quebec, Canada
Noriega-Lopez, L., Ph.D., INCMNSZ, Tlalpan, Mexico
Nowak, D., Ph.D., Cold Spring Harbor Laboratory
Ramsook, C., Ph.D., Brooklyn College, New York
Scherrer S., B.S., Feinstein Institute for Medical Research, Manhasset, New York
Seeliger, J., Ph.D., Stony Brook University, New York
Seeliger, M., Ph.D., Stony Brook University, New York
Stratford, E., Ph.D., Oslo University Hospital, Norway
Troemel, E., Ph.D., University of California, San Diego

SEMINARS

Barker, K., Author, Seattle, Washington: At the helm.
Miller, K., Brown University, Providence, Rhode Island: Science in the public eye.

Protein Purification and Characterization

April 11–24

INSTRUCTORS
R. Burgess, University of Wisconsin, Madison
A. Courey, University of California, Los Angeles
S.-H. Lin, M.D. Anderson Cancer Center/University of Texas, Houston
M. Marr, Brandeis University, Waltham, Massachusetts

ASSISTANTS
J. Cao, University of California, Los Angeles
R. Chumanov, University of Wisconsin, Madison
M. Donovan, Brandeis University, Waltham, Massachusetts
P. Kuhn, University of Wisconsin, Madison
P. Kwong, University of California, Los Angeles
Y.-C. Lee, M.D. Anderson Cancer Center, University of Texas, Houston
C. Olson, Brandeis University, Waltham, Massachusetts
K. Pennington, Brandeis University, Waltham, Massachusetts
A. Ponce, University of California, Los Angeles
M. Spellberg, Brandeis, Waltham, Massachusetts
N. Thompson, University of Wisconsin, Madison

This course was for scientists who were not familiar with techniques of protein isolation and characterization. It was a rigorous program that included laboratory work all day and a lecture with a discussion session every evening. Each student became familiar with each of the major techniques in protein purification by actually performing four separate isolations including (1) a regulatory protein from muscle tissue, (2) a sequence-specific, DNA-binding protein, (3) a recombinant protein overexpressed in *Escherichia coli*, and (4) a membrane-bound receptor. A variety of bulk fractionation, electrophoretic, and chromatographic techniques included precipitation by salts, pH, and ionic polymers; ion exchange, gel-filtration, hydrophobic interaction, and reverse-phase

chromatography; lectin affinity, ligand affinity, oligonucleotide affinity, and immunoaffinity chromatography; polyacrylamide gel electrophoresis, and electroblotting; and high-performance liquid chromatography. Procedures were presented for solubilizing proteins from inclusion bodies and refolding them into active monomeric forms. Methods of protein characterization included 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.

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Adeyo, O., B.S., University of California, Los Angeles
Aquirre-Chen, C., Ph.D., Cold Spring Harbor Laboratory
Bergmann, J., Ph.D., Cold Spring Harbor Laboratory
Dzialo, M., B.S., University of California, Los Angeles
Hooper, C., B.A., University of Wisconsin, Madison
Jackson, S., B.S., University of Wisconsin, Madison
Li, W., B.S., Cold Spring Harbor Laboratory
Martinez, L., Ph.D., Long Island University, Brookville, New York
Ni, S., Ph.D., Cold Spring Harbor Laboratory
Pai, M., B.A., University of California, Los Angeles
Sabari, B., Ph.D., The Rockefeller University, New York
Schneider, L., B.A., University of Wisconsin, Madison
Shi, J., B.S., Cold Spring Harbor Laboratory
Tsou, Wi.-L., B.Sc., Wayne State University School of Medicine, Detroit, Michigan
Wang, A., D.Phil., The Rockefeller University, New York
Woo, J.-R., M.S., University of California, Los Angeles
Yan, D., Ph.D., Harvard Medical School, Boston, Massachusetts

SEMINARS

Burgess, R., University of Wisconsin, Madison: Welcome and introduction to course; Introduction to protein purification; Rapid isolation of weakly binding proteins with the IFAST system.
Chumanov, R., University of Wisconsin, Madison: Halo tag and purification of kinases in mammalian cells.
Courey, A., University of California, Los Angeles: System-wide analyses of Groucho and SUMO in *Drosophila* development.
Lin, S.-H., M.D. Anderson Cancer Center, Houston, Texas: Prostate cancer bone metastasis secretome.
Marr, M., Brandeis University, Waltham, Massachusetts: Stress-induced changes in gene expression.
Thompson, N., University of Wisconsin, Madison: MAbs and immunoaffinity chromatography.

Cell and Developmental Biology of *Xenopus*

April 13–24

INSTRUCTORS **A. Sater,** University of Houston, Texas
G. Thomsen, Stony Brook University, New York

ASSISTANTS **B. Dzamba,** University of Virginia, Charlottesville
T. Nakayama, University of Virginia, Charlottesville
K. Pfister, University of Virginia, Charlottesville

Xenopus is the leading vertebrate model for the analysis of gene function in development. The combination of lineage analysis, gene-knockout strategies, experimental manipulation of the embryo, and genomic/bioinformatic techniques makes it ideal for studies on the molecular control of embryo patterning, morphogenesis, and organogenesis. Moreover, recent advances in *Xenopus* genomics offer new opportunities to integrate computational strategies with experimental approaches. The course combined intensive laboratory training with daily lectures from recognized experts in the field. Students learned both emerging technologies and classical techniques to study gene function in *Xenopus* development. An important element was the informal interaction between students and course faculty.

Technologies covered included oocyte and embryo culture, lineage analysis and experimental manipulation of embryos, time-lapse imaging of morphogenesis, gain- and loss-of-function analyses using mRNAs and antisense oligonucleotides, whole-mount in situ hybridization, immunocytochemistry, genomics and bioinformatics, chromatin immunoprecipitation, preparation of transgenic embryos, and use of *Xenopus tropicalis* for genetic analyses. This course was designed

for those new to the *Xenopus* field, as well as for those wanting a refresher course and/or exposure to the emerging technologies. The course was open to investigators from all countries.

This course was supported with funds provided by the National Institute of Child Health and Human Development, the National Science Foundation, and the Howard Hughes Medical Institute.

PARTICIPANTS

Bouissou, C., Ph.D., MRC National Institute for Medical Research, London, United Kingdom
Ciarleglio, C., Ph.D., Brown University, Providence, Rhode Island
Diaz, B., B.Pharm., UNAM, Mexico City, Mexico
Fuentealba, J., M.S., Universidad de Concepcion, Chile
Gonzalez, S., Ph.D., University College London, United Kingdom
Kuo, H.-C., M.S., University of Alaska, Anchorage
Lee, M., B.S., University of Texas/M.D. Anderson Cancer Center, Houston, Texas
Lobikin, M., B.SE., Tufts University, Medford, Massachusetts
Louza, M., M.S., Cincinnati Children's Hospital, Ohio
Oomen-Hajagos, J., B.S., Stony Brook University, New York
Ota, Y., Ph.D., University of Maryland, Baltimore
Rosinbum, S., Ph.D., Food and Drug Administration, Bethesda, Maryland
Schuler, F., B.S., Tufts University, Medford, Massachusetts
Spruce, T., Ph.D., MRC National Institute for Medical Research, London, United Kingdom
Xu, M., Ph.D., University of Miami, Florida
Yang, Y., B.S., University of Michigan, Ann Arbor

SEMINARS

Blythe, S., University of Princeton, New Jersey: Chromatin immunoprecipitation in *Xenopus laevis.*
El-Hodiri, H., Ohio State University, Columbus: The *retinal homeobox* (*Rx*) gene plays essential roles in the normal and regenerating retina.
Gilchrist, M., National Institute for Medical Research, London, United Kingdom: *Xenopus:* Genomes, genomics, and computational biology.
Keller, R., University of Virginia, Charlottesville: Gastrulation and morphogenesis.
Khokha, M., Yale University School of Medicine, New Haven, Connecticut: Analysis of congenital heart disease genes in *Xenopus*.
Klein, P., HHMI/University of Pennsylvania, Philadelphia: Signaling and pattern before the MBT.
Nakayama, T., University of Virginia, Charlottesville: Approaches for manipulating gene expression in *Xenopus*.
Robert, G., University of Virginia, Charlottesville: Analysis of eye formation in *Xenopus:* Model system for studies of determination, induction, and organogenesis.
Sater, A., University of Houston, Texas: *Xenopus* as a model system: History and prospects; Adventures in Ago-RNP isolation from *Xenopus* embryos.
Slack, J., University of Minnesota, Minneapolis: Stimulation of *Xenopus* limb regeneration by cell transplants; Regeneration of the *Xenopus* tadpole tail.
Thomsen, G., Stony Brook University, New York: The *Xenopus* organizer.
Wallingford, J., University of Texas, Austin: The awesome power of live imaging in *Xenopus.*
Zimmerman, L., MRC National Institute for Medical Research, London, United Kingdom: Genetic tools in *Xenopus*.

Workshop on Schizophrenia and Related Disorders

June 6–12

INSTRUCTORS **J. Hall,** University of Edinburgh, United Kingdom
A. Law, National Institute of Mental Health/NIH, Bethesda, Maryland
A. Malhotra, The Zucker Hillside Hospital, Glen Oaks, New Jersey [**Au: check City/state**]

ASSISTANT **K. Olive**, Columbia University, New York

This workshop provided students with the most current understanding of the molecular, cellular, and neural systems underlying the disturbances in brain function in these devastating illnesses. During the 7-day workshop, students learned about the clinical aspects of schizophrenia, schizoaffective disorder, and bipolar disorder, and also explored in detail the genetic and neurobiological underpinnings of these complex psychiatric disorders. The Workshop included sessions focused on The Clinical Syndrome, Basic Neurobiology, Cognitive Neuroscience, Neuroimaging, Genetics and Genomics, Endophenotypes, and Gene Expression and Gene Modulation. In addition to hearing about the most recent research in these areas, controversial topics and challenges to basic assumptions in the field were explored and discussed. A diverse faculty brought the most up-to-date results and theories to the students, making this workshop a valuable resource for young researchers starting out in this fast-moving and expansive field. Not only did it help them build the foundation for their future research, it also introduced them to many potential collaborators working to understand schizophrenia and similar disorders from different perspectives. Although these 7 days featured intense lecture sessions throughout, students also had free time for reading, informal discussions, and recreation on the beautiful campus of the Banbury Center, which includes a beach, a pool, and a tennis court.

PARTICIPANTS

Atkin, T., Ph.D., Columbia University, New York
Butt, M., B.S., University of Edinburgh, United Kingdom
Pereira, F.C.R., B.S., Friedrich Miescher Institute, Basel, Switzerland
Garvey, M., Ph.D., National Institute of Mental Health/NIH, Rockville, Maryland
Goonawardena, A., Ph.D., SRI International, Menlo Park, California
Guha, S., Ph.D., Zucker Hillside Hospital, Glen Oaks, New York
Heremes, G., B.S., Yale University, New Haven, Connecticut
Keane, B., B.S., Robert Wood Johnson Medical School, Piscataway, New Jersey
Manley, W., B.S., Rutgers University, Piscataway, New Jersey
McCollum, L., B.S., University of Alabama, Birmingham
Miller, B., Ph.D., The Scripps Research Institute, Florida
Mukherjee, S., B.S., Zucker Hillside Hospital, Glen Oaks, New York
Paterson, C., B.S., National Institutes of Health, Bethesda, Maryland
Perez, S., B.S., University of Texas, San Antonio
Peters, B., Ph.D., Hillside Hospital, Glen Oaks, New York
Pouget, J., B.S., University of Toronto, Ontario, Canada
Rodrigue, A., B.S., University of Georgia, Athens
Salum, C., Ph.D., Federal University of ABC, Santo Andre, Brazil
Sauer, A., B.S., Max-Planck Institute of Brain Research, Frankfurt, Germany
Sprooten, E., B.S., University of Edinburgh, United Kingdom
Thomases, D., B.S., Rosalind Franklin University, Chicago, Illinois
Vieker, H., Ph.D., University Hospital, Goettingen, Germany
Wilkins, H., B.S., Washington University, St. Louis, Missouri
Zamberletti, E., B.S., University of Insurbia, Busto Arsizio, Italy

SEMINARS

Abi-Dargham, A., Columbia University, New York: PET imaging in schizophrenia.
Anderson, S., Weill Cornell Medical College, New York: GABA systems in schizophrenia.
Brennand, K., University of California, San Diego: Neurogenesis and iPS in schizophrenia.
Cornblatt, B., Zucker Hillside Hospital, Glen Oaks, New York: Cognitive and psychosocial risk factors.
Fletcher, P., University of Cambridge, United Kingdom: A cognitive neuroscientific model of the positive symptoms of schizophrenia.
Glahn, D., Yale University, New Haven, Connecticut: Cognition of schizophrenia.
Grace, A., University of Pittsburgh, Pennsylvania: The dopaminergic system.
Hall, J., University of Edinburgh, United Kingdom: Introduction of meeting and participants; Functional MR in schizophrenia.
Jones, J., University of Cambridge, United Kingdom: Epidemiology and environmental risk factors.
Kane, J., Zucker Hillside Hospital, Glen Oaks, New York: Treatment strategies.
Kleinman, J., National Institute of Mental Health/NIH, Bethesda, Maryland: Postmortem gene expression in neurodevelopment.
Law, A., National Institute of Mental Health/NIH, Bethesda, Maryland: Genetics of neurodevelopment, gene regulation, and risk for schizophrenia. Lessons from the NRG/ErbB4-PI3 K/AKT pathway.
Lencz, T., Zucker Hillside Hospital, Glen Oaks, New York: Rare variants/CNVs and sequencing in schizophrenia.
Malhotra, A., Zucker Hillside Hospital, Glen Oaks, New York: Pharmacogenomics in schizophrenia.
Malhotra, A., Zucker Hillside Hospital, Glen Oaks, New York; Law, A., National Institute of Mental Health/NIH, Bethesda, Maryland
McIntosh, A., University of Edinburgh, United Kingdom: Structural imaging in schizophrenia.
Moghaddam, B., University of Pittsburgh, Pennsylvania: The glutamatergic system.
Murray, R., Kings College, London, United Kingdom: The clinical syndrome.
Petronis, A., University of Toronto, Canada: Epigenetic approaches to mental illness.
Purcell, S., Mount Sinai School of Medicine, New York: Candidate gene and whole-genome approaches to schizophrenia.
Thomson, P., University of Edinburgh, United Kingdom: Introduction to schizophrenia genetics and the DISC1 example.
Weinberger, D., National Institute of Mental Health/NIH, Bethesda, Maryland: Intermediate phenotypes and schizophrenia-associated genes.

Single-Cell Analysis

June 6–19

INSTRUCTORS **J. Eberwine,** University of Pennsylvania Perelman School of Medicine, Philadelphia
C. McMurray, University of California, Berkeley/Lawrence Berkeley Laboratory

ASSISTANTS **P. Buckley,** University of Pennsylvania Medical School, Philadelphia
C. Canaria, Lawrence Berkeley National Laboratory, California
D.-Y. Lee, Lawrence Berkeley National Laboratory, California
J. Morris, University of Pennsylvania School of Medicine, Philadelphia

The goal of this new 2-week course was to familiarize students with the most recent cutting-edge technologies for characterization of cells. Important in this process was highlighting the advantages to analysis of single cells in isolation and in their natural microenvironment. Sections of the course were taught by scientists who are experts in particular areas of single-cell analysis. Topics covered included quantitative single-cell analysis by RNA-Seq, genomic DNA analysis, epigenetics, RNA localization analysis, proteomics, protein translation, and metabolomics. The techniques included real-time live-cell quantification where appropriate. In addition, multiple techniques to isolate specific cell populations and individual cells were taught. The course included the use of model systems such as *Caenorhabditis elegans*, *Drosophila,* and mouse.

PARTICIPANTS

Daniele, T., M.S., IGBMC, France
Engelstoft, M., M.S., Copenhagen University, Denmark
Erdel, F., Dipl., BioQuant/DKFZ Heidelberg, Germany
Fedorenko, I., B.S., University of South Florida, Tampa

Frank, A.-K., M.Sc., University of Copenhagen, Denmark
Glenn, H., Ph.D., Arizona State University, Tempe
Hughes, A., B.S., University of California, Berkeley
Kim, H., Ph.D., New York University, New York
Li, Q., M.S., Karolinska Institutet, Sweden
Park, J., B.S., Cold Spring Harbor Laboratory
Pfisterer, U., M.S., Lund University, Lund, Sweden
Prudhomme, J., B.Sc., Institut Pasteur, France
Reed, J., Ph.D., University of California, Los Angeles
Shruti, S., Ph.D., Brandeis University, Boston, Massachusetts
van Wolfswinkel, J., M.S., Whitehead Institute/ Massachusetts Institute of Technology, Cambridge
Varma, S., Ph.D., Children's Hospital, Los Angeles, California
Wood, L., Ph.D., Johns Hopkins University School of Medicine, Baltimore, Maryland

SEMINARS

Brenner, D., Columbia University, New York: Irradiating and monitoring single cells and their neighbors, in vitro and in vivo.
Eberwine, J., University of Pennsylvania, Philadelphia: Functional consequences of single-cell transcriptome variability.
Gratton, E., University of California, Irvine: Cellular dynamics using phosphor and FLIM imaging.
Hicks, J., Cold Spring Harbor Laboratory: Single-cell methods in cancer.
Lao, K., Life Technologies, Foster, California: Development and applications of single-cell RNA-Seq analysis.
Larabell, C., University of California, Berkeley: Single-cell CT scans at 50-nm resolution.
Lippincott-Schwartz, J., National Institutes of Health, Bethesda, Maryland: Nanoscopic imaging with photoactivatable fluorescent proteins: Windows into molecular organization and dynamics within cells.
McMurray, C., University of California, Berkeley: The basis for region-specific neuronal death in Huntington's disease.
Petronis, A., Krembil Family Epigenetics Laboratory, Toronto, Canada: Epigenomics of complex disease.
Sweedler, J., University of Illinois, Urbana: Using mass spectrometry to measure the brain's chemistry a cell at a time.

Advanced Bacterial Genetics

June 6–26

INSTRUCTORS **D. Hughes,** Uppsala University, Sweden
B. Lazazzera, University of California, Los Angeles
F. Yildiz, University of California, Santa Cruz

ASSISTANTS **J. Bergman,** Uppsala Biomedical Center, Sweden
A. Cheng, University of California, Santa Cruz
S. Hoover, University of California, Los Angeles

This course presented logic and methods used in the genetic dissection of complex biological processes in diverse bacteria. Laboratory methods included classical mutagenesis using transposons, mutator strains, and chemical mutagens; recombineering with single- and double-stranded DNA; detection of gene expression changes using various reporter genes; the mapping of mutations using genetic and physical techniques; modern approaches to the generation and analysis of targeted gene disruptions and fusions using PCR and cloning methods; epitope insertion mutagenesis; site-directed mutagenesis; and fluorescence microscopy. Key components of the course were the use of sophisticated genetic methods in the analysis of model bacteria (including *Escherichia coli, Salmonella, Bacillus subtilis,* and *Vibrio cholerae*) and the use of the wealth of new genomic sequence information to motivate these methods. Invited lecturers presented various genetic approaches to study bacterial mechanisms of metabolism, development, and pathogenesis.

The course admits 16 students, both foreign and United States residents, from diverse backgrounds and career levels for intensive (but fun) instruction in microbial genetics.

This course was supported with funds provided by the National Science Foundation.

PARTICIPANTS

Adler, M., M.S., Uppsala University, Sweden
Akabayov, B., Ph.D., Harvard Medical School, Cambridge, Massachusetts
Banning, E., Ph.D., Marine Biological Laboratory, Woods Hole, Massachusetts
Boyle, K., B.S., Weill Cornell Medical College, New York
Chevereau, G., Ph.D., Institute of Science and Technology, Klosterneuberg, Austria
Colavin, A., B.S., Stanford University, California
Croney, C., B.S., University of Alabama, Birmingham
Kath, J., B.A., Harvard Medical School, Cambridge, Massachusetts
Levinson, K., B.S., Wadsworth Center/University at Albany, Ohio
Shkundina, I., Ph.D., Institute of Molecular Genetics, Prague, Czech Republic
Snitkin, E., Ph.D., National Human Genome Research Institute, Bethesda, Maryland
Steinrueck, M., B.S., Institute of Science and Technology, Austria
Taheri-Araghi, S., B.Sc., Harvard University, Cambridge, Massachusetts
Vtyurina, N., M.S., Delft University of Technology, the Netherlands
Wang, N.,, B.A., Northwestern University, Chicago, Illinois
Wu, Y., Ph.D., Harvard University, Cambridge, Massachusetts

SEMINARS

Britton, R., Michigan State University, East Lansing: Recombineering in gram-positive bacteria.
Hughes, K., University of Utah, Salt Lake City: The biogenesis of the bacterial flagellum and coupled gene regulatory mechanisms; A genetic approach to measure the speed of ribosome translation through specific codon pairs.
Ibba, M., Ohio State University, Columbus: Adaptation of the translation quality control machinery to amino acid stress.
Klose, K., University of Texas Health Science Center, San Antonio: Genetic techniques in *Vibrio cholera* and *Francisella tularensis.*
Roth, J., University of California, Davis: Selection and mutation in genetic analysis (the tyranny of the obvious).
Ruiz, N., Ohio State University, Columbus: Envelope biogenesis in *E. coli.*
Salama, N., University of Washington, Seattle: Next-generation mutant hunts in *Helicobacter pylori.*
Sawitzske, J., National Institutes of Health, Frederick, Maryland: An update on recombineering mechanisms.

Ion Channels and Synaptic Transmission

June 6–26

INSTRUCTORS
S. Brenowitz, NIDCD/National Institutes of Health, Bethesda, Maryland
I. Duguid, University of Edinburgh, United Kingdom
P. Kammermeier, University of Rochester Medical Center, Rochester, New York

ASSISTANTS
C. Bladen, University of Calgary, Alberta, Canada
A. Graves, Northwestern University, Evanston, Illinois
V. Lu, NIH/NIAAA/LMP, Rockville, Maryland
J. Lueck, University of Iowa Carver College of Medicine, Iowa City, Iowa
M. Rigby, University College London, United Kingdom
Z. Rosen, Columbia University, New York
V. Stempel, Charite Berlin, Germany

The primary goal of this course was to investigate, through lectures and laboratory work, the properties of ion channels that allow neurons to carry out their unique physiological functions in a variety of neural systems. Areas of particular interest included channels that (1) are activated by neurotransmitter at central and peripheral synapses, (2) are activated by voltage changes in axons and dendrites, (3) respond to neuromodulators with changes in functional properties, (4) are developmentally required and regulated, or (5) are light gated and engineered to express in specific neural subtypes. The research interests of guest lecturers reflected these areas of emphasis.

The laboratory component of the course introduced students to state-of-the-art electrophysiological approaches for the study of ion channels in their native environments. Hands-on exercises included patch-clamp recording of ion channel activity in acutely isolated or cultured cells or neurons in brain slice preparations. Different recording configurations were used (e.g., whole-cell,

cell-attached, and dendritic patches and use of voltage- and current-clamp configurations) to examine macroscopic or single-channel activity. Similarly, various methods of ligand and drug application were demonstrated. The advantages and disadvantages of each method, preparation, and recording technique were considered in relation to the specific scientific questions being asked. Admissions priority was given to students and postdocs with a demonstrated interest, specific plans, and a supportive environment to apply these techniques to a defined problem.

This course was supported by the Howard Hughes Medical Institute.

PARTICIPANTS

Ball, J., Ph.D., National Institutes of Health, Bethesda, Maryland
Griffith, T., B.A., Northwestern University, Boston, Massachusetts
Herold, C., M.S., University of California, Berkeley
Hyser, J., Ph.D., Baylor College of Medicine, Houston, Texas
Kougioumtzidou, E., MSc., University College London, United Kingdom
Kuchibhotla, K., Ph.D., Harvard Medical School, Boston, Massachusetts
Meikle, L, Ph.D., University of Edinburgh, United Kingdom
Ng, D, Ph.D., Columbia University, New York
Penrod-Martin, R, B.S., University of Minnesota, Twin Cities
Ruka, K, B.S., University of Michigan, Ann Arbor
Rutlin, M, Ph.D., Brandeis University, Boston, Massachusetts
Vlasits, A, B.A., University of California, Berkeley

SEMINARS

Brenowitz, S., NIDCD/National Institutes of Health, Bethesda, Maryland: V-clamp-Rs/Analysis.
Farrant, M., University College London, United Kingdom: Synapses/GABA/IPSCs.
Freidman, M., State University of Buffalo, New York: Synapses, EPSCs.
Hausser, M., University College London, United Kingdom: Synaptic integration.
Ikeda, S., National Institute on Alcohol Abuse and Alcoholism, Bethesda, Maryland: Modulation; RGK Proteins and Calcium Channel Function.
Kaczmarek, L., Yale University School of Medicine, New Haven, Connecticut.
Kammermeier, P., University of Rochester Medical Center, New York; Duguid, I., University of Edinburgh, United Kingdom: Introduction/Resting potential.
Khaliq, Z., National Institutes of Health, Bethesda, Maryland: Voltage-gated sodium channels; Spontaneous and high-frequency firing in central neurons.
Martens, J., University of Michigan, Ann Arbor: Voltage-gated channels.
Miller, C., Brandeis University, Waltham, Massachusetts: Chloride channels: What are they good for, anyway?; The CLC family of chloride transporters and channels: Weird and weirder.
Nolan, M., University of Edinburgh, United Kingdom: HCN channel structure, physiology, and function; Circuit mechanisms for oscillations and representation of space in the medial entorhinal cortex.
Plested, A., Leibnitz Institute for Molecular Pharmacology, Germany: Glutamate receptors.
Shuttleworth, T., University of Rochester, New York: Store-operated calcium entry: Stim/oral channels.
Sjöstrom, J., Montreal General Hospital, Canada: Synaptic Plasticity and LTP.
Stanley, E., Toronto Western Research Institute, Canada: Ca channels.
Strowbridge, B., Case Western Reserve University, Cleveland, Ohio: Intrinsic plasticity, microcircuits.
Zador, A., Cold Spring Harbor Laboratory: In vivo electrophysiology.
Zhang, F., Massachusetts Institute of Technology, Cambridge, Massachusetts: Channel engineering.

Molecular Embryology of the Mouse

June 6–26

INSTRUCTORS	**K. Hadjantonakis,** Memorial Sloan-Kettering Cancer Institute, New York **J. Rivera-Perez,** University of Massachusetts Medical School, Worcester
CO-INSTRUCTORS	**R. Johnson,** M.D. Anderson Cancer Center, Houston, Texas **X. Sun,** University of Wisconsin, Madison
ASSOCIATE CO-INSTRUCTOR	**D. Escalante-Alcalde,** Institute of Cellular Physiology-UNAM, Mexico
ASSISTANTS	**Y. Furuta**, M.D. Anderson Cancer Center, Houston, Texas **E. Hines,** University of Wisconsin, Madison **M. Kang**, Memorial Sloan-Kettering Cancer Institute, New York **J. Mager**, University of Massachusetts, Amherst **S. Nowotschin**, Memorial Sloan-Kettering Cancer Institute, New York

This intensive laboratory and lecture course was designed for biologists interested in using mouse models to study mammalian development, stem cells, and cancer. Lectures provided the conceptual basis for contemporary research in embryogenesis, organogenesis, embryonic, adult, and induced pluripotent stem cells, and cancer biology. Laboratory practicals provided extensive hands-on introduction to engineering of mouse models, phenotyping, and stem cell technologies. Experimental techniques included isolation, in vitro culture and manipulation of pre- and post-implantation embryos, embryo transfer, genetic manipulation of embryonic stem cells, production of chimeras by embryo aggregation and by embryonic stem cell injection, and transgenesis

by pronuclear microinjection. The course also introduced the generation and differentiation of embryonic stem cells as well as induced pluripotent stem cells, isolation of mouse embryonic fibroblasts, focus formation assay, teratoma formation assay, time-lapse microscopy of early embryos, and organ cultures. In addition, this year's practicals featured increased emphasis on phenotypic analysis of mutants, including vibratome and cryosectioning, in situ hybridization, immunostaining, skeletal preparation, tissue recombination, and confocal and ultrasound imaging.

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Benitez Hernandez, J., Ph.D., Ludwig Institute for Cancer Research, La Jolla, California
Fetting, J., Ph.D., Maine Medical Center Research Institute, Scarborough
Grindheim, J., B.S., University of Pennsylvania, Philadelphia
Hansen, M., B.S., Hagedorn Research Institute, Gentofte, Denmark
Laugesen, A., M.Sc., University of Copenhagen, Denmark
Lay, K.W.J., B.Sc., The Rockefeller University, New York
Ohlstein, B., Ph.D., Columbia University Medical Center, New York
Olsen, R., Ph.D., St. Jude Children's Research Hospital, Memphis, Tennessee
Savov, V., M.Sc., Uppsala University, Sweden
Siegrist, S., Ph.D., University of California, Berkeley
Tiklova, K., Ph.D., Ludwig Institute for Cancer Research, Stockholm, Sweden
Vardam, T., Ph.D., Roswell Park Cancer Institute, Buffalo, New York
Zhong, X., Ph.D., The Johns Hopkins University, Baltimore, Maryland
Zhou, W., M.S., University of Texas/M.D. Anderson Cancer Center, Houston

SEMINARS

Capel, B., Duke University Medical Center, Durham, North Carolina: Germ cells and gonadal development.
Duncan, S., Medical College of Wisconsin, Milwaukee: iPS cells and applications.
Egeblad, M., Cold Spring Harbor Laboratory: Live imaging in mouse models of cancer.
Flores, E., University of Texas/MD Anderson Cancer Center, Houston: DNA repair, mouse models, and cancer.
Hadjantonakis, K., Memorial Sloan-Kettering Cancer Center, New York: Mouse embryo imaging: Confocal approaches.
Johnson, R., M.D. Anderson Cancer Center, Houston, Texas: Liver development and hippo signaling.
Li, J., University of Connecticut Health Center, Farmington: Brain development.
Lovell-Badge, R., MRC National Institute for Medicine, London, United Kingdom: Sex determination.
Mager, J., University of Massachusetts, Amherst: Epigenetics.
Martin, J., Baylor College Medicine, Houston, Texas: Heart development.
Mills, A., Cold Spring Harbor Laboratory: Chromosome engineering and cancer.
Pevny, L, University of North Carolina, Chapel Hill: Retinogenesis and eye development.
Rivera, J., University of Massachusetts Medical School, Worcester: Early postimplantation development: Morphogenesis and cell lineage.
Shen, M., Columbia University Medical Center, New York: Prostate development, stem cells, and cancer.
Solter, D., Institute of Medical Biology, Singapore: Preimplantation development.
Soriano, P., Mount Sinai School of Medicine, New York: Gene targeting, gene traps, and recombinases; Pdgf and Eph signaling.
Sun, X., University of Wisconsin, Madison: Lung development.
Tam, P., Children's Medical Research Institute, Australia: Gastrulation and establishment of the germ layers; Neural induction and patterning.
Threadgill, D., North Carolina State University, Raleigh: Historical overview of the mouse as a model system; Quantitative genetic traits and genomics.
Trainor, P, Stowers Institute for Medical Research, Kansas City, Missouri: Craniofacial development and the neural crest.
Wellik, D., University of Michigan Medical Center, Ann Arbor: Kidney development; Hox genes, development, and cancer.

Workshop on Cognitive Aging

June 13–17

INSTRUCTORS **A. Gazzaley,** University of California, San Francisco
S. Small, Columbia University, New York

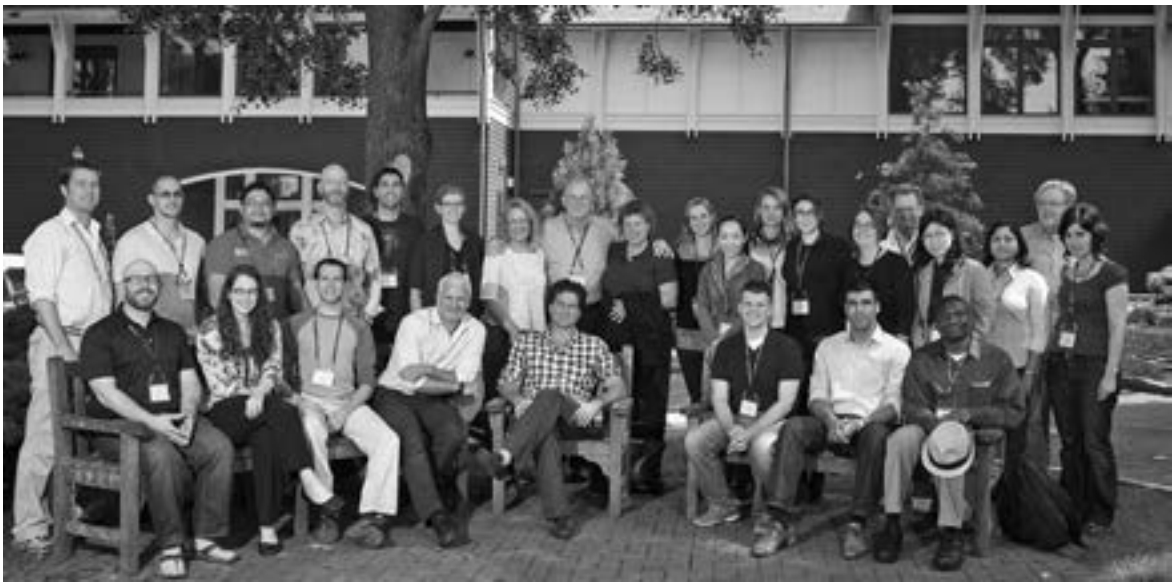
ASSISTANT **U. Khan,** Columbia University Medical Center, New York

This workshop provided students with the most current understanding of the cognitive aging field by bringing together researchers who apply complementary analytical approaches to, and offer diverse biological perspectives on, research in neuroscience and aging. Topics included the hippocampal formation, the frontal lobe, human neuroimaging techniques, animal models, the molecular cell biology of cognitive aging, emerging interventions and therapies, and the bioethics of cognitive enhancement.

This workshop was generously supported with funds provided by the Ellison Medical Foundation, the McKnight Brain Research Foundation®, and the Alzheimer's Drug Discovery Foundation.

PARTICIPANTS

Arum, O., Ph.D., Southern Illinois University School of Medicine, Springfield
Baqri, R., Ph.D., Harvard University, Cambridge, Massachusetts
Bender, A., M.A., Wayne State University, Detroit, Michigan
Berman, D., M.S., Columbia University, New York
Browning, P., Ph.D., Mount Sinai School of Medicine, New York
Das, D., Ph.D., Australian National University, Canberra
Faraco, C., M.S., University of Georgia, Athens
Gordon, B., Ph.D., Washington University, St. Louis, Missouri
Guerreiro, M., Ph.D., Maastricht University, the Netherlands
Harrison, F., B.Sc., Vanderbilt University Medical Center, Nashville, Tennessee



Lo, J., Ph.D., Duke–NUS Graduate Medical School, Singapore
Maass, A., M.Sc., OvG University Magdeburg, Magdeburg, Germany
Monti, J., M.A., University of Illinois, Urbana Champaign
Puccioni, O., M.S., SISSA–International School for Advanced Studies, Italy
Puigdellivol Canadell, M del Mar, M.A., University of Barcelona, Spain
Reaves, Z., B.S., Georgia Institute of Technology, Atlanta
Seaman, K., The Catholic University of America, Washington, D.C.
Villarreal, A., B.S., INDICASAT-AIP, Panama
Xuan, Z., B.S., Mount Sinai School of Medicine, New York

SEMINARS

Arnsten, A., Yale University, New Haven, Connecticut: Molecular mechanism of age-related frontal lobe dysfunction.
Barnes, C., University of Arizona, Tucson: The aging hippocampal formation in animal models.
Brickman, A., Columbia University, New York: The neuroscience of aging.
Buckner, R., Harvard University, Cambridge, Massachusetts: Imaging the aging brain.
Buxton, R., University of California, San Diego, La Jolla: Controversies in fMRI.
Cabeza, R., Duke University, Durham, North Carolina: The cognitive neuroscience of aging.
Gazzaley, A., University of California, San Francisco: The functions of the frontal lobe; Imaging the aging frontal lobes.
Hen, R., Columbia University, New York: Neurogenesis and aging.
Morrison, J., Mount Sinai School of Medicine, New York: Keynote lecture.
Rapp, P., National Institute on Aging/NIH, Baltimore, Maryland: Controversies in animal models.
Small, S., Columbia University, New York; Stark, C., University of California, Irvine: Imaging the aging hippocampal formation II; Isolating molecular mechanisms of hippocampal dysfunction.

Computational Neuroscience: Vision

June 20–July 3

INSTRUCTORS **G. Boynton,** University of Washington, Seattle
G. Horwitz, University of Washington, Seattle
S. Treue, German Primate Center, Goettingen, Germany

ASSISTANTS **J. Freeman**, New York University, New York
L. Jepson, Salk Institute for Biological Studies, La Jolla, California

TECHNICIAN **R. Dotson,** New York University, New York

Computational approaches to neuroscience will produce important advances in our understanding of neural processing. Prominent success will come in areas where strong inputs from neurobiological, behavioral, and computational investigation can interact. The theme of the course was that an understanding of the computational problems, the constraints on solutions to these problems, and the range of possible solutions can help guide research in neuroscience. Through a combination of lectures and hands-on experience with MATLAB-based computer tutorials and projects, this intensive course examined visual information processing from the retina to higher cortical areas, spatial pattern analysis, motion analysis, neuronal coding, and decoding, attention, and decision making.

PARTICIPANTS

Azevedo, F.A., B.S., Max-Planck Institute for Biological Cybernetics, Tuebingen, Germany
Benson, N., Ph.D., University of Pennsylvania, Philadelphia
Cutrone, E., B.S., New York University, New York
Glasser, D., B.S., University of Rochester, New York
Goddard, E., Ph.D., The University of Sydney, Australia

Isola, P., B.S., Massachusetts Institute of Technology, Cambridge
Kohler, P., B.S., Dartmouth College, Hanover, New Hampshire
Meister, M., B.S., University of Texas, Austin
Mineault, P., B.S., McGill University, Montreal, Canada
Oleskiw, T., B.S., University of Washington, Seattle
Pagan, M., B.S., University of Pennsylvania, Philadelphia
Popovic, M., B.S., Brandeis University, Waltham, Massachusetts
Purcell, B., B.S., Vanderbilt University, Nashville, Tennessee
Radonjic, A., Ph.D., University of Pennsylvania, Philadelphia
Schwedhelm, P., B.S., German Primate Center, Goettingen, Germany
So, N., B.S., University of Washington, Seattle
Sprague, T., B.S., University of California, San Diego, La Jolla
Urgen, B., B.S., University of California, San Diego, La Jolla
Ziemba, C., B.S., New York University, New York
Zuiderbaan, W., B.S., Utrecht University, Utrecht, the Netherlands

SEMINARS

Boynton, G., University of Washington, Seattle: fMRI in the LGN and V1; Psychophysics and signal detection theory.
Boynton, G. and Horwitz, G., University of Washington, Seattle; Treue, S., German Primate Center: Welcome.
Brainard, D., University of Pennsylvania, Philadelphia: Color vision.
Carandini, M., University College London, United Kingdom: V1.
Carrasco, M., New York University, New York: Psychophysics of attention.
Chichilnisky, E.J., The Salk Institute, La Jolla, California: Retina.
Cohen, M., Harvard Medical School, Boston, Massachusetts: Attention and population coding.
DeAngelis, G., University of Rochester, New York: MT: Motion and depth.
Fine, I., University of Washington, Seattle: Retinal prosthesis.
Geisler, W., University of Texas, Austin: Filter estimation from perceptual data.
Heeger, D., New York University, New York: Contrast normalization.
Horwitz, G., University of Washington, Seattle: White noise analysis.
Kohn, A., Albert Einstein College of Medicine, Bronx, New York: Population coding: Dorsal stream.
Kriegeskorte, N., Medical Research Council, Cambridge, United Kingdom: fMRI of higher-level vision.
Maunsell, J., Harvard Medical School, Boston, Massachusetts: Physiology of attention II: Normalization, correlation, and oscillation.
Moore, T., Stanford University, California: Frontal influences on sensory information processing.
Movshon, A., New York University, New York: What vision (and the course) is all about.
Rust, N., University of Pennsylvania, Philadelphia: Population coding: Ventral stream.
Shadlen, M., University of Washington, Seattle: Decision making.
Simoncelli, E., New York University, New York: Linear systems.
Simoncelli, E., New York University, New York: Optimal encoding and decoding.
Treue, S., German Primate Center, Goettingen, Germany: Physiology of attention I: Single cells.

Statistical Analysis of Genome Scale Data

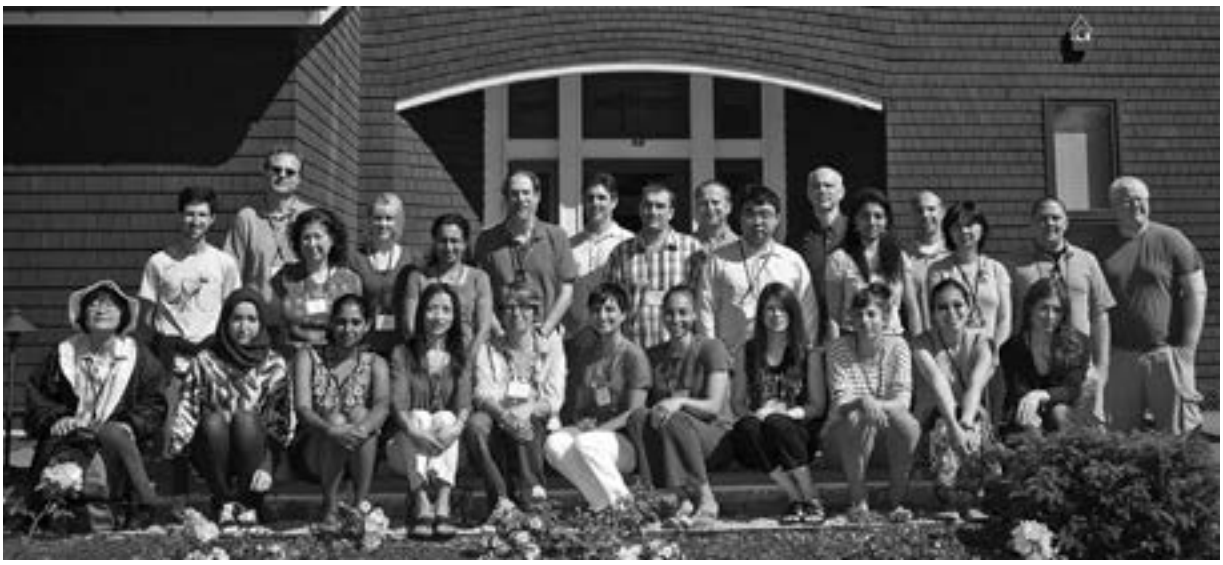
June 22–July 3

INSTRUCTORS
N. Altman, Penn State University, University Park
H. Bussemaker, Columbia University, New York
O. Elemento, Weill Cornell Medical College, New York
S. Horvath, University of California, Los Angeles
M. Reimers, VCU School of Medicine, Richmond, Virginia

ASSISTANTS
M. Behnke, VCU School of Medicine, Richmond, Virginia
R. Braun, Northwestern University, Chicago, Illinois
E. Giannopoulou, Weill Cornell Medical College, New York
P. Gomez-Alcala, Columbia University, New York
A. Lazarovici, Columbia University, New York
P. Manser, VCU School of Medicine, Richmond, Virginia
T. Riley, Columbia University, New York
A. Wolen, VCU School of Medicine, Richmond, Virginia

High-throughput genomics assays have become pervasive in modern biological research. To properly interpret these data, experimental and computational biologists need to have a firm grasp of statistical methodology. This course was designed to build competence in quantitative methods for the analysis of high-throughput molecular biology data. Detailed lectures and presentations by guest speakers in the morning and evening were combined with hands-on computer tutorials in the afternoon. The methods covered in the lectures were applied to public high-throughput data sets, primarily human, and mouse and yeast data. Students were expected to have a basic familiarity with the R programming language at the start of the course.

The following topics were covered: review of R and introduction to the Bioconductor; review of statistical methods for genomics; microarray technologies; high-throughput sequencing



technologies; basic analysis (quality control, normalization); analysis using predefined gene sets; *cis*-regulatory sequence analysis; modeling of transcriptional networks; DNA methylation assays and DNase I footprinting; expression profiling by RNA-Seq; analysis of ChIP-chip and ChIP-Seq data; integration of multiple data types; and expression QTL analysis.

This course was supported with funds provided by the National Institute of General Medical Sciences.

PARTICIPANTS

Akagi, K., Ph.D., Ohio State University, Columbus
Alshafai, K., M.Sc., Qatar Foundation, Doha
Bowman, R., B.A., Memorial Sloan-Kettering Cancer Center, New York
Garrett-Bakelman, F., Ph.D., Weill Cornell Medical College, New York
Gibney, P., Ph.D., Princeton University, New Jersey
Golenberg, E., Ph.D., Wayne State University, Detroit, Michigan
Kantor, D., Ph.D., Children's Hospital Boston, Massachusetts
Kruczyk, M., M.Sc., Uppsala University, Sweden
Krug, L., Ph.D., Stony Brook University, New York
Liscovitch, N., B.Sc., Bar IIan University, Ramat Gan, Israel
Liu, Y., Ph.D., Lovelace Respiratory Research Institute, Albuquerque, New Mexico
Miranda, T., Ph.D., National Institutes of Health, Bethesda, Maryland
Mukamel, E., Ph.D., University of California, San Diego, La Jolla
Nair, S., Ph.D., University of Texas, Brownsville
Nikulova, A., M.S., Lomonosov Moscow State University, Russia
Pai, S., Ph.D., Centre for Addiction and Mental Health, Toronto, Canada
Qin, J., Ph.D., Duke University, Durham, North Carolina
Rotenberg, D., Ph.D., Kansas State University, Manhattan, Kansas
Schetter, A., Ph.D., Center for Cancer Research, NCI, Bethesda, Maryland
Shepherd, J., B.S., Baylor College of Medicine, Houston, Texas
Singh, N., B.A., Massachusetts General Hospital, Charlestown
Sun, X., Ph.D., Columbia University, New York
Vaka, D., M.S., Stanford University, California
Yang, W., Ph.D., Johns Hopkins University School of Medicine, Baltimore, Maryland

SEMINARS

Elemento, O., Weill Cornell Medical College, New York: Modeling the impact of transcription factor binding, chromatin marks, and DNA looping on gene expression.
Futcher, B., Stony Brook University, New York: Lessons learned from a successful microarray experiment.
Hughes, T., University of Toronto, Canada: Exploring the sequence specificity of eukaryotic DNA and RNA binding proteins.
Ingolia, N., Carnegie Institution, Baltimore, Maryland: Genome-wide profiling of translation initiation and protein synthesis.
Leslie, C., Memorial Sloan-Kettering Cancer Center, New York: New insights into regulatory transcriptomics with next-generation sequencing.
Mardis, E., Washington University School of Medicine, St. Louis, Missouri: New-generation sequencing and analysis of the cancer genome.
Reimers, M., VCU School of Medicine, Richmond, Virginia: The BrainSpan project.
Stamatoyannopoulos, J., University of Washington, Seattle: Mapping and analysis of human regulatory DNA.

Frontiers and Techniques in Plant Science

June 29–July 19

INSTRUCTORS
S. Harmer, University of California, Davis
R. Last, Michigan State University, E. Lansing
J. Maloof, University of California, Davis

ASSISTANTS
S. Bush, University of California, Davis
J. Corwin, University of California, Davis
C. Peng, Michigan State University, E. Lansing
A. Schilmiller, Michigan State University, E. Lansing

This course provided an intensive overview of topics in plant physiology, biochemistry, and development, focusing on genomic, analytical, computational, and other high-throughput approaches to understanding plant biology. It emphasized recent results from model organisms including *Arabidopsis*, maize, and tomato, as well as a variety of other plants, and provided an introduction to current methods used in basic and applied plant biology. It is designed for scientists with some experience in molecular techniques or in plant biology who wish to work with plants using the latest molecular, genomic, and computational technologies. The course consisted of a vigorous lecture series, a hands-on laboratory, and informal discussions. The instructors and a stellar group of invited speakers, acknowledged leaders in their fields, presented up-to-the-moment research on a wide range of topics in plant research.

These seminars included plant evolution, morphology, and anatomy; various topics in plant development (including development of meristems, gametophytes, and roots); light perception and photomorphogenesis; cell wall biosynthesis and biofuels; function and perception of hormones; small RNAs; biotic and abiotic interactions; and applications addressing current agronomic problems. Lectures describing bioinformatics tools available to the plant community and the resources

provided by plant genome projects were also included. Speakers provided expert overviews of their fields, followed by in-depth discussions of their own work. The laboratory sessions provided exposure to cutting-edge techniques currently used in plant research. These included studies of plant development and genome evolution, transient gene expression, applications of fluorescent proteins, automated phenotyping, analysis of polysomal mRNA, analysis of global gene expression data (microarray and short-read sequencing), construction of genetic networks, and metabolome analysis. Students were introduced to leading computational environments and programs including R, Bioconductor, and ImageJ. The course also included several short workshops on important themes in plant research.

This course was supported with funds provided by the National Science Foundation.

PARTICIPANTS

Bendix, C., B.A., University of California, Berkeley
De Souza, A., Ph.D., Centro de Citricultura Sylvio Moreira–IAC, Brazil
Franke, K., Ph.D., University of Delaware, Newark
Jones, V., B.A., University of Oxford, United Kingdom
Kapitzky, L., Ph.D., Mendel Biotechnology, Inc., Hayward, California
Lee, S., Ph.D., University of Massachusetts, Amherst
Lor, V., Ph.D., University of Minnesota, Twin Cities
Moss, B., Ph.D., University of Washington, Seattle
Nielsen, B., Ph.D., Brigham Young University, Provo, Utah
Novikova, P., B.S., Gregor Mendel Institute of Molecular Plant Biology, Vienna, Austria
Pabon Mora, N., B.S., City University of New York, New York
Ires, I., M.S., New York University, New York
Rattner, R., B.S., University of California, Riverside
Smith, L., Ph.D., Central Baptist College, Conway, Arizona
Urton, J., Ph.D., University of Washington, Seattle
Wilson, M., B.A., University of Minnesota, Twin Cities

SEMINARS

Alonso, J., Salk Institute for Biological Studies, La Jolla, California: Functional genomics and recombineering.
Brady, S., University of California, Davis: Root development.
Dinesh-Kumar, S., University of California, Davis: Plant immune responses.
Hall, A., University of Liverpool, United Kingdom: Modern approaches to crop genetics.
Heard, J., Monsanto Company, Chesterfield, Missouri: Plant yield: An industry perspective.
Poethig, S., University of Pennsylvania, Philadelphia: Introduction to anatomy.
Harmer, S., University of California, Davis: Circadian rhythms.
Kellogg, E., University of Missouri, St. Louis: Molecular systematics.
McSteen, P., University of Missouri, Columbia: Development of the apical meristem.
Nemhauser, J., University of Washington, Seattle: Interactions between hormone signaling networks.
Niyogi, K., University of California, Berkeley: Photosynthesis.
Noel, J., HHMI/Salk Institute for Biological Sciences, La Jolla, California: Structural approaches to understanding metabolism.
Pappin, D., Cold Spring Harbor Laboratory: Mass spectrometry: Theory and practice.
Oldroyd, G., John Innes Centre, Norwich, United Kingdom: Plant/rhizobial interactions.
Provart, N., University of Toronto, Canada: Genomic approaches to plant biology.
Shiu, S.-H., Michigan State University, East Lansing: Evolutionary genomics.
Spalding, E., University of Wisconsin, Madison: Plant growth.
Timmermans, M., Cold Spring Harbor Laboratory: RNA interface pathways.
Walling, L., University of California, Riverside: Plant/pest interactions.

Drosophila Neurobiology: Genes, Circuits, and Behavior

July 2–19

INSTRUCTORS **G. Macleod,** University of Texas Health Science Center, San Antonio
K. O'Connor-Giles, University of Wisconsin, Madison
A. Rothenfluh, University of Texas Southwestern, Dallas

ASSISTANTS **D. Gonzalez,** University of Texas Southwestern, Dallas
S. Gratz, University of Wisconsin, Madison
A. Rossano, University of Texas Health Science Center, San Antonio
M. Zwart, University of Cambridge, United Kingdom

This laboratory/lecture course was intended for researchers at all levels from beginning graduate students through established primary investigators who wanted to use *Drosophila* as an experimental system for nervous system investigation. The two and a half-week course was designed to introduce students to a wide variety of topics and techniques, including the latest approaches to study nervous system development, connectivity, and activity. Daily research seminars presented comprehensive overviews of specific subfields of nervous system function or focused on specific techniques and approaches to study fly neurobiology. Expert guest lecturers discussed their findings and approaches and brought along their own assays and techniques for students to learn in the laboratory part of the course. The hands-on portion of the course was centered around student-led projects, where gene mutants were discovered and analyzed throughout the course utilizing the different morphological and physiological measurements and behavioral paradigms at hand. These included electrophysiological and in vivo calcium recordings, anatomical examination and circuit mapping, and numerous quantitative behavioral measures. Collectively, the course provided a comprehensive and practical introduction to modern experimental methods for studying the *Drosophila* nervous system.

This course was supported with funds provided by the National Institute of Drug Abuse, the National Institute of Neurological Disorders and Stroke, and the National Science Foundation.

PARTICIPANTS

Dunlap, A., Ph.D., University of Arizona, Tucson
Gala, U., B.S., Baylor College of Medicine, Waco, Texas
Jordanova, A., Ph.D., VIB and University of Antwerp, Belgium
Krench, M., B.S., Massachusetts Institute of Technology, Cambridge
Li, J., B.S., University of Michigan, Ann Arbor
MacNamee, S., B.A., University of Arizona, Tucson
Quiroz, E., Ph.D., Universidad Central del Caribe, Puerto Rico
Robinson, J., Ph.D., University of California, San Diego
Antonio Sanchez-Alcaniz, J.A., M.S., University of Lausanne, Switzerland
Schneider-Mizell, C., Ph.D., ETH/University of Zurich, Switzerland
Schwarz, O., M.Sc., Friedrich Miescher Institute for Biomedical Research, Switzerland
Warren, T., Ph.D., University of California, San Francisco

SEMINARS

Allada, R., Northwestern University, Evanston, Illinois: Circadian rhythms.
Daniels, R., University of Wisconsin, Madison: NMJ physiology.
Dubnau, J., Cold Spring Harbor Laboratory: Learning and memory.
Kravitz, E., Harvard Medical School, Boston, Massachusetts: Aggression.
Lee, C.-H., National Institute of Child Health and Human Development/NIH, Bethesda, Maryland: Circuit development in the visual system.
Levitan, E., University of Pittsburgh, Pennsylvania: Imaging; Imaging neuropeptide release.
MacLeod, G., University of Texas Health Science Center, San Antonio pH1uorin imaging; Ca^{2+} imaging.
McKellar, C., Janelia Farm Research Campus, Sterling, Virginia: Mapping the circuits underlying motor behavior.
O'Connor-Giles, K., University of Wisconsin, Madison: NMJ development/synapse formation.
Pulver, S., Janelia Farms Research Campus, Sterling, Virginia: CNS physiology.
Reist, N., Colorado State University, Fort Collins: Mechanisms of NT release.
Rolls, M., Pennsylvania State, University Park: Cell biology of the neuron.
Rothenfluh, A., University of Texas Southwestern, Dallas: Ethanol screen.
Rothenfluh, A., University of Texas Southwestern, Dallas; MacLeod, G., University of Texas Health Science Center, San Antonio; O'Connor-Giles, K., University of Wisconsin, Madison; Lee, C.-H., National Institute of Child Health and Human Development/NIH, Bethesda, Maryland: Genetic screens.
Ruta, V., The Rockefeller University, New York: Circuit mapping.
Simpson, J., HHMI/Janelia Farm Research Campus, Sterling, Virginia: Neuroanatomy; Circuit mapping and manipulation.
Turner, G., Cold Spring Harbor Laboratory: Whole-cell patch clamp.
Yoshihara, M., University of Massachusetts, Worcester: *Drosophila* feeding circuit.

Advanced Techniques in Molecular Neuroscience

July 3–19

INSTRUCTORS
C. Lai, Indiana University, Bloomington
R. Lansford, California Institute of Technology, Pasadena
J. Loturco, University of Connecticut, Storrs
B. Stevens, Children's Hospital Boston/Harvard Medical School, Boston, Massachusetts

ASSOCIATE INSTRUCTORS
L. Fenno, Stanford University, California
K. Haas, University of British Columbia, Canada

TEACHING ASSISTANTS
B. Benazeraf, Institut de Genetique Biologie Moleculaire et Cellulaire, France
A. Brosius Lutz, Stanford University, California
A. Che, University of Connecticut, Storrs
F. Chen, University of Connecticut, Storrs
M. Girgenti, University of Connecticut, Storrs
D. Huss, California Institute of Technology, Pasadena
A. Kaur, Indiana University, Bloomington
B. Maher, Johns Hopkins Medical School, Baltimore, Maryland
E. Munroe, Columbia University, New York
K. Podorski, University of British Columbia, Canada
A. Rosen, Harvard Medical School, Boston, Massachusetts

This laboratory and lecture course was designed to provide neuroscientists at all levels with a conceptual and practical understanding of several of the most advanced techniques in molecular neuroscience. The course curriculum was divided into three sections: an extensive and up-to-date set

of laboratory exercises, daily lectures covering the theoretical and practical aspects of the various methods used in the laboratory, and a series of evening research seminars. The informal and interactive evening lectures were given by leading molecular neuroscientists and served to illustrate the ways in which the various experimental approaches have been used to advance specific areas of neurobiology. In this year's course, the laboratory portion included topics such as an introduction to the design and use of animal virus vectors in neurobiology; the use of small interfering RNAs (siRNAs) for regulating the expression of specific genes in neurons; practical exercises in gene delivery systems including mammalian cell transfection protocols and single-cell electroporation techniques for targeted gene transfer in vivo; an introduction to overall strategies, use and design of 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.

This course was supported with funds provided by the Howard Hughes Medical Institute.

PARTICIPANTS

Baysinger, A., M.Sc., Yale University, New Haven, Connecticut
Cedernaes, J., M.D., Uppsala University, Sweden
Chen, M., B.A., Stanford University, California
Dang, C., B.S., Fred Hutchinson Cancer Research Center, Seattle, Washington
Ekin Demir, I., M.D., Technische Universitat Muenchen, Germany
DeWitt, S., B.S., Emory University, Atlanta, Georgia
Fuchs Lokensgard, R., Ph.D., University of North Carolina, Chapel Hill
Garcia-Olivares, J., Ph.D., University of Pittsburgh, Pittsburgh, Pennsylvania
Herrmann, U., M.D., University Hospital Zurich, Switzerland
Hormigo, A., Ph.D., University of Rochester Medical Center, New York
Alexandre Kihara, A., Ph.D., Universidade Federal do ABC, Santo Andre, Brazil
Moore, A., B.S., Brandeis University, Waltham, Massachusetts
Munch, A., M.A., University of Copenhagen, Denmark
Nagy, C., B.Sc., Douglas Mental Health University Institute, Verdun, Canada
Tabor, K., Ph.D., University of Washington, Seattle
Zhang, C., Ph.D., The Rockefeller University, New York

SEMINARS

Barres, B., Stanford University School of Medicine, California: What do astrocytes do?
Darnell, R.B., The Rockefeller University, New York: Maps of the RNA world.
Eberwine, J., University of Pennsylvania Perelman School of Medicine, Philadelphia: Functional consequences of single-cell transcriptome variability.
Huang, Z.J., Cold Spring Harbor Laboratory: Genetic dissection of GABAergic circuits in the neocortex: Chandeliers light up the path.
Lichtman, J., Harvard University, Cambridge, Massachusetts: Using connectomics to reveal circuit motifs.
Schnitzer, M., Stanford University, California: Visualizing the dynamics of the neuronal orchestra in awake behaving animals.
Stevens, B., Children's Hospital Boston/Harvard Medical School, Boston, Massachusetts: Pruning CNS synapses: An active role for glia and the complement cascade.
Trainor, P., Stowers Institute for Medical Research, Kansas City, Missouri: Distinguished Alumni Lecture: Studies of head development while wearing tight speedos.

Proteomics

July 7–July 22

INSTRUCTORS
M. Bereman, University of Washington, Seattle
M. Cilia, U.S. Department of Agriculture/Agriculture Research Service, Ithaca, New York
I. Cristea, Princeton University, New Jersey
C. Dufresne, Thermo Fisher Scientific, West Palm Beach, Florida
D. Pappin, Cold Spring Harbor Laboratory

ASSISTANTS
S. DeBlasio, U.S. Department of Agriculture/Agriculture Research Service, Ithaca, New York
B. Diner, Princeton University, New Jersey
T. Greco, Princeton University, New Jersey
T. Li, Princeton University, New Jersey
S. Peacock, Cold Spring Harbor Laboratory
K. Rivera, Cold Spring Harbor Laboratory
A. Roberts, Thermo Fisher Scientific, West Palm Beach, New York
E. Rowland, Cornell University, Ithaca, New York
C. Ruse, Cold Spring Harbor Laboratory
J. Wilson, Cold Spring Harbor Laboratory

This intensive laboratory and lecture course focused on cutting-edge proteomic approaches and technologies. Students gained practical experience purifying and identifying protein complexes and posttranslational modifications. In a section focused on quantitative whole-proteome analyses or top-down proteomics, students gained hands-on experience using two-dimensional gel electrophoresis and mass spectrometry analysis. Students used differential in-gel electrophoresis (DIGE) for gel-based protein quantification. Differentially expressed proteins were recognized by statistical methods using advanced gel analysis software and identified using MALDI mass spectrometry.

For shotgun proteomic analysis sections or bottom-up proteomics, students used label-free and covalent isotopic-labeling quantitative approaches to differentially profile changes in protein complexes and whole proteomes. Students were trained in high-sensitivity microcapillary liquid chromatography coupled with nanospray-ESI and tandem mass spectrometry analysis. Students learned both single-dimension and multidimensional separation methods. In a section focused on targeted proteomics, students learned to analyze and process shotgun proteomic data for the development of SRM/MRM assays that accurately identify and quantify targeted proteins. Students designed transitions for selected peptides and performed SRM/MRM mass spectrometry assays. They learned to process and interpret the acquired data to measure changing quantities of targeted proteins in a variety of biological samples. For all sections of the course, a strong emphasis was placed on data analysis. A series of outside lecturers discussed various proteomics topics including imaging by mass spectrometry, de novo sequence analysis, advanced mass spectrometry methods, protein arrays, and functional proteomics. The aim of the course was to provide each student with the fundamental knowledge and hands-on experience necessary for performing and analyzing proteomic experiments. The overall goal was to train students to identify new opportunities and applications for proteomic approaches in their biological research.

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Ayoglu, B., M.Sc., Royal Institute of Technology–KTH, Stockholm, Sweden
Bumgardner, E., B.S., Newport Laboratories, Worthington, Minnesota
Carter, D., B.S., Medical College of Wisconsin, Milwaukee
Chan, A., Ph.D., J. Craig Venter Institute, La Jolla, California
Chaturvedi, R., Ph.D., University of North Texas, Denton
Fleming, R., Ph.D., Durham VAMC and Duke University Medical Center, North Carolina
Garnett, S., B.Sc., University of Cape Town, South Africa
Grzmil, M., Ph.D., Friedrich Miescher Institute, Switzerland
Kaczorowski, C., Ph.D., Medical College of Wisconsin, Milwaukee
James Koerber, J., Ph.D., University of California, San Francisco
Freddyson Martinez, F., B.S., University of Puerto Rico, San Juan
Jason Maynard, J., Ph.D., National Institute of Environmental Health Sciences/NIH, Bethesda, Maryland
Kathleen Noon, K., Ph.D., Medical College of Wisconsin, Milwaukee
Jiqing Peng, J., Ph.D., Iowa State University, Ames
Rooney, M., B.S., Massachusetts Institute of Technology, Cambridge
Semba, R., M.D., Johns Hopkins University School of Medicine, Baltimore, Maryland
Sivagnanam, K., B.Tech., McGill University, Montreal, Canada

SEMINARS

Bereman, M., University of Washington, Seattle: Making columns for nano-scale liquid chromatography; Introduction to mass spectrometry.
Chait, B., The Rockefeller University, New York: Proteomics analysis of protein complexes.
Cilia, M., Boyce Thompson Institute and U.S. Department of Agriculture/Agriculture Research Service, Ithaca, New York: Course introduction; Introduction to 2-D DIGE.
Clauser, K., Broad Institute of Massachusetts Institute of Technology and Harvard University, Cambridge, Massachusetts: De novo interpretation of tandem mass spectra.
Conlon, F., University of North Carolina, Chapel Hill: Directed proteomics approaches in stem cell and developmental biology.
Costello, C., Zaia, J., Boston University, Massachusetts: Deciphering glycosylation: The most intriguing PTM.
Cristea, I., Princeton University, New Jersey: Studying protein complexes using mass spectrometry.
MacCoss, M., University of Washington, Seattle: Targeted proteomics using the Skyline software environment and selection reaction.
Medzihradszky, K., University of California, San Francisco: How to interpret/evaluate MS/MS spectra: Focus on PTM assignments.

Muddiman, D., North Carolina State University, Raleigh: Mass-spectrometry-based absolute protein quantification; MALDI, ESI, and hybrids of the two.
Nesvizhskii, A., University of Michigan, East Lansing: Analysis of AP-MS protein–protein interaction data.
Pappin, D., Cold Spring Harbor Laboratory: Identification of peptides and proteins using mass spectrometry; Peptide and protein quantities using mass spectrometry.
Smolks, M., Cornell University, Ithaca, New York: Studying cell signaling networks with quantitative mass spectrometry.
Wolf-Yadlin, A., University of Washington, Seattle: Introduction of phosphoproteomics.

Workshop on Biology of Social Cognition

July 11–17

INSTRUCTORS **J. Mitchell,** Harvard University, Cambridge, Massachusetts
D. Skuse, University College London, United Kingdom

ASSISTANT **C. Startin,** University College London, United Kingdom

The past few years have seen remarkable advances in our knowledge of the genetic, molecular, and neural factors that contribute to social behavior. At the same time, sophisticated analytical and theoretical approaches have helped to make sense of the data. This week-long workshop aimed to provide a comprehensive overview of these topics. Although the emphasis was on social cognition in humans, there were also study days dedicated to state-of-the-art presentations on comparative approaches and evolutionary models. Finally, all themes were related to the clinical consequences of dysfunctional social cognition, and the role of translational research. The course included introductory seminars on key themes, offered every morning. There were afternoons devoted largely to practical sessions that provided hands-on experiments in consultation with seminar leaders, rounded out by after-supper keynote lectures by leading scientists that reflected cutting-edge and future views related to our theme for that day. Ample breaks allowed time not only for informal interactions between lecturers and students, but also for reading, swimming, tennis, and other outdoor activities available at the Banbury Center.

PARTICIPANTS

Aparicio Betancourt, M., B.S, University of Illinois, Urbana-Champaign

Bastiaansen, J., Ph.D., University Medical Center Groningen, the Netherlands

Elision, J., Ph.D., California Institute of Technology, Pasadena
Fisher, G., B.S., California Institute of Technology, Pasadena
Goddings, A.-L., B.S., University College London, United Kingdom
Ha, C., B.S., University of Houston, Texas
Hillebrandt, H., B.S., University College London, United Kingdom
Kim, J., B.S., Dartmouth College, Hanover, New Hampshire
Kim, Y., Ph.D., Cold Spring Harbor Laboratory
Lapate, R., B.S., University of Wisconsin, Madison
Mattek, A., B.S., Dartmouth College, Hanover, New Hampshire
Mills, K., B.S., National Institutes of Health, Bethesda, Maryland
Palmer, A., B.S., Dartmouth College, Hanover, New Hampshire
Raio, C., B.S., New York University, New York
Sullivan, N., B.S., California Institute of Technology, Pasadena, California
Tritt, S., B.S., University of Toronto, Toronto, CANADA
Venta, A., B.S., University of Houston, Houston, TX
Von Der H., Ph.D., Temple University, Conshohocken, Pennsylvania
Wolf, L., B.S., University College London, United Kingdom
Yoon, J., B.S., Stanford University, California
Youssef, F., B.S., University of the West Indies, St. Augustine, Trinidad and Tobago

SEMINARS

Binder, E., Emory University, Atlanta, Georgia: Biology of stress, epigenetics, and depression.
Blakemore, S.-J., University College London, United Kingdom: Social cognition in adolescence.
Brennand, K., Mount Sinai School of Medicine, New York: Modeling schizophrenia using human-induced pluripotent stem cells.
Buckholtz, J., Princeton University, New Jersey: Neuroscience of self-control.
Cushman, F., Brown University, Providence, Rhode Island: Moral development.
de Waal, F., Emory University, Atlanta, Georgia: The age of empathy.
Giedd, J., National Institute of Mental Health/NIH, Bethesda, Maryland: Neurodevelopment of the brain.
Haig, D., Harvard University, Cambridge, Massachusetts: Genomic imprinting and the divided brain.
Mitchell, J., Harvard University, Cambridge, Massachusetts: Investigating theory of mind; Welcome and introduction to the course.
Munafo, M., University of Bristol, United Kingdom: Gene–environment interactions.
Murray, R., King's College London, United Kingdom: Social cognition in major mental illness.
Navarro, A., Institute of Evolutionary Biology, University of Pompeu Fabrue, Barcelona, Spain: Introduction to genetics.
O'Doherty, J., California Institute of Technology, Pasadena: Decision making.
Santos, L., Yale University, New Haven, Connecticut: Economic models of comparative cognition.
Schultz, R., Children's Hospital of Philadelphia, Pennsylvania: Autism and the brain.
Shaw, P., National Institute of Mental Health/NIH, Bethesda, Maryland: Functional neuroimaging of social cognition.
Simmons, J., National Institute of Mental Health/NIH, Bethesda, Maryland: NIMH and social neuroscience.
Skuse, D., University College London, United Kingdom: X-linked genes and social cognition.
Szekely, T., Bath University, United Kingdom: Cross-species studies of cooperative behavior.
Whalen, P., Dartmouth College, Hanover, New Hampshire: Anxiety and the amygdala.
Young, L., Emory University, Atlanta, Georgia: Neuropeptides and social bonding.
Zaki, J., Stanford University, California: Empathic accuracy, social influences, and altruism.

Brain Tumors

July 19–25

INSTRUCTORS **Gutmann, D.**, Washington University School of Medicine, St. Louis, Missouri
Holland, E., Memorial Sloan-Kettering Cancer Center, New York
Majumder, S., University of Texas M.D. Anderson Cancer Center, Houston

ASSISTANT **Sathyan, P.**, University of Texas M.D. Anderson Cancer Research Center, Houston

This 1-week discussion course provided a clinical overview of brain tumors and emphasized molecular mechanisms involved in the growth and development of brain tumors, with special emphasis on neural differentiation, signaling mechanisms, DNA replication, chromatin modulation, stem cells, mouse models, genomics, imaging techniques, genetically modified mouse techniques, nanotechnology, mechanism-based therapeutic strategies, and biobanks and ethical concerns. Attendees were able to interact with senior investigators on a one-to-one basis in an informal environment. All nonfaculty students were provided with a generous stipend depending on stated need. Applications were invited from medical and graduate students, postdoctoral fellows, faculty, clinicians, and scientists.

PARTICIPANTS

Blacher, E., Ph.D., Tel-Aviv University, Israel
Colquhoun, A., B.Sc., University of Sao Paulo, Brazil
Coutinho, F., B.S., University of Toronto/Hospital for Sick Children, Canada
Garancher, A., Ph.D., Curie Institute, Orsay Cedex, France
Hamerlik, P., M.Sc., Danish Cancer Society Research Center, Copenhagen, Denmark
Khuong Quang, D.A., Ph.D., McGill University, Montreal, Canada

Kim, Y., Ph.D., Cleveland Clinic Lerner Research Institute, Ohio
Kimmell, K., B.A., University of Rochester Medical Center, New York
Lin, C.-T., M.D., University of Vermont, Burlington
Liu, X., B.S., McGill University, Longueuil, Canada
Maherally, Z., Ph.D., University of Portsmouth, United Kingdom
Marisetty, A., Ph.D., University of Texas/M.D. Anderson Cancer Center, Houston
Matlaf, L., Ph.D., California Pacific Medical Center, San Francisco
Pattwell, S., Ph.D., Weill Cornell Medical College, New York
Rakopoulos, P., Ph.D., University of Toronto, Canada
Semenkow, S., Ph.D., Johns Hopkins Medical Institute, Baltimore, Maryland
Sin, W.C., B.Sc., University of British Columbia, Vancouver, Canada
Srinivasan, V., M.D., University of Rochester, New York
Stringer, B., Ph.D., Queensland Institute of Medical Research, Brisbane, Australia
Sturm, D., M.D., German Cancer Research Center, Heidelberg, Germany
Tang, Y., Ph.D., Stanford University, California
Veo, B., Ph.D., University of Texas/M.D. Anderson Cancer Center, Houston
Xie, Q., Ph.D., Van Andel Research Institute, Grand Rapids, Michigan

SEMINARS

Baker, S., St. Jude Children's Research Hospital, Memphis, Tennessee: Pediatric brain tumors.
Berens, M., Translational Genomics Research Institute, Phoenix, Arizona: Right drug…right glioma patient.
Bondy, M., Baylor College of Medicine, Houston, Texas: Molecular and genetic epidemiology of brain tumors: State of the science.
Brennan, C., Memorial Sloan-Kettering Cancer Center, New York: Genomic analysis of glioblastoma.
Eberhart, C., Johns Hopkins University School of Medicine, Baltimore, Maryland: Notch signaling in brain tumors.
Enikolopov, G., Cold Spring Harbor Laboratory.
Fuller, G., University of Texas/M.D. Anderson Cancer Center, Houston: Clinical aspects of brain tumors: Neuropathology.
Furnari, F., University of California, San Diego: EGF receptors in glioma biology: Therapeutic targets and multifaceted drivers of pathogenicity.
Gladson, C., Cleveland Clinic, Ohio: Regulation of angiogenesis by TNF-α/TNFR1 in glioblastoma.
Gutmann, D., Washington University School of Medicine, St. Louis, Missouri: Using neurofibromatosis-1 to understand pediatric brain tumors.
Holland, E., Chan, T., Memorial Sloan-Kettering Cancer Center, New York: Epigenomic drivers of glioma oncogenesis.
James, D., University of California, San Francisco: Engraftment approaches and models for the study of brain tumors.
Kenney, A.M., Harvard Medical School/Dana Farber Cancer Institute, Boston, Massachusetts: Signaling pathway interactions in the developing brain and medulloblastoma.
Kornblum, H., University of California, Los Angeles: If a brain tumor stem cell falls in the woods, will anybody hear it?
Majumder, S., University of Texas/M.D. Anderson Cancer Center, Houston; Rich, J., Cleveland Clinic, Ohio
Mietz, J., National Cancer Institute/NIH, Bethesda, Maryland: Funding opportunities at the National Cancer Institute.
Pieper, R., University of California, San Francisco: The multiple roles of ubiquitination in the development and treatment of brain cancer.
Reilly, K., National Cancer Institute/NIH, Bethesda, Maryland: Cross-species comparisons to better understand epistatic relationships in brain tumor susceptibility.
Roussel, M., St. Jude Children's Research Hospital, Memphis, Tennessee: Molecular networks and cell of origin in pediatric medulloblastoma.
Sawaya, R., University of Texas/M.D. Anderson Cancer Center, Houston: Clinical aspects of brain tumors and clinical trials.
Snyder, E., Sanford-Burnham Medical Research Institute, La Jolla, California: The role of neural stem cell biology in the genesis and treatment of neoplasms of the CNS.
Verma, I., The Salk Institute, La Jolla, California: Transdifferentiation of gliomas into endothelial cells.
Yu, J., Cedars-Sinai Medical Center, Los Angeles, California: Immunotherapy for brain tumors.

Computational Cell Biology

July 24–August 13

INSTRUCTORS **R. Albert,** Pennsylvania State University, University Park
L. Loew, University of Connecticut Health Center, Farmington
S. Scarlata, Stony Brook University, New York
G. Smith, The College of William and Mary, Williamsburg, Virginia

ASSISTANTS **L. Harris,** University of Pittsburgh School of Medicine, Pennsylvania
M. Huertas, University of Texas Medical School of Houston
O. Marchenko, University of Connecticut Health Center, Farmington
O. Walch, University of Michigan, Ann Arbor
X. Wang, The College of William and Mary, Williamsburg, Virginia

Computational cell biology is the field of study that applies the mathematics of dynamical systems, together with computer simulation techniques, to the study of cellular processes. The field encompasses several topics that have been studied long enough to be well established in their own right, such as calcium signaling, molecular motors and cell motility, the cell cycle, and gene expression during development. In addition to providing a recognizable larger community for topics such as these, this course provided a base for the development of newer areas of inquiry, for example, the dynamics of intracellular second-messenger signaling, of programmed cell death, of mitotic chromosome movements, and of synthetic gene networks. Unlike computational genomics or bioinformatics, computational cell biology is focused on simulation of the molecular machinery (genes-proteins-metabolites) that underlies the physiological behavior (input-output characteristics) of living cells.

This 3-week course incorporated a series of didactic lectures on the mathematics of dynamical systems, computational simulation techniques, cell biology, and molecular biology. Practicing theoreticians and experimentalists rotated in for 1–3-day visits during the course to give lectures and interact with the students. Midway through the course, students selected an area for independent

study, and the focus of the last week of the course was largely on these projects, supplemented by continued visiting lecturers.

This course was supported with funds provided by the National Science Foundation.

PARTICIPANTS

Betizeau, M., B.S., Inserm, Bron, France
Carulli, A., B.S., University of Michigan, Ann Arbor
Chu, L., B.S., Johns Hopkins University, Baltimore, Maryland
Fink, M., B.A., Long Island University, Brookville, New York
Ikeda, F., B.S., College of William and Mary, Williamsburg, Virginia
Liu, Y., Ph.D., University of Duisburg-Essen, Germany
Maitra, A., Ph.D., Stony Brook University, New York
Nilsson, E., M.Sc., Karolinska Institutet, Stockholm, Sweden
Perez, N., M.S., Cinvestav, Ecatepec de Morelos, Mexico
Ropski, A., B.S., University of Illinois, Chicago
Rubio Garcia, A., B.Sc., Technical University of Denmark, Lyngby
Sambarey, A., M.S., Indian Institute of Science, Bangalore, India
Sedzinski, J., Ph.D., University of Texas, Austin
Selem, N., M.S., Langebio, Irapuato Guanajuato, Mexico
Sharifai, N., B.S., University of Miami Miller School of Medicine, Coral Gables, Florida
Stefan, D., B.S., INRIA, Montbonnot, St. Ismier, France
Tikhonov, M., Ph.D., Institute of Gene Biology RAS, Moscow, Russia
Tokarev, A., M.S., Federal Research and Clinical Centre of Pediatrics, Moscow, Russia
Wang, S., B.S., Duke University, Durham, North Carolina
Watanabe, K., B.S., The University of Tokyo, Japan
Waters, R., Ph.D., Montana State University, Bozeman
Weinberg, S., Ph.D., College of William and Mary, Williamsburg, Virginia
Wu, F., B.S., Delft University of Technology, the Netherlands
Zhu, C., B.S., University of Iowa, Iowa City

SEMINARS

Albert, R., Pennsylvania State University, University Park: Discrete dynamic modeling of signal transduction networks.
Cohen D., Asawri, S., The Mathworks, Inc., Natick, Massachusetts: An overview of MATLAB and the SimBiology toolbox.
Cytrynbaum, E., University of British Colombia, Vancouver, Canada: Michaelis Menten enzyme kinetics.
Feinberg, M., Ohio State University, Columbus: Chemical reaction network theory.
Harris, L., University of Pittsburg, Pennsylvania: Rule-based modeling.
Hucka, M., California Institute of Technology, Pasadena: The Systems Biology Markup Language (SBML): Model databases and translation.
Iyengar, R., Mount Sinai School of Medicine, New York: Models of cell signaling.
Keener, J., University of Utah, Salt Lake City: Cardiac models.
Lechleiter, J., University of Texas, San Antonio: Calcium signaling.
Ma'ayan, A., Mount Sinai School of Medicine, New York: Network analysis in systems biology.
Mendes, P., University of Manchester, United Kingdom: Optimization and parameter estimation with COPASI; COPASI and parameter estimation: Part 1; COPASI and parameter estimation: Part 2.
Moraru, I., Loew, L., University of Connecticut, Storrs: Modeling with virtual cell; Virtual cell and ODEs.
Moraru, I., Schaff, J., University of Connecticut, Storrs: Virtual cell, stochastic reaction networks, and stochastic spatial modeling.
Phair, R., Integrative Bioinformatics, Mountain View, California: Molecular cell biology and ODEs; Fluorescent proteins and tracer kinetics.
Sachs, K., Stanford University, Palo Alto, California: Bayesian analysis of signal transduction networks.
Scarlata, S., Stony Brook University, New York: G-protein signaling; Experimental methods.
Schaff, J., Loew, L., University of Connecticut, Storrs: Virtual cell and PDEs; Actin and dendritic nucleation.
Smith, G.D., College of William and Mary, Williamsburg, Virginia: Cellular biophysics and modeling: Part 1; Cellular biophysics and modeling: Part 2; Random walks and diffusion; Stochastic modeling in cell biology: Chemical reaction networks.
Sobie, E., Mount Sinai Medical College, New York: Calcium sparks and local control of excitation contraction coupling in cardiac myocytes.
Terman, D., Ohio State University, Columbus: Dynamical systems: Part 1; Dynamical systems, Part 2.
Tyson, J., Virginia Polytechnic Institute and State University, Blacksburg: Regulation of the cell cycle: Part 1; Regulation of the cell cycle: Part 2.

Eukaryotic Gene Expression

July 24–August 13

INSTRUCTORS **J. Espinosa**, HHMI/University of Colorado, Boulder
W.L. Kraus, University of Texas Southwestern Medical Center, Dallas
A. Shilatifard, Stowers Institute for Medical Research, Kansas City, Missouri
D.Taatjes, University of Colorado, Boulder

ASSISTANTS **B. Gibson,** University of Texas Southwestern Medical Center, Dallas
J. Nichol, McGill University, Montreal, Canada
N. Mohaghegh, Stowers Institute for Medical Research, Kansas City, Missouri
S.-c. Lin, University of Colorado, Boulder

This course was designed for students, postdocs, and principal investigators who have recently ventured into the exciting area of gene regulation. The course focused on state-of-the-art strategies and techniques used in the field. Emphasis was placed on both in vitro and in vivo protein–DNA interactions and on novel methodologies to study gene regulation. Students made nuclear extracts, performed in vitro transcription reactions, and measured RNA levels using primer extension. Characterizations of the DNA-binding properties of site-specific transcription factors were carried out using electrophoretic mobility-shift and DNase I footprinting assays. In addition, students learned techniques for the assembly and analysis of chromatin in vitro, including transcription assays, chromatin footprinting, and chromatin remodeling assays. During the past few years, the gene regulation field has developed in vivo approaches to study gene regulation. Students were exposed to the chromatin immunoprecipitation technique. They also used 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

Ask, K., M.S., BRIC, Copenhagen, Denmark
Bednarova, K., M.S., CEITEC–Masaryk University, Brno, Czech Republic
Benjamin, J., B.S., Johns Hopkins Medical Institute, Baltimore, Maryland
Bukowski, J.-P., B.Sc., University of Copenhagen, Denmark
Cheung, K.L., Ph.D., Mount Sinai Hospital, New York
Christianson, M., Ph.D., Duke University, Durham, North Carolina
Ferguson, M., B.S., National Cancer Institute/NIH, Bethesda, Maryland
Fernando, T., B.S., Weill Cornell Medical College, New York
Frasca, A., B.A., Mount Sinai School of Medicine, New York
Hames, M., B.S., University of Alabama, Huntsville
Pedersen, K., Ph.D., University of Maryland Greenebaum Cancer Center, Baltimore
Rengasamy, M., M.S., Mount Sinai School of Medicine, New York
Ripkens, K., B.S., University Duisburg-Essen, Germany
Siggers, T., Ph.D., Brigham & Woman's Hospital/Harvard Medical School, Boston, Massachusetts
Soltau, W., B.S., Purdue University, West Lafayette, Indiana
Tarradas, A., B.S., University of Girona, Spain

SEMINARS

Adelman, K., National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina: Probing the dynamics and function of promoter-paused RNA polymerase II.
Boyer, L., Massachusetts Institute of Technology, Cambridge: Dynamic and coordinate regulation of gene expression programs in lineage commitment.
Cairns, B., University of Utah, Salt Lake City: Regulations and mechanisms of chromatin remodeling.
Crabtree, G., Stanford University, California: Chromatin regulation: New methods, new concepts, and human disease.
Henikoff, S., Fred Hutchinson Cancer Research Center, Seattle, Washington: Histone variants, nucleosome dynamics, and epigenetic inheritance.
Jones, K., Salk Institute, La Jolla, California: Transcription elongation in cell signaling and disease.
Kingston, R., Harvard University, Boston, Massachusetts: Chromatin-based mechanisms and their regulation by lncRNAs.
Levine, M., University of California, Berkeley: Transcriptional precision in the *Drosophila* embryo and the importance of gene regulation in animal evolution.
Liu, X., University of Texas Southwestern, Dallas: Molecular basis of transcription initiation by RNA pol II.
Meyer, B., University of California, Berkeley: Chromosome-wide repression via molecular machines.
Panning, B., University of California, San Francisco: Chromatin regulation in embryonic stem cells.
Pugh, F., Penn State University, University Park: Global organization of chromatin, their regulators, and the transcription machinery.
Roeder, R., The Rockefeller University, New York: Biochemical studies of transcriptional regulatory mechanisms in animal cells.
Rogatsky, I., Hospital for Special Surgery/Weill Cornell, New York: Transcriptional mechanisms underlying anti-inflammatory actions of glucorticoids.
Roy, A., Tufts University School of Medicine, Boston, Massachusetts: Proliferative functions of the transcription factor TFII-I.
Tyler, J., University of Texas/M.D. Anderson Cancer Center, Houston: Chromatin assembly and disassembly.

Imaging Structure and Function in the Nervous System

July 24–August 13

INSTRUCTORS **D. DiGregorio,** Institut Pasteur, France
J. Waters, Northwestern University, Chicago, Illinois

ASSISTANTS **G. Moneron,** Institut Pasteur, Paris, France
F. Munoz-Cuevas, University of California, San Francisco
A. Scimemi, National Institutes of Health, Bethesda, Maryland
R. Thereau, Bordeaux University, France
A. Tran-Van-Minh, Institut Pasteur, Paris, France

Advances in light microscopy, digital image processing, and the development of a variety of powerful fluorescent probes presented expanding opportunities for investigating the nervous system, from synaptic spines to networks in the brain. This intensive laboratory and lecture course provided participants with the theoretical and practical knowledge to utilize emerging imaging technologies. The primary emphasis of the course was on vital light microscopy. Students learned the principles of light microscopy, as well as use of different types of electronic cameras, laser-scanning systems, functional fluorophores, delivery techniques, and digital image-processing software. In addition to transmitted light microscopy for viewing cellular motility, the course examined a variety of molecular probes of cell function, including calcium-sensitive dyes, voltage-sensitive dyes, photo-activated ("caged") compounds, and exocytosis tracers. Issues arising in the combination of imaging with electrophysiological methods were covered. Particular weight was given to multiphoton laser-scanning microscopy and to newly available biological fluorophores, especially green fluorescent protein (GFP) and its variants. A spectrum of neural and cell biological systems were used, including living animals, brain slices, and cultured cells. Applicants had a strong background in the neurosciences or in cell biology. In their personal statements, applicants specified (1) their experience with optical techniques, (2) how they applied optical methods in their current projects, (3) the microscope systems available to them, and (4) their long-term goals in learning more about optical methods.

This course was supported with funds provided by the Howard Hughes Medical Institute, Carl Zeiss Microscopy, and Coherent, Inc.

PARTICIPANTS

Akbergenova, Y., M.A., Massachusetts Institute of Technology, Cambridge
Fedorchak, A., B.S., University of California, Berkeley
Grienberger, C., M.D., Technical University Munich, Germany
Iurilli, G., M.Sc., Italian Institute of Technology, Genova, Italy
Kim, Y., Ph.D., Cold Spring Harbor Laboratory
Lefort, S., B.A., Brandeis University, Waltham, Massachusetts
Macé, E., B.A., Institut de la Vision, Paris, France
Maurer, C., Ph.D., Max-Planck Institute for Medical Research, Heidelberg, Germany
Palmer, L., M.S., University of Bern, Switzerland
Phillips, M., Ph.D., University of Wisconsin, Madison
Scholl, B., B.S., University of Texas, Austin
Smith, K., Ph.D., Northwestern University, Chicago, Illinois

SEMINARS

Canepari, M., INSERM, Grenoble, France: VSDs.
De Grand, A., Olympus America, Inc., Center Valley, Pennsylvania: BX51 optics and objective cleaning.
Deisseroth, K., Stanford University, California; Weninger, K., North Carolina State University, Raleigh; University of California, Los Angeles: Single-molecule FRET.
DiGregorio, D., Institut Pasteur, Paris, France: Flash photolysis.
DiGregorio, D., Institut Pasteur, Paris, France; Waters, J., Northwestern University, Chicago, Illinois: Projects–Examples.
Dittman, J., Weill Cornell Medical College, New York: Photoactivable XFPs.
Dombeck, D., Northwestern University, Evanston, Illinois: Awake imaging in hippocampus.
Emiliani, V., CNRS, INSERM, University Paris Descartes, Paris, France: SLM.
Engert, F., Harvard University, Cambridge, Massachusetts: Shot noise; Image J and Fiji; 2P microscopy.
Feller, M., University of California, Berkeley: Imaging in the retina.
Griesbeck, O., Max-Planck Institute of Neurobiology, Martinsried, Germany: XFP calcium indicators.
Huebener, M., Max-Planck Institute of Neurobiology, Martinsried, Germany: Intrinsic imaging.
Kilborn, K., Intelligent Imaging Innovations, Inc., Denver, Colorado: Deconvolution; Optogenetics.
Lanni, F., Carnegie Mellon University, Pittsburgh, Pennsylvania: Basic microscopy.
Lichtman, J., Harvard University, Cambridge, Massachusetts: Confocal microscopy.
Mertz, J., Boston University, Massachusetts: Phase-contrast microscopy.
Moneron, G., Institut Pasteur, Paris, France: STED.
Moomaw, L.; Pritchard, K., Hamamastu Photonic Systems, Spring Branch, Texas: Cameras and PMTs.
Naegerl, V., Max-Planck Institute of Neurobiology, Munich, Germany: Imaging spines with STED.
Nimmerjahn, A., Salk Institute, La Jolla, California: Acquisition and automated analysis of large-scale calcium imaging data.
Prakriya, M., Northwestern University, Chicago, Illinois: FRET and FLIM.
Shih, A., University of California, San Diego, La Jolla: Imaging blood flow.
Tsai, P., University of California, San Diego, La Jolla: Optics bench lab exercises: Basics Koehler; Ray matrices; Optics bench lab exercises: Basics, Koehler; Scanning and fluorescence; Optics bench lab exercises: Scanning; Saturation; Optics bench lab exercises: Confocal.
Waters, J., Northwestern University, Chicago, Illinois: Week one recap; Organic calcium indicators; Homebrew microscope including laser safety.
Wilbrecht, L., University of California, San Francisco: GFP applications spine morphology.
Winifred, D., Max-Planck Institute for Medical Research, Heidelberg, Germany: Extended 2P imaging and block-face EM.
Witkowski, J., Cold Spring Harbor Laboratory: Ethics.
Zhuang, X., Harvard University, Cambridge, Massachusetts: PALM/STORM.
Zito, K., University of California, Davis: 2P photolysis and applications.

Yeast Genetics and Genomics

July 24–August 13

INSTRUCTORS **M. Dunham,** University of Washington, Seattle
S. Jaspersen, Stowers Institute for Medical Research, Kansas City, Missouri
J. Smith, University of Virginia Health System, Charlottesville

ASSISTANTS **A. Brosius,** University of Washington, Seattle City, Missouri
J. Chen, Stowers Institute for Medical Research, Kansas
M. Wierman, University of Virginia Health System, Charlottesville

This course is a modern, state-of-the-art laboratory course designed to teach students the full repertoire of genetic approaches needed to dissect complex problems in the yeast *Saccharomyces cerevisiae*. Combinations of classical genetic approaches were emphasized, including the isolation and characterization of mutants, tetrad analysis, complementation, and mitotic recombination. Molecular genetic techniques, including various types of yeast transformations, gene replacement with plasmids and PCR, construction and analysis of gene fusions, and generation of mutations in cloned genes, were emphasized. Classical and molecular approaches allowed students to gain experience in identifying and interpreting various kinds of genetic interactions including suppression and synthetic lethality. Students were immersed in yeast genomics and performed and interpreted experiments with DNA arrays. They gained first-hand experience in modern cytological approaches such as epitope tagging and imaging yeast cells using indirect immunofluorescence, GFP–protein fusions, and a variety of fluorescent indicators for various subcellular organelles. Lectures on fundamental aspects of yeast genetics were presented along with seminars given by outside speakers on topics of current interest.

This course was supported with funds provided by National Human Genome Research Institute and the Howard Hughes Medical Institute.

PARTICIPANTS

Aksnes, H., M.S., University of Bergen, Norway
Balakrishnan, L., Ph.D., University of Rochester School of Medicine, New York
Beierlein, J., Ph.D., Brandeis University, Waltham, Massachusetts
Bouklas, T., B.S., Albert Einstein College of Medicine, Bronx, New York
Budin, I., Ph.D., Harvard University, Boston, Massachusetts
Conlin, P., B.S., University of Washington, Seattle
Freschi, L., M.Sc., Laval University, Quebec, Canada
Garde, C., Ph.D., Technical University of Denmark, Kgs. Lyngby, Denmark
Georgiev, D., Ph.D., University of West Bohemia in Pilsen, Czech Republic
Goetz, A., Ph.D., University of Helsinki, Finland
Kim, J., Ph.D., Tufts University, Medford, Massachusetts
Langerak, A., M.Sc., Heineken, Zoeterwoude, The Netherlands
Lindberg, L., M.S., Chalmers University of Technology, Gothenburg, Sweden
Schreiber, A., Ph.D., ETH Zurich, Switzerland
Stavrou, E., Ph.D., University of Cyprus, Nicosia
Zhang, Y., M.S., Stowers Institute for Medical Research, Kansas City, Missouri

SEMINARS

Amon, A., HHMI/Massachusetts Institute of Technology, Cambridge: Causes and consequences of aneuploidy.
Andrews, B., University of Toronto, Ontario, Canada: Using yeast functional genomics to explore biological pathways and networks.
Burke, D., University of Virginia, Charlottesville: Mitotic regulation in *Saccharomyces cerevisiae*.
Caudy, A., University of Toronto, Ontario, Canada: Discovering new metabolic pathways using small-molecule metabolomics and the awesome power of yeast genetics.
Cherry, M., Stanford University, California: Discover using the *Saccharomyces* Genome Database.
Gresham, D., New York University, New York: Watching evolution in real time.
Hieter, P., University of British Columbia, Canada: Chromosome instability in yeast and cancer.
Li, R., Stowers Institute for Medical Research, Kansas City, Missouri: Fluorescence correlation spectroscopy as a tool for in vivo analysis of protein dynamics and interaction.
Nislow. C.; Giaever, G., University of Toronto, Ontario, Cananda: Chemogenomic profiling: Exploring the yeast druggable genome; Chromatin architecture and gene expression, mutants, drugs, and evolution.
Pugh, F., Penn State University, University Park: Genome interplay between chromatin and the transcription machinery.
Strathern, J., National Cancer Institute/NIH, Bethesda, Maryland: The fidelity of transcription.
Winey, M., University of Colorado, Boulder: Centrosome assembly as viewed from budding yeast.

Stem Cells

July 27–August 5

INSTRUCTORS **McKay, R.**, Lieber Institute, Baltimore, Maryland
Shen, M., Columbia University Medical Center, New York
Tanaka, E., University of Dresden, Germany

ASSISTANT **Chenoweth, J.**, Lieber Institute, Baltimore, Maryland

Stem cells construct organs and tissues in development. They sustain tissues in the adult and restore them after injury. Because of these properties, isolating and manipulating stem cells have become major new elements in biomedical science. This workshop course covered a series of biological subjects relating to stem cells and regenerative medicine. Topics included embryology and development, reprogramming, degenerative disease, cancer stem cells, and human genetics.

This 10-day-long discussion course brought together leading researchers in the stem cell field with a small group of international students. The purpose of the workshop was to provide participants with an opportunity to achieve an advanced understanding of the scientific and clinical importance of stem cells. A major aim of the workshop was to discuss in considerable detail the relationship between stem cells and disease. The significance of this relationship with respect to developing new approaches to treating and understanding human disease was explored.

A key feature of the course is easy access to the workshop leaders and the invited lecturers for informal discussion. The course was held at the Laboratory's Banbury Conference Center located on the North Shore of Long Island. All participants stayed within walking distance of the Center, close to a tennis court, pool, and private beach.

PARTICIPANTS

Battistoni, G., B.S., Scuola Normale Superiore, Pisa, Italy
Battula, V., B.S., M.D. Anderson Cancer Center, Houston, Texas
Bell, D., M.D., M.D. Anderson Cancer Center, Houston, Texas
Bertacchi, M., B.S., Scuola Normale Superiore, Pisa, Italy
Carey, J., Ph.D., M.D. Anderson Cancer Center, Houston, Texas
Dequeant, M.-L., Ph.D., Harvard Medical School, Boston, Massachusetts
Eichler, F., M.D., Massachusetts General Hospital, Boston
Han, L., Ph.D., M.D. Anderson Cancer Center, Houston, Texas
Huang, L., B.S., University of Texas/M.D. Anderson Cancer Center, Houston
Iommelli, F., B.S., National Research Council (CNR), Naples, Italy
Kim, S.-K., B.S., Lieber Institute for Brain Development, Baltimore, Maryland
McMahon, R., Ph.D., M.D. Anderson Cancer Center, Houston, Texas
Medelnik, J.-P., B.S., Technical University, Dresden, Germany
Mishra, S., B.S., University of Texas Health Science Center, San Antonio
Raykova, D., B.S., Uppsala University, Sweden
Seo, S., B.S., Mayo Clinic, Rochester, Minnesota
Sgualdino, J., B.S., European Institute of Oncology, Milan, Italy
Shutova, M., Ph.D., Vavilov Institute of General Genetics, RAS, Moscow, Russia
Stemann Andersen, M., B.S., Harvard University, Cambridge, Massachusetts
Thompson, H., B.S., University of California, Merced
Zhang, Y., B.S., Regeneron Pharmaceuticals Inc., Tarrytown, New York

SEMINARS

Aznar Benitah, S., Center for Genomic Regulation, Barcelona, Spain: Adult stem cells.
Brickman, J., University of Edinburgh, United Kingdom: Lineage priming in embryonic stem cells.
Butler, J., Weill Cornell Medical College, New York: Endothelial-derived angiocrine signals in stem cell maintenance, organ regeneration, and tumor growth.
Hadjantonakis, K., Memorial Sloan-Kettering Cancer Institute, New York: Imaging lineage commitment and morphogenesis of early mouse embryos.
Klein, K., Harvard Medical School, Boston, Massachusetts: Principles of clonal analysis and patterns of stem cell fate in adult tissues.
Konrad Hochedlinger, K., Harvard Medical School, Boston, Massachusetts: Strategies to study cellular reprogramming to pluripotency.
McKay, R., Lieber Institute, Baltimore, Maryland: Stem cells in science and medicine.
Meissner, A., Broad Institute, Cambridge, Massachusetts: Epigenetics, stem cells, and development.
Mills, A., Cold Spring Harbor Laboratory: Chromatin-mediated dynamics and stem cells.
Park, C., Memorial Sloan-Kettering Cancer Center, New York: Cancer stem cells: Opportunities and controversies.
Robertson, E., University of Oxford, United Kingdom: Early mouse development.
Shen, M., Columbia University Medical Center, New York; Prostate development, stem cells, and cancer.
Tanaka, E., University of Dresden, Germany: Principles of vertebrate regeneration.
Tesar, P., Case Western Reserve University School of Medicine, Cleveland, Ohio: Derivation and utilization of distinct pluripotent stem cell states.
Testa, G., European Institute of Oncology, Milan, Italy: The epigenetic basis of genome programming and reprogramming.
Xiao, A., Yale University School of Medicine, New Haven, Connecticut: Epigenetic regulation in pluripotent stem cells, development, and behavior.
Young, R., Whitehead Institute/Massachusetts Institute of Technology, Cambridge: Transcriptional control of cell state.

Genetics of Complex Human Diseases

August 7–13

INSTRUCTORS **A. Al-Chalabi,** Kings College, London, United Kingdom
L. Almasy, Southwest Foundation for Biomedical Research, San Antonio, Texas

Complex diseases are conditions that are influenced by the actions of multiple genes and their interactions with each other and with the environment. This lecture course considered the difficulties in studying the genetic basis of complex disorders such as diabetes, cardiovascular disease, cancer, Alzheimer's disease, schizophrenia, and epilepsy. We discussed genetic-epidemiologic study designs, including family, twin, case/control, and adoption studies, as well as methods for quantifying the strength of the genetic influences on a disease. A major focus was the identification of specific gene effects using both linkage and association analysis and their variants. We discussed the efficiency and robustness of different designs for such analysis and how evidence from epidemiologic studies informs both the design and interpretation of molecular genetic studies. Study design and methods for analysis of quantitative risk factors related to complex diseases were covered as well as haplotype mapping, SNP tagging, meta-analysis, and gene-environment interaction. An overview of high-throughput laboratory methods was given to provide participants with insight into the applications of these techniques. Illustrations were provided through discussion of results from ongoing studies of a variety complex diseases and related risk factors.

PARTICIPANTS

Chen, G., B.S., Stowers Institute for Medical Research, Kansas City, Missouri
Crutchley, R., Pharm.D., University of Houston, College of Pharmacy, Texas
Deik, A., M.D., Beth Israel Medical Center, New York
Feng, Y., Ph.D., Columbia University, New York
Glerup, S., Ph.D., Aarhus University, Aarhus, Denmark

Jones, A., Ph.D., Institute of Psychiatry, King's College London, United Kingdom
Jones, G., Ph.D., Frederick National Laboratory for Cancer Research, Maryland
Knox, R., Ph.D., University of California, San Francisco
Østergaard, S., M.D., Unit for Psychiatric Research, Aalborg, Denmark
Perea, C., M.Sc., Universidad Antonio Nariño, Bogotá, Colombia
Puccio, A., Ph.D., University of Pittsburgh, Pennsylvania
Quillen, E., Ph.D., Texas Biomedical Research Institute, San Antonio
Scott, K., M.Phil., University of Cambridge, United Kingdom
Scott, R., Ph.D., Addenbrooke's Hospital, Cambridge, United Kingdom
Vinson, A., Ph.D., Oregon Health & Science University, Beaverton
Xu, H., Ph.D., National Institutes of Health, Bethesda, Maryland
Zeevi, D., Ph.D., Weizmann Institute of Science, Rehovot, Israel

SEMINARS

Sinsheimer, J., University of California, Los Angeles: Statistics 101.
Borecki, I., Washington University School of Medicine, St. Louis, Missouri: Introduction to genetic epidemiology.
Loos, R., Mount Sinai School of Medicine, New York: Gene–environment interaction and common disease.
Petronis, A., University of Toronto, Ontario, Canada: Epigenomics of complex disease.
Knight, J., Centre of Addiction and Mental Health, Toronto, Canada: GWAS and principles of association.
Nickerson, D., University of Washington, Seattle: Sequencing: The final frontier in genetic analysis.
Sham, P., University of Hong Kong, China: Power calculations for genetic studies.
Purcell, S., Mount Sinai School of Medicine, New York: Analysis of data from next-generation sequencing studies: Population-based targeted resequencing and PLINK/SEQ practical.
Neale, B., Massachusetts General Hospital, Boston: Exome arrays and analyzing de novo variation.
MacArthur, D., Massachusetts General Hospital, Boston.
Breen, G., King's College London, United Kingdom: Linkage analysis in human complex disorders: Examples from current studies in neuropsychiatry; miRNAs: An introduction and their role in human diseases.

X-Ray Methods in Structural Biology

October 15–30

INSTRUCTORS **W. Furey,** V.A. Medical Center & University of Pittsburgh, Pennsylvania
G. Gilliland, Janssen R&D, LLC, Johnson & Johnson, Radnor, Pennsylvania
A. McPherson, University of California, Irvine
J. Pflugrath, Rigaku Americas Corporation, The Woodlands, Texas

ASSISTANT **R. Cardoso,** Janssen R&D, LLC, Johnson & Johnson, 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, membrane proteins, nucleic acids, and complexes), crystal characterization, X-ray sources and optics, synchrotrons, crystal freezing, data collection, data reduction, multiple isomorphous replacement, multiwavelength anomalous diffraction, molecular replacement, solvent flattening, noncrystallographic symmetry averaging, electron density interpretation, molecular graphics, structure refinement, structure validation, coordinate deposition, and structure presentation. Participants learned through extensive hands-on experiments. One or more proteins were crystallized and the structure(s) determined by several methods, in parallel with lectures on the theory and informal discussions behind the techniques.

This course was supported with funds provided by the National Institute of General Medical Sciences.

PARTICIPANTS

Baker, P., Ph.D., The Hospital for Sick Children, Toronto, Canada
Basilio, D., Ph.D., Weill Cornell Medical College, New York
Bedford, R., B.S., University of North Carolina, Chapel Hill
Chappie, J., Ph.D., National Institute of Diabetes and Digestive and Kidney Disease/NIH, Bethesda, Maryland
Cuello, L., Ph.D., Texas Tech University Health Sciences Center, Lubbock
Deis, S., B.A., Indiana University, Bloomington
Duerr, K., Ph.D., Oregon Health and Science University, Portland
Feklistov, A., Ph.D., The Rockefeller University, New York
Kassube, S., M.Sc., University of California, Berkeley
Lin, D., B.S., California Institute of Technology, Pasadena
Malinauskaite, L., B.S., Aarhus University, Aarhus, Denmark
Mikaelsson, C., M.S., Karolinska Institute, Stockholm, Sweden
Rouge, L., M.S., Genentech, South San Francisco, California
Soumana, D., Ph.D., University of Massachusetts Medical School, Worcester
Wangkanont, K., B.S., University of Wisconsin, Madison
Winkler, D., Ph.D., HHMI/Colorado State University, Fort Collins

SEMINARS

Caffrey, M., Trinity College Dublin, Ireland: Crystallizing membrane proteins for structure-function studies using lipidic mesophases.
Chen, Y.-h., Columbia University, New York: Crystallization of TehA membrane protein.
Cohen, A., Stanford Linear Accelerator Center, California: Remote synchrotron data collection.
Emsley, P., University of Glasgow, Oxford, United Kingdom: Model-building tools in coot.
Furey, W., V.A. Medical Center & University of Pittsburgh, Pennsylvania: Anomalous data collection consideration; Patterson group therapy; Isomorphous replacement and anomalous scattering; Solvent flattening/phase combination; Noncrystallographic symmetry averaging; MAD phasing: A classical approach. The role of direct methods in macromolecular crystallography.
Furukawa, H., Cold Spring Harbor Laboratory: Structural dissection of NMDA receptor pharmacology.
Gilliland, G., Janssen R&D, LLC, Johnson & Johnson, Radnor, Pennsylvania: Welcome and introduction; Course overview: The structure determination of biological macromolecules; Maximizing crystallization success through seeding.
Hendrickson, W., Columbia University, New York: MAD and SAD phasing.
Holton, J., University of California, San Francisco: Signal vs. noise: Where they come from and how to tip the balance in your favor; Tips and tricks for improving diffraction.
Kleywegt, G., European Bioinformatics Institute, Cambridge, United Kingdom: Just because it's in Nature, doesn't mean it's true (macromolecular structure validation).
McPherson, A., University of California, Irvine: Crystallization of macromolecules I; Crystallization of macromolecules II; Symmetry, periodicity, unit cells, space groups, miller planes, and lattices; Waves, vectors, and complex numbers; Fundamental diffraction relationships and Bragg's Law; Diffraction patterns, reciprocal space, and Ewald's Sphere; Fourier transforms and the electron density equation; Patterson methods.
Perrakis, A., The Netherlands Cancer Institute, Amsterdam: Automated model building and rebuilding: From ARP/WARP to PDB REDO; Macromolecular animation.
Pflugrath, J., Rigaku Americas Corporation, The Woodlands, Texas: Data collection: Design and setup; Cryocrystallography; HKL2000 tutorial; Scaling and merging synchrotron data.
Ready, R., University of Cambridge, United Kingdom: Using SAD data in phase; Molecular replacement: New structures from old.
Richardson, D., Duke University Medical Center, Durham, North Carolina: Structure presentation; Detection and repair of model errors using MolProbity.
Sweet, R., Brookhaven National Laboratory, Upton, New York: A slightly different view of fundamental crystallography; X-ray sources and optics.
Terwilliger, T., Los Alamos National Laboratory, New Mexico: Automated structure solution and model-building.
Tronrud, D., Oregon State University, Corvalis: Macromolecular refinement I; Macromolecular refinement II; Electron density maps.

Programming for Biology

October 15–30

INSTRUCTOR **S. Prochnick,** DOE-Joint Genome Institute, Berkeley, California

CO-INSTRUCTOR **S. Robb,** University of California, Riverside

ASSISTANTS **J. Bredeson,** University of California, Berkeley
S. Kocher, Harvard University, Cambridge, Massachusetts
D. Messina, Cofactor Genomics, St. Louis, Missouri
R. Ramamurthy, University of California, Riverside
E. Ross, Stowers Institute for Medical Research/HHMI, Kansas City, Missouri
S. Rynearson, University of Utah, Salt Lake City
D. Triant, University of Virginia, Charlottesville

A computer is already an indispensable tool for database searches, but the use of web-based tools alone is not enough for today's biologist who needs to access and work with data from myriad sources in disparate formats. This need will become ever more important as new technologies increase the already exponential rate at which biological data is generated. Designed for students and researchers with little or no prior programming experience, this 2-week course gave biologists the

bioinformatics skills necessary to exploit this abundance of biological data. The course was based around the Perl scripting language because of its ease of learning and incredible wealth of ready-built modules such as Bioperl, which was designed to solve common biological problems. Starting with introductory coding and continuing with a survey of available biological libraries and practical topics in bioinformatics, students end by learning how to construct and run powerful and extensible analysis pipelines in a straightforward manner. The course combined formal lectures with hands-on sessions in which students worked to solve problem sets covering common scenarios in the acquisition, validation, integration, analysis, and visualization of biological data. For their final projects, which run during the second week of the course, students were posed problems using their own data and worked with each other and the faculty to solve them. Final projects have formed the basis of publications as well as public biological websites (see, e.g., http://bio.perl.org/wiki/Deobfuscator).

The prerequisite for the course was basic knowledge of UNIX. Lectures and problem sets covering this background material were available online from previous years 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 recommend the Computational and Comparative Genomics course.

This course was supported by the National Human Genome Research Institute.

PARTICIPANTS

Andino, G., B.S., Purdue University, W. Lafayette, Indiana
Bazzini, A., Ph.D., Yale University School of Medicine, New Haven, Connecticut
Borhan, H., Ph.D., AAFC, Saskatoon, Canada
Boundy, K., M.D., University of Massachusetts Medical School, Worcester
Cavinder, B., Ph.D., University of California, Riverside
Hall, M., B.S., Pennsylvania State University, University Park
Hartmaier, R., Ph.D., University of Pittsburgh, Pennsylvania
Law, M.-Y., Ph.D., University of Utah, Salt Lake City
Leung, M., B.S., University of Texas/M.D. Anderson Cancer Center, Houston
Low, L., Ph.D., Malaysian Palm Oil Board, Kajang, Malaysia
Martin, L., M.S., Cornell University, Ithaca, New York
Murray, P., B.A., Yale University, New Haven, Connecticut
Ngara, M., M.Sc., International Institute of Tropical Agriculture, Nairobi, Kenya
Nickel, N., Ph.D., Case Western Reserve University, Cleveland, Ohio
Oetjens, M., Ph.D., Vanderbilt University, Nashville, Tennessee
Panda, K., M.S., Ohio State University, Columbus
Seda Miro, J., Ph.D., Pontifical Catholic University of Puerto Rico, Ponce
Shah, S., Memorial Sloan-Kettering Cancer Center, New York
Tasdemir-Yilma, O., B.S., University of Massachusetts Medical School, Worcester
Zinovyeva, A., Ph.D., University of Massachusetts Medical School, Worcester

SEMINARS

Cain, S., Ontario Institute of Cancer Research, Canada: Gbrowse genome browser tutorial and workshop.
Chia, J.-M., Cold Spring Harbor Laboratory: Databases and SQL.
Haas, B., Broad Institute, Cambridge, Massachusetts: RNA-Seq II: Lecture: RNA-Seq assembly and differential expression; RNA-Seq III: Workshop: RNA-Seq assembly.
Hide, W., Harvard University, Boston, Massachusetts: RNA-Seq I: mRNA sequencing and assembly.
J. Tisdall, DuPont Experimental Station, Wilmington, Delaware: Scientific computing.
Marques, T., Universitat Popeu Fabra, Barcelona, Spain: DNA sequence III: Structira; variation.
Marth, G., Boston College, Chestnut, Massachusetts: DNA sequence II: Haplotypes, variants.
Pearson, W., University of Virginia, Charlottesville: Protein sequence I. Lecture: Sequence searching, blast, homology; Protein sequence II. Workshop: Sequence searching.
Schatz, M., Cold Spring Harbor Laboratory
Stajich, J., University of California, Riverside: DNA sequence I: Handling NGS data, filtering, aligning, SNP calling.
Thomas, P., University of Southern California, Los Angeles: Protein function prediction and annotation.

Antibody, Engineering, and Phage Display

October 17–30

INSTRUCTORS **C. Barbas,** The Scripps Research Institute, La Jolla, California
D. Siegel, Perelman School of Medicine at the University of Pennsylvania, Philadelphia
G. Silverman, New York University School of Medicine, New York

ASSISTANTS **S. Kacir,** University of Pennsylvania, Philadelphia
C. Noren, New England Biolabs, Ipswich, Massachusetts
K. Noren, New England Biolabs, Ipswich, Massachusetts
L. Novinger, University of Vermont College of Medicine, Burlington

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 to the construction of synthetic antibody libraries. Antibodies were selected from the library by panning. Production, purification, and characterization of Fab fragments expressed in *Escherichia coli* were also covered. Epitopes were selected from peptide libraries and characterized.

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, yeast and cell display libraries, the immunobiology of the antibody response, and recent results on the use of antibodies in therapy for cancer,

HIV, and Alzheimer's disease. The theory and practical implications for selection from phage-displayed libraries of random peptides, cDNA products, and semisynthetic proteins were also explored.

This course was supported with funds provided by the Howard Hughes Medical Institute.

PARTICIPANTS

Blosser, T., Ph.D., Technical University of Delft, the Netherlands
Freire, M., Ph.D., Harvard University, Cambridge, Massachusetts
George, M., B.S., University of South Florida, Tampa
Gilgunn, S., B.Sc., Dublin City University, Ireland
Hammond, M., M.Sc., Uppsala University, Sweden
Jores, J., Ph.D., University of Alberta, Edmonton, Canada
Kanje, S., M.S., Royal Institute of Technology, Stockholm, Sweden
Lu, X., Ph.D., Celgene, Warren, New Jersey
Matochko, W., Ph.D., University of Alberta, Edmonton, Canada
Pohl, M.A., Albert Einstein College of Medicine, Bronx, New York
Ribiero, J., Ph.D., New York University, New York
Sabetzadeh, B.S., B.S., University of Sydney, Australia
Sall, S., M.Sc., Lund University, Sweden
Seijsing, J., M.Sc., Royal Institute of Technology, Stockholm, Sweden
Tafreshi, N., B.S., Moffitt Cancer Center and Research Institute, Tampa, Florida
Volk, A.-L., M.S., Royal Institute of Technology, Stockholm, Sweden

SEMINARS

Barbas, C., The Scripps Research Institute, La Jolla, California: New approaching to therapeutic antibodies.
Chung, J., Seoul National University School of Medicine, South Korea: Anti-hapten antibody as a carrier of peptides or aptamers; Intracellular expression of antiphosphotyrosine residue antibody.
Derda, R., University of Alberta, Edmonton, Canada: Organic chemistry on phage, evolution of reactivity, and genetically encoded libraries of nonpeptidic molecules.
Noren, C., New England BioLabs, Beverly, Massachusetts: Phage peptide libraries: The Ph.D. for peptides Ipswich.
Rader, C., The Scripps Research Institute, Jupiter, Florida: Monoclonal antibody drug and target discovery for cancer therapy.
Scholler, N., Perelman School of Medicine at the University of Pennsylvania, Philadelphia: *Saccharomyces cerevisiae* for display and expression of tagged antibodies.
Sidhu, S., University of Toronto, Ontario, Canada: Synthetic proteins: New tools for new biology.
Siegel, D., Perelman School of Medicine at the University of Pennsylvania, Philadelphia: Use of antibody phage display to study human immune repertoires.
Silverman, G., New York University School of Medicine, New York: Overview of innate and adaptive immune systems.
Stahl, S., KTH Royal Institute of Technology, Stockholm, Sweden: Affibody molecules and their applications in cancer and Alzheimer's disease.
Stansfield, R., The Scripps Research Institute, La Jolla, California: Structural biology of the immune system.

Advanced Sequencing Technologies and Applications

October 18–30

INSTRUCTORS
E. Mardis, Washington University School of Medicine, St. Louis, Missouri
G. Marth, Boston College, Chestnut Hill, Massachusetts
W. McCombie, Cold Spring Harbor Laboratory
A. Quinlan, University of Virginia, Charlottesville
M. Zody, Broad Institute, Cambridge, Massachusetts

ASSISTANTS
A. Farrell, Boston College, Chestnut Hill, Massachusetts
M. Griffith, Washington University School of Medicine, St. Louis, Missouri
O. Griffith, Washington University School of Medicine, St. Louis, Missouri
M. Kramer, Cold Spring Harbor Laboratory
V. Magrini, Washington University School of Medicine, St. Louis, Missouri
S. McGrath, Washington University School of Medicine, St. Louis, Missouri
J. Walker, Washington University School of Medicine, St. Louis, Missouri

During the last decade, large-scale DNA sequencing has markedly impacted the practice of modern biology and is beginning to affect the practice of medicine. With the recent introduction of several advanced sequencing technologies, costs and timelines have been reduced by orders of magnitude, encouraging investigators to conceptualize and perform sequencing-based projects that had been impractical. Furthermore, the application of these technologies to answer questions previously not experimentally approachable is broadening their impact and application. This intensive 2-week course explored applications of next-generation sequencing technologies, with a focus on commercially available methods. Students were instructed in the detailed operation of several revolutionary sequencing platforms, including sample preparation procedures, general data handling through pipelines, and in-depth data analysis. A diverse range of biological

questions was explored, including DNA resequencing of human genomic regions (using cancer samples as a test case), de novo DNA sequencing of bacterial genomes, and the use of these technologies in studying small RNAs, among others. Guest lecturers highlighted their own applications of these revolutionary technologies. We encouraged applicants from a diversity of scientific backgrounds including molecular evolution, development, neuroscience, cancer, plant biology, and microbiology.

This course was supported by the National Human Genome Research Institute, Illumina & Life Technologies, and with major equipment provided by Illumina & Life Technologies.

PARTICIPANTS

Altinas, A., Ph.D., Technical University of Denmark, Kongens Lyngby, Denmark
Aruda, A., Ph.D., MIT/WHOI Joint Program in Oceanography, Woods Hole, Massachusetts
Augustine, R., B.S., University of Wisconsin, Madison
Barnard-Kubow, K., Ph.D., University of Virginia, Charlottesville
Cembrowski, M., Ph.D., Howard Hughes Medical Institute, Ashburn, Virginia
Dali, R., Ph.D., McGill University, Montreal, Canada
Finberg, K., M.D., Duke University Medical Center, Durham, North Carolina
Fink, S., Ph.D., Case Western Reserve University, Cleveland, Ohio
Hu, W., Ph.D., Whitehead Institute for Biomedical Research, Cambridge, Massachusetts
Justice IV, J., Ph.D., Johns Hopkins University, Baltimore, Maryland
Pereira, J., Ph.D., Fox Chase Cancer Center, Philadelphia, Pennsylvania
Platt, M., M.D., Massachusetts General Hospital, Boston
Estrada, V., Ph.D., The Rockefeller University, New York
Reuther, J., Ph.D., M.D. Anderson Cancer Center, Houston, Texas
Schrader, K., Ph.D., Memorial Sloan-Kettering Cancer Center, New York
Thomas, R., Ph.D., USMHRP, Walter Reed Army Institute of Research, Silver Spring, Maryland

SEMINARS

Brayer, J., Oxford Nanopore Technologies, Essex, United Kingdom: Nanopore sequencing.
Goldstein, T., University of California, Santa Cruz: PARADIGM, DIPSC, and PATHMARK software in pathway-based analyses.
Griffith, M., Washington University School of Medicine, St. Louis, Missouri: RNA-Seq.
Haas, B., The Broad Institute of MIT and Harvard, Cambridge, Massachusetts: Trinity RNA-Seq assembly software.
Hall, I., University of Virginia, Charlottesville: Structural variant detection.
Mardis, E., Washington University School of Medicine, St. Louis, Missouri: NGS in cancer genomics studies.
McCombie, W., Cold Spring Harbor Laboratory
Quinlan, A., University of Virginia, Charlottesville: Mining the genome.
Robertson, A., Life Technologies, Inc., Carlsbad, California: Ion torrent and ion proton sequencers.
Robinson, J., The Broad Institute of MIT and Harvard, Cambridge, Massachusetts: The IGV viewer.
Schadt, E., Mount Sinai School of Medicine, New York: Build better models of disease in the era of big data.
Schatz, M., Cold Spring Harbor Laboratory: Assembly of NGS data.
Sisneros, N., Pacific Biosciences, Menlo Park, California: SMRT sequencing with the Pacific Biosciences Sequencer.
Smith, A., University of Southern California, Los Angeles: Methylation by NGS bisulfite data analysis.
Smith, G., Illumina, Chesterford, United Kingdom: Illumina presentation.
Stolovitsky, G., IBM, Yorktown Heights, New York: Nanopore sequencing development.
Yandell, M., University of Utah, Salt Lake City: VAAST and VAAST pedigree analysis.

Computational and Comparative Genomics

November 28–December 4

INSTRUCTORS **W. Pearson,** University of Virginia, Charlottesville
L. Stubbs, University of Illinois, Urbana-Champaign

ASSISTANTS **X. Cao,** University of Illinois, Urbana-Champaign
L. Mills, University of Virginia, Charlottesville

This course presented a comprehensive overview of the theory and practice of computational methods for the identification and characterization of functional elements from DNA sequence data. The course focused on approaches for extracting the maximum amount of information from protein and DNA sequence similarity through sequence database searches, statistical analysis, and multiple sequence alignment. Additional topics included alignment and analysis of "next-gen" sequencing data, with applications from metagenomic, RNA-Seq, and ChIP-Seq experiments; the Galaxy environment for high-throughput analysis; regulatory element and motif identification from conserved signals in aligned and unaligned sequences; integration of genetic and sequence information in biological databases; and the ENSEMBL genome browser and BioMart.The course combined lectures with hands-on exercises; students were encouraged to pose challenging sequence analysis problems using their own data. The course was designed for biologists seeking advanced training in biological sequence and genome analysis, computational biology core resource directors and staff, and for individuals in other disciplines (e.g., computer science) who wish to survey current research problems in biological sequence analysis. Advanced programming skills were not required. The primary focus of this course was the theory and practice of algorithms in computational biology, with the goals of using current methods more effectively for biological discovery and developing new algorithms. Students more interested in the practical aspects of

software development were encouraged to apply to the course on Programming for Biology. Students who liked in-depth training in the analysis of next-generation sequencing data (e.g., SNP calling and the detection of structural variants) should apply to the course on Advanced Sequencing Technologies and Applications.

This course was supported by the National Human Genome Research Institute.

PARTICIPANTS

Bjoerklund, S., B.S., University of Oslo, Radium Hospital, Norway
Copin, R., Ph.D., New York University School of Medicine, New York
Dali, R., B.Sc., McGill University, Montreal, Canada
Davis, M., B.S., Yale Medical and Graduate School, New Haven, Connecticut
DeHart, J., B.S., The Salk Institute, La Jolla, California
Edgington, N., Ph.D., Southern Connecticut State University, New Haven
Froekjaer Jensen, C., Ph.D., University of Utah, Salt Lake City
Ingram, C., Ph.D., University of Virginia, Charlottesville
Kazmierczak, B., Ph.D., Yale University School of Medicine, New Haven, Connecticut
Labo, N., M.P.H., Frederick National Laboratories for Cancer Research, Maryland
Lam, D., Ph.D., Ohio University, Athens
Malone, J., Ph.D., Florida State University, Tallahassee
Meihls, L., Ph.D., The Boyce Thompson Institute for Plant Research, Ithaca, New York
Rehan, S., Ph.D., University of Pennsylvania, Philadelphia
Sandel, M., B.S., University of Alabama, Birmingham
Sawicka, A., M.S., Medical University of Vienna, Austria
Sleckman, B., Ph.D., Washington University School of Medicine, St. Louis, Missouri
Stojanova, Z., M.S., House Research Institute, Los Angeles, California
Tavera-Mendoza, L.E., Ph.D., Dana-Farber Cancer Institute/Harvard Medical School, Boston, Massachusetts
Zacharias, A., Ph.D., University of Pennsylvania, Philadelphia

SEMINARS

Mackey, A., University of Virginia, Charlottesville: Genome annotation (HMM basics); Sequencing technologies/tools; SNP discovery and variation; Whole-genome de novo assembly; RNA-Seq.
Ouellette, F., Ontario Institute for Cancer Research, Toronto, Canada: Databases for computational biology; The Ensembl database of genomes; Review/panel discussion: Ensembl/UCSC–Which browser when?; ENSEMBL/BioMart; Ensembl API; Gene lists, interaction, and pathway databases.
Pearson, W., University of Virginia, Charlottesville: Protein evolution and sequence similarity sequencing; Alignment algorithms and scoring matrices; Multiple sequence alignment, PSSMs, HMMs, and Pfam; Phenotype prediction PolyPhen/SIFT.
Pearson, W., University of Virginia, Charlottesville; Stubbs, L., University of Illinois, Urbana-Champaign: Introduction and overview.
Sinha, S., University of Illinois, Urbana-Champaign: Identifying functional DNA motifs; Tying functional elements to biology.
Sinha, S., University of Illinois, Urbana-Champaign; Zhong, S., University of California, San Diego: Integrating genomics data sets for biological inference.
Stubbs, L., University of Illinois, Urbana-Champaign: Introduction to genome biology.
Taylor, J., Emory University, Atlanta, Georgia; Mills, L., University of Virginia, Charlottesville; Ouellette, F., Ontario Institute for Cancer Research, Toronto, Canada: The Galaxy computing environment; Galaxy for high-throughput analysis.
Zhong, S., University of California, San Diego: Functional elements: II–Epigenomics.

The Genome Access Course

April 29–May 1, November 12–14

TRAINERS

A. Gordon, Cold Spring Harbor Laboratory
G. Howell, The Jackson Laboratory
B. King, Mount Desert Island Biological Laboratories
C. Lambert, Cold Spring Harbor Laboratory

This course is an intensive 2-day introduction to bioinformatics that was held twice in 2012 and trained almost 70 participants in total. The core of the course was designed to cover the manipulation and analysis of sequence information. The course was broken into modules designed to give a broad overview of a given topic, with ample time for examples chosen by the instructors. Each module included three parts, consisting of a discussion of theory and methods, coverage of software and web resources, and use of selected tools with examples (including those supplied by the students). The modular design allowed the instructors to tailor the presentation to the interests of the students. Modules included Electronic Sequence Information; Pairwise Sequence Comparisons; Multiple Sequence Alignments; Gene Prediction; Genome Analysis; Sequence Variation; Protein Classification and Structural Analysis; Proteomics; and Phylogenetic Analysis. Applications to the course were open to all on a first-come-first-served basis, subject to basic eligibility requirements. 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 29 –May 1

Biais, N., Columbia University, New York
Boncristiani, H., University of North Carolina, Greensboro
Brownstein, C., Boston Children's Hospital, Massachusetts
Castellano-Torres, L., Universidad de Guanajuato, Guanajuato, Mexico
Cheung, H.-W., Dana-Farber Cancer Institute, Boston, Massachusetts
Cimmino, L., New York University, New York
Cooper, M., Washington University, St. Louis, Missouri
Fabius, A., Memorial Sloan-Kettering Cancer Center, New York
Fakhro, K., Cornell University, New York
Feng, J., Mount Sinai School of Medicine, New York
Guryanova, O., Memorial Sloan-Kettering Cancer Center, New York
Hurwitz, M., Yale University School of Medicine, New Haven, Connecticut
Kosova, G., Northwestern University, Chicago, Illinois
Lebowitz, J., BioMarin Pharmaceutical, Novato, California
Lopez, D., U.S. Department of Agriculture/Agricultural Research Service, Beltsville, Maryland
Miller, A., St. Louis University, Missouri
Ostrovnaya, I., Memorial Sloan-Kettering Cancer Center, New York
Petukhova, L., Columbia University, New York
Qin, W., The Jackson Laboratory, Bar Harbor, Maine
Rabara, R., South Dakota State University, Brookings
Rapp, J., University of Toledo, Ohio
Riddle, S., Life Technologies Corp., Madison, Wisconsin
Schatz, J., Memorial Sloan-Kettering Cancer Center, New York
Simard, L., University of Manitoba, Winnipeg, Canada
Tarbox, J., Washington University, St. Louis, Missouri
Vogel, K, Life Technologies Corp., Madison, Wisconsin
Wang, E., BioMarin Pharmaceuticals Inc., San Francisco, California
Xu, J., Tufts University, North Grafton, Massachusetts
Yu, D., University of Texas/M.D. Anderson Cancer Center, Houston
Zhang, P., University of Texas/M.D. Anderson Cancer Center, Houston

November 12–14

Allen, V., University of Toronto, Ontario, Canada
Basson, M., King's College London, United Kingdom
Belanger, R., Laval University, Quebec, Canada
Colaneri, A., University of North Carolina, Chapel Hill
Dowar, M., Samuel Lunenfeld Research Institute, Mount Sinai, Toronto, Canada
Feinstein, P., Hunter College, CUNY, New York,
Gubbay, J., Public Health Ontario Laboratory, Toronto, Canada

Haffner, M., Johns Hopkins University, Baltimore, Maryland
Harke, M., Stony Brook University, Southampton, New York
Ingraham, S., Nationwide Children's Hospital, Columbus, Ohio,
Jafarifar, F., Cleveland Clinic–Lerner Research Institute, Cleveland, Ohio
Jain, S., M.D. Anderson Cancer Center, Houston, Texas
Ji, Y., Mayo Clinic, Rochester, Minnesota
Lukasiewicz, K., National Institutes of Health, Bethesda, Maryland
Maness, P., University of North Carolina School of Medicine, Chapel Hill
McIvor, E., University of Texas/M.D. Anderson Cancer Center, Smithville
Mootha, V., University of Texas Southwestern Medical Center, Dallas
Napierala, M., University of Texas/M.D. Anderson Cancer Center, Smithville
Palangat, M., National Cancer Institute/NIH, Bethesda, Maryland
Park, H.J., University of Cambridge, United Kingdom
Patel, S., Public Health Ontario, Toronto, Canada
Ragan, S., Amgen, Seattle, Washington
Raznahan, A., National Institute of Mental Health/NIH, Bethesda, Maryland
Reed, D., Monell Chemical Senses Center, Philadelphia, Pennsylvania
Royal, M.A., Rutgers University, Piscataway, New Jersey
Ruhl, S., SUNY Buffalo, New York,
Sanda, T., Dana-Farber Cancer Institute, Boston, Massachusetts
Sayed, S., BRAC University, Dhaka, Bangladesh
Soliman, G., Western Michigan University, Kalamazoo
Swartz, K., Northwestern University, Chicago, Illinois
Tran, N.Q., University of Tennessee Health Science Center, Memphis

The Laboratory acknowledges the generosity of the following companies that loaned equipment and reagents to the various courses:

Agilent Technologies Inc.
Andor Technology
Applied Biosystems
Applied Precision
Art Robbins Institute
ASI Imaging
Astro-Med Inc.
BD Biosciences
Berthold Technologies USA, LLC
Bio-Rad Laboratories
Cairn Research LTD
Caliper Life Sciences
Carl Zeiss Inc.
Chroma Technology Corporation
Coherent Laser
ConOptics
CoolLED
Corning Inc.
Covaris, Inc.
CrystaLaser
Dagan Corporation
Dage
Doric Lenses
Drummond Scientific Company
Eppendorf North America
Fludigm; Fotodyne Inc.
GE Life Sciences
Hamamatsu Photonic Systems
Hamilton Thorne Biosciences
Hampton Research
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Miltenyi Biotec Inc.
Molecular Devices
Nalge Nunc International
Nanodrop Technologies
Narishige International USA Inc.
NeoBiosystems
New England BioLabs Inc.
Newport Instruments
Nikon Inc.
Nonlinear Dynamics
NuGEN
Olympus America Inc.
PerkinElmer Life and Analytical Sciences
Photometrics
Prairie Technologies
Qiagen Inc.
RC Testing Services
Red Shirt Imaging, LLC
Roche Applied Science
Roche Nimblegen
Scientifica Ltd.
SciGene
Singer Instruments
Spectra-Physics Lasers Inc.
Stanford Photonics
Stanford Research Systems
Stratagene
Sutter Instruments
Tecan US, Inc.
Thermo Fisher Scientific
Thor Labs
VDS Vosskuhler GmbH
Warner Instruments
Waters Corporation
World Precision Instruments

SEMINARS

INVITED SPEAKER PROGRAM

Each year, Cold Spring Harbor Laboratory invites speakers from outside the institution to present their research findings. These weekly seminars keep the CSHL staff current on the latest scientific developments and broaden their perspectives. Graduate students and postdoctoral fellows meet with the seminar speakers for lunch immediately after the seminar, providing an opportunity for the exchange of ideas in an informal setting.

	Title	Host
January		
Dr. Mark Bear, Massachusetts Institute of Technology	Synaptic plasticity: From amblyopia to autism.	Stephen Shea
Dr. Bonnie Basler, Princeton University	Manipulating quorum sensing to control bacterial pathogenicity.	Terri Grodzicker
Dr. Rachel Green, Johns Hopkins University School of Medicine	Quality control during translation in bacteria and eukaryotes.	Leemor Joshua-Tor
Dr. Matthew Meyerson, Dana Farber Cancer Institute	Genomic studies of human cancer.	Hongwu Zheng
February		
Dr. Alejandro Sánchez Alvarado, Stowers Institute for Medical Research	Stem cells, regeneration, and the planarian *Schmidtea mediterranea*.	Christopher Hammell
Dr. Ed Luk, Stony Brook University	Mechanism of histone H2A.Z replacement by the SWR1 chromatin remodeling complex.	David L. Spector
Dr. Mike Levine, University of California, Berkeley	Mechanisms of transcriptional precision in the *Drosophila* embryo.	Marja Timmermans
Dr. Carol Robinson, University of Oxford	Mass spectrometry from micelles to motors.	Leemor Joshua-Tor
March		
Dr. Robert Langer, Massachusetts Institute of Technology	Biomaterials and biotechnology: From the discovery of the first angiogenesis inhibitors to the development of controlled drug delivery systems and the foundation of tissue engineering.	Postdocs
Dr. Ronald Breaker, Yale University	New and mysterious riboswitches and ribozymes.	Christopher Hammell
Dr. Guoping Feng, Massachusetts Institute of Technology	Synaptic and circuitry mechanisms of compulsive/repetitive behavior.	Joshua Huang
Dr. Mina Bissell, Lawrence Berkeley National Laboratory	Everything is context dependent: Tales of glucose metabolism, MyC, and other players!	Mikala Egeblad
April		
Dr. Michael Karin, University of California, San Diego	Lymphocyte-produced cytokines and the control of tumor development and metastatic progression.	Mikala Egeblad
May		
Dr. Qiang Wu, Shanghai Jiao Tong University, China	Molecular diversity and genetic analysis of variable and constant gene clusters.	Adrian R. Krainer
October		
Dr. Dirk Schübeler, Friedrich Miescher Institute for Biomedical Research, Switzerland	DNA sequence determinants of the epigenome.	Keerthi Krishnan
Dr. Songhai Shi, Memorial Sloan-Kettering Institute for Cancer Research	Structural and functional development of the neocortex.	Joshua Huang
November		
Craig Thompson, Memorial Sloan-Kettering Cancer Center	Metabolic inputs into cancer genetics.	Lloyd Trotman

	Title	Host
November (*continued*)		
Dr. Ali Shilatifard, Stowers Institute for Medical Research	Chromatin modifications, transcriptional elongation control, and childhood leukemia.	Christopher Vakoc
December		
Dr. John D. Scott, University of Washington School of Medicine	Guiding signals for enzyme complexes.	Nicholas Tonks
Dr. Robert Tjian, Howard Hughes Medical Institute	Controlling cell-type-specific gene expression: An old problem tackled by modern tools to reveal new insights.	Terri Grodzicker

IN-HOUSE SEMINAR PROGRAM

Cold Spring Harbor Laboratory's In-House Seminars were initiated to provide a semiformal avenue for communication among the various research groups at the Laboratory. The seminars also afford a necessary opportunity for the graduate students and postgraduate staff to develop their skills in organizing, presenting, and defending their research.

Speaker	Title
January	
Florin Albeanu	Understanding neuronal circuits underlying mammalian olfaction.
Ivan Iossifov	Molecular function view of the genetics of autism.
Wanhe Li (Dubnau lab)	Small regulatory RNAs in olfactory arousal, memory, and cognitive decline.
February	
Pavel Osten	Serial two-photon (STP) tomography for automated ex vivo mouse brain imaging.
Dan Levy (Wigler lab)	Rare copy-number variation in autistic spectrum disorders.
Justin Kinney	Dissecting, decoding, and reprogramming multiprotein DNA complexes.
March	
Mikala Egeblad	Tumor-associated myeloid cells: The good, the bad, and the ugly.
Alex Krasnitz	Recurrent properties and clustering of cancer genomes: Tools and results.
Tony Zador	Sequencing the connectome.
Molly Hammell	Two tales of small RNA: From plant leaf development to neurodegeneration.
Marja Timmermans	Patterning the leaf: A new StART and old small RNAs.
April	
Zachary Lippman	It's spring! Time to start thinking about tomatoes.
Partha Mitra	Mouse brain architecture project.
October	
Gholson Lyon	Finding and analyzing human genetic variation in neuropsychiatric disorders.
Raffaella Sordella	Resistance to chemotherapy: New treatments and novel insights into an old problem.
Mads Aaboe Jensen (Krainer lab)	Splicing factor SRSF6 regulates somatic stem-cell fate in mouse skin.
November	
Manzar Hossein (Stillman lab)	Meier–Gorlin syndrome mutations disrupt an Orcl CDK inhibitory domain and cause centrosome reduplication.
David Tuveson	Solving cancer at CSHL.
Astrid Haase and Jonathan Ipsaro (Hannon and Joshua-Tor labs)	The structural biochemistry of zucchini implicates it as a nuclease in piRNA biogenesis.
December	
Joshua Dubnau	Neurodegeneration and the transposon storm.
Kate Creasey (Martienssen lab)	Caught in the act: How transposons are controlled when methylation fails.
Jesse Gillis	Network-based interpretation of gene function from postsynaptic protein interaction to schizophrenia coexpression.


BANBURY CENTER

BANBURY CENTER
EXECUTIVE DIRECTOR'S REPORT

Any account of the events of 2012 must begin with what is officially called "posttropical cyclone" Sandy, but more appropriately named "Superstorm Sandy." It reached the north shore of Long Island in the evening and night of Monday, October 29, with devastating effects. Trees fell throughout the area, bringing down power lines and causing extensive blackouts. More than 30 trees fell at Banbury, including one that fell on the roof of Robertson House; fortunately, this was the only building damaged by the storm. We lost power in the evening of October 29, and the Banbury office thus moved into temporary quarters in the Meetings and Courses offices in Grace Auditorium. Because we could not move back to Banbury until November 14, two Banbury Center meetings had to be cancelled—the first time that we have had to do so in at least 25 years, and two meetings were moved to the main campus.

Nevertheless, 2012 was a busy year for Banbury, with 18 meetings, two Watson School of Biological Science courses, six summer courses, and 10 other events. The 517 meeting participants were drawn from 30 states in the U.S.A. and from 19 foreign countries.

There have been a number of Banbury Center meetings that have dealt with science-related issues rather than research topics. Among the most notable are those on patenting. The first meeting, *Patenting of Life Forms,* was held in October 1981, and the second, *Intellectual Property and Biotechnology*, was held in 1991. Norton Zinder was prescient when he spoke on "Using Data from the Human Genome Project" at the 1991 meeting. The patenting of human genes and gene sequences has become an area of great controversy, highlighted by the recent Myriad case involving patents covering the *BRCA* gene and the decision in the Prometheus case. Thus, the 2012 meeting on *Patenting Genes: New Developments, New Questions* was particularly timely, examining the current state of gene patents, especially those covering diagnostic tests and the implications of whole-genome sequencing.

Another meeting relating to human genetics was held in May. Jim Watson, Mila Pollock, and I have been advancing the argument that as the Human Genome Project (HGP) was one of the great scientific accomplishments, it more than justifies serious historical analysis. A major preliminary to such a study would be locating and cataloging materials relating to the HGP. This has been explored in collaboration with the Wellcome Trust Library. Another component must be recording the personal experiences of scientists who worked on key aspects of the HGP, and Watson proposed that this should be done as soon as possible. The Alfred P. Sloan Foundation became interested in the archival project and the book of essays about the HGP. The suggestion was made that we should explore a possible long-term project to produce a history (or histories) of the HGP that will be useful and interesting to the public and scholars. This meeting, *Toward a History of the Human Genome Project*, was funded by the Sloan Foundation with the goal of laying the groundwork for such a book and other media productions. We brought a particularly interesting set of participants for the discussions, including those involved with the HGP (scientists, bioethicists), as well as archivists, historians, and publishers of books and documentaries. The participants helped to define the purpose of the book, what would distinguish it from other HGP books, its audience, and style.

A third meeting that was not directly based on biomedical research was organized by Suzanne Nalbantian: *Interdisciplinary Symposium on Literature, Memory, and Neuroscience*. This was a follow-up to a meeting held in 2007 on *Memory in Neuroscience and the Humanities,* the thesis of which was that 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. The 2012 meeting, held under the auspices of the International Comparative Literature Association, continued this theme, with, for example, papers on "Marcel Proust and Memory: A Neuropsychological Perspective" and "Nonconscious Memory and the Surrealist Mind."

Cancer meetings have long had a large role in the Banbury Center calendar, and 2012 was no exception. The first cancer meeting, *Transcription and Cancer,* examined how the new insights in transcriptional and chromatin biology that have come with the application of modern analytical techniques may pave the way for the development of therapies directed against transcription factors. These have been traditionally thought of as undruggable, but participants in this meeting faced up to the challenge of developing direct-acting inhibitors of gene regulatory complexes.

The metabolism of cancer cells has held a fascination ever since Otto Warburg's observations in the early part of the 20th century that cancer cells metabolized glucose via glycolysis even in the presence of oxygen. This is a general metabolic feature of cancer cells, but its causal relationship to the origins of cancer cells and cancer progression is still unclear. However, understanding this process better could lead to the identification of new therapeutic targets. The main objectives of the meeting *Regulation of Metabolism in Cancer* were to discuss (1) biophysical and biochemical studies of the unique metabolic requirements and pathway utilizations of transformed cells, (2) emerging sequencing and computational technologies that can rapidly analyze cancer genomes and transcriptional profiles, and (3) biomedical informatic and physical approaches to integrating the metabolic, genomic, and transcriptional interactions of cancer.

It is generally thought that a key event in the development of cancer is the transformation of cells from an epithelial state to having the properties of mesenchymal cells. This epithelial-mesenchymal transition (EMT) is accompanied by the loss of epithelial cell junction proteins leading to weakening of cell adhesion and an increase in cell motility. Furthermore, cellular sensitivity to multiple targeted therapies, chemotherapy, and radiotherapy has been shown to be governed by the extent to which cells have undergone an EMT transition. Resistance associated with cellular plasticity and heterogeneity has been observed in multiple systems derived from adenocarcinomas and squamous carcinomas. Participants in the meeting *Cell Plasticity in Cancer Evolution* discussed data on the molecular and pathobiological significance of cellular plasticity in carcinomas, and how to explore, and exploit for treatments, the signaling pathways that promote cell plasticity.

The year of Superstorm Sandy was a hard one for us all. Janice Tozzo and Pat Iannotti continued the work of the Banbury office while Basia Polakowski had to cope with the tree that came crashing down on the Robertson House roof. It was the grounds crew of Sonny Leute, Fredy Vasquez, and Joseph McCoy, assisted by reinforcements from the main campus, who bore the brunt of the effects of the storm, and three months later, they were still removing tree trunks and branches. Jon Parsons and Connie Brukin continue to be indispensable for AV and photographs, respectively, and Culinary Services and Housekeeping cope admirably with the rapid turnover of guests.

Jan Witkowski
Executive Director

A stump remains

Tree brought down by storm

Clearing the mess

Downed power lines

Cutting up trees

Reduced to wood chips

BANBURY CENTER MEETINGS

Communicating Science

February 10–15

FUNDED BY **Boehringer Ingelheim Fonds Foundation for Basic Research in Medicine,** Heidesheim, Germany

ARRANGED BY **S. Schedler,** Boehringer Ingelheim Fonds, Heidesheim, Germany
C. Walther, Boehringer Ingelheim Fonds, Heidesheim, Germany

The Boehringer Ingelheim Fonds (BIF) has an international program of support for Ph.D. fellowships. It first brought its fellows to the Banbury Center for their annual North American retreat in 2005, and it has been a great pleasure to have them return. Their 2012 stay at Banbury was the fifth occasion that they have been here. At Banbury, fellows receive intensive instruction in matters such as giving presentations and writing papers, topics usually learned by default (and often poorly) during graduate research.

Opening Remarks and All About BIF: C. Walther, Boehringer Ingelheim Fonds, Heidesheim, Germany

SESSION 1: First Writing Assignment

W. Wells, Global Alliance for TB Drug Development, New York: Writing techniques and how to structure papers

SESSION 2: Presentation of Graphic Information and How to Prepare and Deliver a Scientific Talk

W. Tansey, Vanderbilt University Medical Center, Nashville, Tennessee

SESSION 3

Group A: Four-minute PowerPoint presentations and reviews.

SESSION 4: Second Writing Assignment

SESSION 5

Group B: Four-minute PowerPoint presentations and reviews.
Group A: Preparation of 3-minute PowerPoint presentations.

SESSION 6

Group B: Preparation of 3-minute PowerPoint presentation.

SESSION 7

Groups A and B: Three-minute PowerPoint presentations and reviews.

SESSION 8: What Makes Success in Science?
G. Hannon, Cold Spring Harbor Laboratory

SESSION 9: How to Design Figures
M. Hansen and M. Corral, *Nature* Publishing Group, New York

SESSION 10: Walking Tour of Cold Spring Harbor Laboratory Campus

Students hard at work

W. Tansey reviewing student presentations

Leading Science Workshop

February 24–27

FUNDED BY **The American Express Foundation**, New York, New York

ARRANGED BY **C.M. Cohen,** Science Management Associates, Newton, Massachusetts
D. Kennedy, Worklab Consulting LLC, New York

This workshop, the second in a series supported by the American Express Foundation, brought together life scientists making, or recently having made, the transition to a leadership or managerial position in academia, not-for-profit organizations, or the private sector. It focused on the techniques, situations, and challenges that relate specifically to leading and managing in the scientific workplace. Participants were able to share their experiences and challenges with one another and to receive feedback and guidance from others with similar experience. The workshop helped participants identify areas where they needed guidance, as well as how to capitalize on areas of strength. Participants learned and developed the necessary skills to lead and interact effectively with others in both one-on-one and group settings.

SESSION 1: Who We Are

Participants read 50–100-word essay "Who I am and what do I hope to get from this workshop?" aloud to the entire group.

SESSION 2: Introduction: What Is Leadership and What Makes a Great Scientist/Leader?

Small groups proposed attributes of leadership, especially in a scientific context, and discussed examples of effective and ineffective leadership based on their own experience and observations. This was followed by the entire group deciding on attributes of excellent leaders.

SESSION 3: Difficult Conversations and Interactions

The group reviewed the types of situations and interactions that scientists find difficult as they transition into leadership positions. There was discussion of the fundamental tools needed for negotiating difficult conversations with difficult people.

SESSION 4: Keynote Speaker: K. Barker

The author and laboratory management expert spoke of her experiences.

SESSION 5: Science in the Public Eye

Facilitator: K.R. Miller, Brown University, Providence, Rhode Island

Dr. Miller reviewed what he has learned debating complex scientific issues in sometimes contentious circumstances. He led the group in an exercise simulating a lively public interchange about the teaching of evolution in public schools. Participants got first-hand experience in dealing with a public audience and received valuable guidance and pointers on how to comport themselves in such circumstances.

SESSION 6: Group Dynamics and Meetings

- How to run and lead meetings
- How to structure and encourage open discussion, ensuring participation
- How to deal with silence and nonparticipants
- How to recognize and manage impediments to effective group problem solving

SESSION 7: Projecting Leadership

Volunteers were selected to deliver a "pitch" about their institution, department, or group, with participants providing feedback in the context of what had been learned so far in the workshop.

SESSION 8: Case Studies

Attendees were instructed to bring a one-page case study describing a difficult management situation or leadership challenge they faced or are facing. In small groups, each attendee read their case aloud. A structured discussion guide was used to elicit comments, discussion, and suggestions from the group, which then selected one case that best illustrated a key leadership challenge for presentation in summary to meeting.

SESSION 9: Concluding Group Discussion

Participants reviewed the definition of leadership constructed at the beginning of the workshop and asked, "What did we learn?" and "What didn't we learn that we would have liked to learn?"

Entrance to the Banbury Center

The Fourth NIMH-Sponsored Brain Camp

March 16–18

FUNDED BY **National Institute of Mental Health**, Bethesda, Maryland

ARRANGED BY **M. Akil,** National Institute of Mental Health, Bethesda, Maryland
T. Insel, National Institute of Mental Health, Bethesda, Maryland

Once again, we were delighted to host the National Institute of Mental Health (NIMH)-sponsored "Brain Camp." The goal of the Brain Camp is to identify areas of neuroscience that are of interest and relevance to psychiatrists and to communicate these to a small group of outstanding psychiatry residents and research fellows. Some of the most distinguished and thoughtful neuroscientists in the country came as guest speakers to the meeting. The goal of the series of meetings is to develop a neuroscience curriculum that can eventually be shared with psychiatry training programs around the country.

Opening Remarks: M. Akil, National Institute of Mental Health, Bethesda, Maryland

SESSION 1: Developmental Neurobiology

F. Lee, Weill Cornell Medical College, New York: Role of neurotrophins in psychiatric disorders: A neurodevelopmental approach.

D. Amaral, University of California, Davis: Neurobiological and neuroimmune approaches to understanding autism.

Introduction and Charge: T. Insel, National Institute of Mental Health, Bethesda, Maryland

Special Lecture: Studies of Rett Syndrome and MeCP2 and Their Relevance to Neuropsychiatric Disorders
H. Zoghbi, Baylor College of Medicine, Houston, Texas

SESSION 2: Innovators in Psychiatry

S. Lisanby, Columbia University, New York: Innovations in brain stimulation: Game changer for clinical neuroscience.

X. Castellanos, New York University Child Study Center, New York: Spontaneous neural/BOLD fluctuations reveal intrinsic functional connectivity circuits.

SESSION 3: The Rational Development of Novel Therapeutics

K. Ressler, Emory University, Atlanta, Georgia: Targeting neural plasticity to treat fear and anxiety.

R. Duman, Yale University School of Medicine, New Haven, Connecticut: Keeping neurons alive, healthy, and connected.

Special Lecture: Cognitive Neuroscience: Tools that Facilitate Research on Novel Therapeutics

B. Cuthbert, National Institute of Mental Health, Rockville, Maryland;
New cognitive neuroscience tools for novel therapeutics: Dimensions and data sets.

SESSION 4: Neuroscience and Psychiatry

J. Krystal, Yale University School of Medicine, New Haven, Connecticut: Glutamatergic treatment strategies for schizophrenia: A translational neuroscience perspective.

Round Table Discussion with All Speakers and NIMH Staff

How to sustain the research careers of physician scientists in psychiatry.

SESSION 5: Development of Novel Therapeutics

S. Paul, Weill Cornell Medical College, New York: Drug discovery and development: Current challenges and opportunities. "It is the worst of times—It is the best of times."

SESSION 6: Development of Novel Therapeutics

C. Austin, National Human Genome Research Institute, Bethesda, Maryland: Translational therapeutics development at the NIH.

H. Zoghbi

Envisioning the Future of Science Libraries at Academic Research Institutions

April 1–3

FUNDED BY **The Alfred P. Sloan Foundation and the Rockefeller University**, New York, New York

ARRANGED BY **C. Feltes,** the Rockefeller University, New York
D. Gibson, Memorial Sloan-Kettering Cancer Center, New York
C. Norton, Marine Biological Laboratory, Woods Hole, Massachusetts
L. Pollock, Cold Spring Harbor Laboratory

As a result of social, economic, and technological factors, the role of libraries in society and academia is changing rapidly and significantly. As key service providers, libraries are expected to be up-to-date technologically and to adapt to changing circumstances and the changing needs of their users. This is particularly true of libraries catering to scientists where the changes in science publishing have been remarkable, and where the users are more likely to expect the latest technologies. This has been a source of conflict, uncertainty, and concern at many institutions. For this reason, this meeting brought together librarians, researchers, administrators, and experts in various topics relating to future developments in library science to discuss the future of science libraries at academic research institutions.

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
Introduction: L. Pollock, Library and Archives, Cold Spring Harbor Laboratory

SESSION 1: Overview of Scientific Research Libraries

Chairperson: C. Rinaldo, Ernst Mayr Library of the Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts

C. Feltes, the Rockefeller University, New York and F. Norman, National Institute for Medical Research, Mill Hill, London: The current state of scientific research libraries in the U.S. and the U.K.

K. Douglas, California Institute of Technology, Pasadena: Economic factors affecting scientific research libraries.

A. Raymond-Denise, Pasteur Institute, Paris, France: Research libraries in France: A future in progress.

K. Holmes, Washington University, St Louis, Missouri: Understanding research impact on the individual, group, and organization level: A critical role for libraries.

R. James King, National Institutes of Health Library, Bethesda, Maryland: Facilitating collaboration with researchers and clinicians.

SESSION 2: Our Changing System of Scholarly Communication

Chairperson: R. Akerman, National Research Council National Science Library, Ontario, Canada

T.S. Plutchak, University of Alabama at Birmingham: What is the true value of scientific literature?

J. Neal, Columbia University, New York: Disseminating new scientific and medical findings.

M. Ackerman, National Library of Medicine, Bethesda, Maryland: The image as the future primary research data source.

SESSION 3: Transforming Scientific Research Libraries

Chairperson: K. Chad, Kenchad Consulting, United Kingdom

Moderators: P. Mitra, Cold Spring Harbor Laboratory, and P. Thibodeau, Duke University, Durham, North Carolina

Discussion: Researchers' Expectations for Future Library Collections and Services

M. Tennant, University of Florida, Gainesville: Looking forward: A critical role for the library in research, education, and assessment.

R. Luce, Emory University, Atlanta, Georgia: Transforming research library roles into workflow support.

SESSION 4: Envisioning the Future of Scientific Research Libraries

Chairperson: D. Gibson, Memorial Sloan-Kettering Cancer Center, New York

M. Marlino, National Center for Atmospheric Research, Boulder, Colorado: Seven times around Jericho: How do we bring down the walls?

F. Heath, University of Texas, Austin: A model for scientific research libraries of the future.

T. Hickerson, University of Calgary, Alberta, Canada: Shared mission, converged programs: Libraries, archives, and the scientific record.

H. Miller, Marine Biological Laboratory, Woods Hole Oceanographic Institute, Woods Hole, Massachusetts: Data and informatics: A new realm for libraries.

General Discussion and Future Direction

M. Pollock, Cold Spring Harbor Laboratory

C. DeRosa, H. Miller

M. Marlino, R. Luce

Transcription and Cancer

April 9–12

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **J. Bradner,** Dana-Farber Cancer Institute, Boston, Massachusetts
R. Young, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts

Genetic alterations that alter signaling, transcription, and chromatin are hallmarks of cancer. New insights in transcriptional and chromatin biology, coupled with technical advances in discovery chemistry, have allowed unprecedented progress toward therapeutics that target this traditionally undruggable class of proteins. Motivated by the historic and pressing challenge of developing direct-acting inhibitors of gene regulatory complexes, participants in this meeting included leaders in the fields of transcriptional biology, chromatin biology, protein biochemistry, and cancer drug discovery.

A. Koehler, J. Bradner

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Introductory Remarks: J.D. Watson, Cold Spring Harbor Laboratory

SESSION 1: Biology of Transcription in Cancer

M. Ptashne, Memorial Sloan-Kettering Cancer Center, New York: Nucleosomes and the logic of gene regulation.

K. Adelman, National Institute of Environmental Health, Research Triangle Park, North Carolina: Pol II pausing at genes involved in cell proliferation.

K. Jones, Salk Institute for Biological Studies, La Jolla, California: SKIP connects signaling to P-TEFb elongation and splicing.

J. Espinosa, University of Colorado, Boulder, Colorado: The role of mediator in oncogenesis.

A. Shilatifard, Stowers Institute for Medical Research, Kansas City, Missouri: Trithorax/MLL (COMPASS) family of H3K4 methylases in cancer.

S. Orkin, Harvard Medical School, Boston, Massachusetts: Polycomb complex and cancer.

SESSION 2: Targeting Transcription Factors

A. Mapp, University of Michigan, Ann Arbor: Small-molecule transcriptional modulators.

A. Koehler, Broad Institute of Harvard and Massachusetts Institute of Technology, Cambridge, Massachusetts: Small-molecule probe development for transcription factors.

J. Toretsky, Georgetown University, Washington, D.C.: Small-molecule targeting of fusion protein transcription factors and the biochemistry of intrinsically disordered proteins.

SESSION 3: Myc: A Master Regulator of Cancer Pathogenesis

G. Evan, University of Cambridge, United Kingdom: The role of Myc in tumor maintenance.

R. Young, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Transcriptional amplification in tumor cells with elevated c-Myc.

SESSION 4: Epigenetic Reader Proteins

J. Bradner, Dana-Farber Cancer Institute, Boston, Massachusetts: Targeting epigenetic reader proteins.

S. Frye, University of North Carolina, Chapel Hill: Chemical biology of methyl lysine.

SESSION 5: Structural Insights into Gene Regulatory Complexes

C. Arrowsmith, University of Toronto, Canada: Structural and chemical biology of the readers and writers of histone marks.

D. Patel, Memorial Sloan-Kettering Cancer Center, New York: Structural biology of maintenance DNA methylation in mammals.

C. Wolberger, Johns Hopkins School of Medicine, Baltimore, Maryland: Structural insights into the assembly and activation of SAGA.

M. Lei, University of Michigan Medical Center, Ann Arbor: The same pocket in menin binds both MLL and JunD but oppositely regulates transcription.

M. Luo, Memorial Sloan-Kettering Cancer Center, New York: Profile nonhistone targets of protein methyltransferases.

K. Adelman

D. Weinberg, M. Ptashne, J. Darnell, R. Young

SESSION 6: Identification and Targeting of Tumor Dependencies

C. Vakoc, Cold Spring Harbor Laboratory: RNAi screening to identify roles for chromatin regulators in cancer.

S. Armstrong, Dana-Farber Cancer Institute, Boston, Massachusetts: Targeting DOT1L in MLL-rearranged leukemias.

V. Richon, Epizyme, Inc., Cambridge, Massachusetts: Targeting histone methyltransferases.

J. Grembecka, University of Michigan, Ann Arbor: Therapeutic targeting of MLL fusion proteins in leukemia.

J. Jin, University of North Carolina, Chapel Hill: Discovery of chemical probes for histone methyltransferases.

Review and Summary

A presentation at the meeting

Phage and Phage-Based Therapies

April 15–17

FUNDED BY **GangaGen, Inc.**, Newark, California

ARRANGED BY **S. Adhya,** National Cancer Institute, Bethesda, Maryland
J. Ramachandran, GangaGen Inc., Palo Alto, California
G. Schoolnik, Stanford University Medical Center, Palo Alto, California

The first Banbury conference on phage therapy was held in November 2002. Since that discussion of the potential value of phage therapy and the challenges it faced, there has been much progress in both phage science and the development of phage-based therapies. As more and more pathogens are developing resistance to the current antibiotics, there is a pressing and ever-increasing need for new therapies. This second conference on Phage Therapy was organized to review the progress in phage science, the preclinical development of phage-based therapies, and clinical experience.

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Introduction: J.D. Watson, Cold Spring Harbor Laboratory

SESSION 1: Phage Genomics and Evolution

Chairperson: D. Court, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

A. Kuchment, *Scientific American*, New York: Cowboy medicine.

G. Hatfull, University of Pittsburgh, Pennsylvania: Phage genomics and evolution.

M. Krupovic, Institute Pasteur, Paris, France: Diversity of prokaryotic viruses.

S. Moineau, Université Laval, Quebec, Canada: Phage resistance.

J. Gill, Texas A&M University, College Station: Informing phage therapy with phage genomics.

I. Connerton, University of Nottingham, Leicestershire, United Kingdom: The ecology of campylobacter phages and the carrier state.

E. Semenova, Waksman Institute, Piscataway, New Jersey: CRISPR/Cas: Bacterial adaptive immunity and memory system guided by short RNAs.

SESSION 2: Efficacy of Phage-Derived Products

Chairperson: S. Adhya, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

J. Ramachandran, GangaGen Inc, Palo Alto, California: Lysis-deficient phages and phage tails: Potential therapeutics.

B. Sriram, GangaGen Biotechnologies Pvt Ltd., Bangalore, India: Development of a novel phage-derived antistaphylococcal protein.

V.A. Fischetti, the Rockefeller University, New York: Exploiting a billion years of phage evolution to develop novel anti-infectives.

B. Peddie, University of Maryland, College Park: Engineering phage as a drug delivery vector.

R. Danner, National Institutes of Health, Bethesda, Maryland: Hospital outbreak of *Klebsiella pneumoniae* producing carapenemase (KPC).

SESSION 3: Phage Therapy

Chairperson: G.K. Schoolnik, Stanford University, California

R. Adamia, Eliava Institute, Tbilsi, Georgia: Prospects of phage therapy: East and west.

E. Stibitz, Food and Drug Administration, Bethesda, Maryland: FDA's perspective on phage therapy and specific issues involved in these proposals.

H. Brussow, Nestle Research Center, Lausanne, Switzerland: Toward a treatment of *E. coli* diarrhea with T4 phages.

A. Gorski, Polish Academy of Sciences, Warsaw, Poland: Immunomodulating effects of phage: Their implications for therapy.

J.A. Fralick, Texas Tech University Health Sciences Center, Lubbock: Applemans protocol for the generation of therapeutic bacteriophages.

B. Biswas, Naval Medical Research Center, Fort Detrick, Maryland: Applications of phage therapy in military medicine.

General Discussion and Future Directions

E. Ching, S. Subramani

E. Semenova

Interdisciplinary Symposium on Literature, Memory, and Neuroscience

April 19–21

FUNDED BY **Haig R. Nalbantian**, New York, New York
The Satenik and Adom Ourian Educational Foundation, New York, New York
Mr. and Mrs. Howard Phipps, Jr., Westbury, New York
The Daniel and Joanna S. Rose Fund, Inc., New York, New York

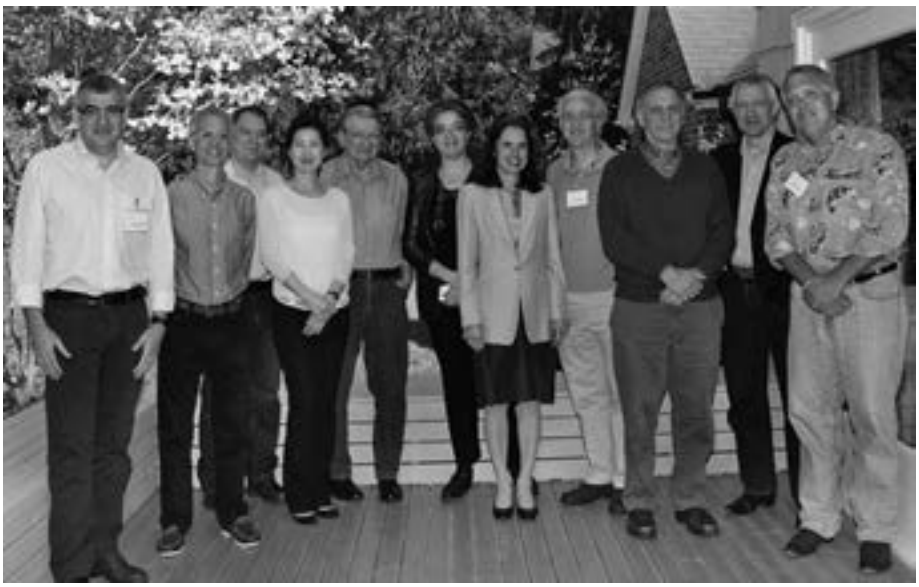
ARRANGED BY **S. Nalbantian,** Long Island University, Brookville, New York

It is now more than 50 years since C.P. Snow gave his controversial Reith Lectures on *The Two Cultures,* discussing the gulf, as Snow saw it, between the humanities and science. Snow was making specific reference to the British education system, but the phrase soon came into widespread use. This meeting, held under the auspices of the International Comparative Literature Association, might be regarded as a contribution to uniting the two cultures. Its purpose was to create cross-disciplinary exchange and collaboration between neuroscientists and literary scholars on topics of memory, emotion, consciousness, and creativity.

Keynote Speaker: S. Park, University of Oxford, United Kingdom: Cognitive Literary Criticism and the Two Cultures: With Reference to T.S. Eliot, Virginia Woolf, and Samuel Beckett.

Introduction to the Banbury Center: J.A. Witkowski, Director, Banbury Center, Cold Spring Harbor Laboratory

Background and Introduction to the Meeting: S. Nalbantian, Chair of ICLA Research Committee on Literature & Neuroscience, Long Island University, Brookville, New York



SESSION 1

L. Squire, University of California, San Diego: Conscious and unconscious memory systems of the mammalian brain.

H. Mancing, Purdue University, West Lafayette, Indiana: Struggling with memory in Spanish fiction: Miguel de Unamuno, Camilo José Cela, and Carmen Martin Gaite.

R. Epstein, University of Pennsylvania, Philadelphia: Marcel Proust and memory: A neuropsychological perspective.

SESSION 2

P. Matthews, GlaxoSmithKline, United Kingdom, and Imperial College, London, United Kingdom: Alzheimer's disease and fragmentation of the self.

J. Bickle, Mississippi State University, Mississippi State: Manipulating brain genes and proteins to affect social learning and memory.

F. Vidal, Max-Planck Institute for the History of Science, Berlin, Germany and F. Ortega, State University of Rio de Janeiro, Brazil: Brains in literature/literature in the brain: Memory and identity in Anglo-American neurofiction.

Visit to Cold Spring Harbor Laboratory Main Campus

SESSION 3

R. Stickgold, Harvard University, Boston, Massachusetts: Nonconscious memory processing in sleep and dreams.

S. Nalbantian, Long Island University, Brookville, New York: Nonconscious memory and the surrealist mind.

G. Starr, New York University, New York: Memory and aesthetics: Probing the role of imagery in literature and the visual arts.

G. Starr, L. Squire

R. Stickgold, S. Nalbantian

Patenting Genes: New Developments, New Questions

April 22–25

FUNDED BY **Baxter Healthcare Corporation of Westlake Village, California**
DRI Capital, Inc., Vancouver, British Columbia, Canada
Eli Lilly & Company, Indianapolis, Indiana
Genentech, Inc., South San Francisco, California
Jones Day LLP , New York, New York
Kaye Scholer LLP , New York, New York
King & Spalding, LLP, New York, New York
Novartis Pharma AG, Basel, Switzerland
Novo Nordisk Inc., Princeton, New Jersey
Ropes & Gray

ARRANGED BY **K. Sonnenfeld,** King & Spalding, LLP, New York
M. Brivanlou, King & Spalding, LLP, New York

In 1981, more than 30 years ago and soon after the Supreme Court's decision in Diamond v. Chakrabarty, the Banbury Center held a discussion meeting called *Patenting of Life Forms*. A second meeting in 1991 was held, and now, 20 years later, many of the very same issues raised at those two meetings continue to be contentious and the subject of intense debate. They have been brought into sharp focus by the recent Myriad case involving patents covering the *BRCA* gene, and so it seemed the right time to convene a third meeting. By bringing together lawyers, judges, clinicians, scientists, academicians, investors, and others who are directly impacted by gene patents, the conference provided a unique opportunity to examine fundamental assumptions that have provided fuel on both sides of the debate for or against gene patents.

Welcoming Remarks: J.A. Witkowski, Director, Banbury Center, Cold Spring Harbor Laboratory

Introduction: K. Sonnenfeld and M. Brivanlou, King & Spalding, New York, and **S. Brenner**, Salk Institute for Biological Studies, San Diego, California

SESSION 1: Gene Patents: Where Are We Now?

Chairperson: L. Coruzzi, Jones Day, LLP, New York

T. Stanek Rea, United States Patent and Trademark Office, Alexandria, Virginia: PTO perspective after Prometheus.
H. Sauer, Biotechnology Industry Organization, Washington, D.C.: Reflections on the right and wrong of gene patenting: Normative considerations in ethics and law.
D. Leonard, Weill Cornell Medical College, New York: SACGHS task force on gene patents.
B. Barrett, Eli Lilly & Company, Indianapolis, Indiana: Patenting genes: A biopharmaceutical company's perspective.

SESSION 2: Gene Patents Covering Diagnostics: Different Approaches

Chairperson: P. Fehlner, Novartis Pharma AG, Basel, Switzerland

R. Cook-Deegan, Duke University, Durham, North Carolina: Gene patents and diagnostics: The many paths not taken.
W. Grody, American College of Medical Genetics, Los Angeles, California: Impact of gene patents on an academic medical center laboratory.
D. Brenner, Dysautonomia Foundation, Inc., New York: Strategic use of patents by nonprofits.
P. Fehlner, Novartis Pharma AG, Basel, Switzerland: Accelerating personalized medicine: Pools, consortia, and open innovation.
R. Marsh, Myriad Genetics, Inc., Salt Lake City, Utah: The Myriad perspective.

Panel: Follow-Up Discussion of Session

SESSION 3: Gene Patents: Relevance to Development

Chairperson: C. Shepherd, DRI Capital Inc., Toronto, Canada

S. Chandrasekharan, Duke University, Durham, North Carolina: The shadow of patent thickets on emerging genomic diagnostics and whole-genome sequencing: What do empirical studies tell us?
F. Toneguzzo, Partners HealthCare, Charlestown, Massachusetts: Gene patents and implementation of diagnostic tests.
J. Elliott, Genentech, South San Francisco, California: Gene patents and business concerns.
C. Shepherd, DRI Capital Inc., Toronto, Canada: Relevance of patent claims to life sciences investing.

Panel: Follow-Up Discussion of Session

SESSION 4: Enforcement of Gene Patents

Chairperson: P. Eagleman, Baxter Healthcare Corporation, Westlake Village, California

K. Sonnenfeld, King & Spalding, LLP, New York: Mayo v Prometheus: Implications of the Supreme Court's decision.
R. Dreyfuss, New York University School of Law, New York: Interpreting the opinions of the Federal Circuit in the Myriad case: Will the Supreme Court grant review?
L. Ben-Ami, Kaye Scholer, LLP, New York: Litigating the genome: What the future holds for patent litigation.

Panel: Follow-Up Discussion of Session

K. Sonnenfeld, S. Brenner

J. Elliott

SESSION 5: Claiming Genes: In the Beginning and Today

Chairperson: M. Brivanlou, King & Spalding, New York

J. Haley, Jr., Ropes & Gray LLP, New York: Changes in claim language since Chakrabarty.

H.-R. Jaenichen, Vossius & Partner, Munich, Germany: The patenting of genes by the European Patent Office.

J. Broughton and R. Bizley, Avidity IP, Epping, United Kingdom: Whose genes are they anyway?

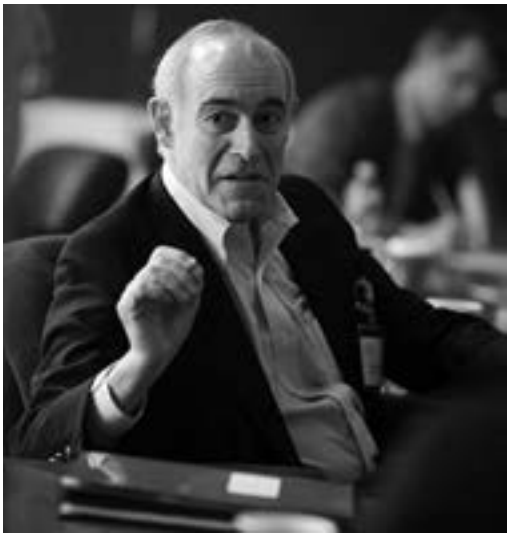
J. Cherry, Freehills Patent & Trade Mark Attorneys, Melbourne, Australia: Gene patents in Australia: Past, present and future.

Panel: Follow-Up Discussion of Session

SESSION 6: Where Next?

Review and Summary

T. Rea



R. Desnick

A History of the Human Genome Project

May 3–5

FUNDED BY **The Alfred P. Sloan Foundation**, New York, New York

ARRANGED BY **L. Pollock,** Cold Spring Harbor Laboratory
J.A. Witkowski, Cold Spring Harbor Laboratory

J.A. Witkowski

The International Human Genome Project (HGP) was one of the great scientific accomplishments, ranking with the Manhattan Project, the Hubble Telescope, and the Large Hadron Collider. However, it is only during the past few years that a movement has begun to lay the groundwork for a history of the HGP. As a first step, CSHL and the Wellcome Trust initiated a project to locate and catalog primary materials relating to the origins of the HGP by holding a meeting at Banbury in 2009. This 2012 meeting reviewed the current state of the HGP history project and plans for producing a book on the HGP, what might be needed for a long-term project, and the goals and organization of long-term projects. Participants included scientists, writers, documentary directors, social scientists, media experts, historians, and archivists.

Welcoming Remarks and Background: J.A. Witkowski, Banbury Center, Cold Spring Harbor

SESSION 1: The History of the HGP and the Public Communication

This session reviewed and discussed current projects under way relating to the history of the HGP and how these are expected to contribute to the long-term public communication about the HGP.

L. Pollock, Cold Spring Harbor Laboratory: HGP original materials.
R. Aspin and J. Shaw, Wellcome Trust, London, United Kingdom: HGP original materials.
R. Cook-Deegan, Duke University, Durham, North Carolina: DNA sequencing: Technology history, sharing practices, and applications to medicine and personal genomics.
J. Durant, Massachusetts Institute of Technology, Cambridge: Museum Genomics Consortium.

General Discussion: The opportunities for long-term public communication on the history of the HGP and its impact.

SESSION 2: General Issues and Current Book Outline

This session began with a brief review of the current book outline and then moved on to examine topics for chapters based on lists made by participants. This was a free-flowing session and each discussion ran for as long as necessary.

G. Weinstock, Washington University, St. Louis, Missouri: Introduction to the book. Discussion of the scope of the book and the current book outline.
C.T. Caskey, Baylor College of Medicine, Houston, Texas: Origins of the HGP in the United States.
J. Weissenbach, Centre National de Sequençage, Evry, France: Origins of the HGP in France.
M. Olson, University of Washington, Seattle: Cloning and mapping.
L. Smith, University of Wisconsin, Madison: Development of automated sequencing.
M. Adams, J. Craig Venter Institute, San Diego, California: Shotgun sequencing.
J. Rogers, Genome Analysis Centre, Norwich, United Kingdom: Completing the genome.
W.R. McCombie, Cold Spring Harbor Laboratory: Computational/bioinformatics.
R. Cook-Deegan, Duke University, Durham, North Carolina: Social-legal issues.
K. Davies, Cambridge Healthtech Institute, Needham, Massachusetts: Current and future genomic science and medicine.

SESSION 3: Wrap-Up and Future Developments

The Sloan Foundation has expressed an interest in a long-term project on the history of the HGP and for bringing the achievements of the HGP and future implications for health to the public. The discussions in the session could focus on the question: What advice would participants give to the Sloan Foundation about what a long-term project might include?

M. Olson, G. Weinstock

J. Rogers, J. Shaw

Regulation of Metabolism in Cancer

May 14–17

FUNDED BY **Brown Cancer Center**, University of Louisville, Kentucky

ARRANGED BY **J. Chesney,** University of Louisville, Louisville, Kentucky
J.D. Watson, Cold Spring Harbor Laboratory

Genetic and epigenetic alterations of transformed cells confer selective advantages that ultimately change their metabolic phenotype. For example, transformed cells transport increased glucose for energetic and anabolic pathways. Approaches to integrate the metabolic with the genomic, epigenetic, and transcriptional alterations of cancer should lead to the identification of novel cancer therapeutic targets. The main objectives of this meeting were to discuss (1) the biophysical and biochemical studies of the unique metabolic requirements and pathway utilizations of transformed cells, (2) emerging sequencing and computational technologies that can rapidly analyze cancer genomes and transcriptional profiles, and (3) biomedical informatics and physical approaches to integrating the metabolic, genomic, and transcriptional interactions of cancer.

R. Kalluri, J. Watson

Introduction: The Basics and Some History: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Welcoming Remarks: Origins of the Banbury Center and Concept Behind Its Conference Center: **J.D. Watson**, Cold Spring Harbor Laboratory

Brief Introductory Comments: J. Chesney, Brown Cancer Center, University of Louisville, Louisville, Kentucky

Comments: Warburg, Keilin, and Energy Metabolism: W. Koppenol, Swiss Federal Institute of Technology, Zurich, Switzerland

SESSION 1: Approaches to Understanding Metabolic Networks in Cancers

Chairperson: D. Miller, University of Louisville, Kentucky

G. Stephanopoulos, Massachusetts Institute of Technology, Cambridge: Linking cancer and metabolism via isotopic labeling and metabolic network analysis.
M. Cascante, Universitat De Barcelona, Barcelona, Spain: Metabolic flux alterations associated with cancer: Application to target discovery.
A. Lane, University of Louisville, Kentucky: Tracer methodologies, platforms, and models for cancer metabolism.
T. Fan, University of Louisville, Kentucky: How can stable isotope-resolved metabolomics bridge bench-to-bedside understanding of human cancer?

SESSION 2: Systems Biology and Epigenomics of Cancer

Chairperson: T. Rouault, National Institute of Child Health and Development, NIH, Bethesda, Maryland

A. Califano, Columbia University Medical Center, New York: Cancer systems biology: Assembling and interrogating the regulatory logic of the cancer cell.
J. Ernst, University of California, Los Angeles: Epigenomic signatures for interpreting disease associated genomic loci.

SESSION 3: Cancer Metabolism I (Imaging, mTOR and H+ATP Synthase)

Chairperson: C. Dang, University of Pennsylvania, Philadelphia

J. Koutcher, Memorial Sloan-Kettering Cancer Center, New York: Noninvasive magnetic resonance studies of tumor metabolism and inhibition.
D. Sabatini, Whitehead Institute, Massachusetts Institute of Technology, Cambridge: Regulation of growth by the mTOR pathway.
J. Manuel Cuezva, Universidad Autonoma de Madrid, Spain: The mitochondrial H+ATP synthase in cancer.

SESSION 4: Cancer Metabolism II (PI3K, Myc, and Rb)

Chairperson: C. Thompson, Memorial Sloan-Kettering Cancer Center, New York

F. Morrish, Fred Hutchinson Cancer Research Center, Seattle, Washington: Metabolic circuit flexibility in triple-negative breast cancer cells.
M. Yuneva, University of California, San Francisco: Glucose and glutamine metabolism as targets for cancer therapy.
C. Dang, University of Pennsylvania, Philadelphia: Targeting oncogenic alterations of glucose and glutamine metabolism.
B. Clem, University of Louisville, Kentucky: Retinoblastoma protein regulation of glucose and glutamine metabolism.
R. Kalluri, Beth Israel Deaconess Medical Center, Boston, Massachusetts: Metabolism and metastasis.
C. Thompson, Memorial Sloan-Kettering Cancer Center, New York: Where does NADPH come from?

SESSION 5: Tumor Microenvironment, Nutrient Sensing, and AMPK

Chairperson: L. Cantley, Harvard Medical School, Boston, Massachusetts

T. Schroeder, Duke University School of Medicine, Durham, North Carolina: The metabolic tumor microenvironment as a synthetic lethal condition.
D. Carling, MRC Clinical Sciences Centre, London, England: Regulation of lipid metabolism and role of AMPK in cancer cells.
D. Ayer, University of Utah, Salt Lake City: Integrating nutrient sensing and growth control.
N. Hay, University of Illinois, Chicago: AMPK regulates NADPH homeostasis to promote tumor cell survival during energy stress.
L. Cantley, Harvard Medical School, Boston, Massachusetts: PI3 K and cancer metabolism.

SESSION 6: Metabolism of Noncancer Cells in Neoplastic Tumors

Chairperson: T. Schroeder, Duke University School of Medicine, Durham, North Carolina

P. Carmeliet, Katholieke Universiteit, Leuven, Belgium: Targeting endothelial cell metabolism.
J. Rathmell, Duke University Medical Center, Durham, North Carolina: Lymphocyte metabolism in immunity and leukemogenesis.

SESSION 7: Fumarate Hydratase, 6-Phosphofructo-Kinase, and Metabolic Therapeutics

Chairperson: J. Eaton, University of Louisville, Kentucky

T. Rouault, National Institute of Child Health and Development, NIH, Bethesda, Maryland: Remodeling of metabolism

in familial renal cancer caused by mutations of fumarate hydratase (HLRCC) and SDHB.

E. Gottlieb, the Beatson Institute for Cancer Research, Glasgow, Scotland: Revealing metabolic adaptations and therapeutic strategies to renal cancer with fumarate hydratase mutations.

S. Telang, University of Louisville, Kentucky: Regulation of glycolysis by fructose-2,6-bisphosphate.

J. Chesney, University of Louisville, Kentucky: Development of small-molecule inhibitors of 6-phosphofructo-2-kinases.

D. Sabatini

J. Nelson, I. Jain

Emerging Approaches in Oncology: A Brainstorming Think Tank

June 13–15

FUNDED BY **University of Southern California, NCI Physical Sciences in Oncology Center**, Los Angeles

ARRANGED BY **D. Hillis,** Applied Minds, Inc. Glendale, California
D. Agus, University of Southern California, Los Angeles
P. Mallick, Stanford School of Medicine, California
T. Tombrello, California Institute of Technology, Pasadena

The goal of this meeting was to identify critical challenges in oncology and to evaluate the potential of innovative approaches for solving them. The meeting had a rather unusual structure for a Banbury Center meeting. Prior to the meeting, each participant was assigned to one of two groups (biological/clinical or technology/engineering) and selected a collaborator from the other who could engage in the project. Unlike traditional meetings, in which people present their findings, it was hoped that this would be an opportunity for participants to share emerging research challenges and to identify and evaluate creative approaches for solving them. Team meetings were held in the Conference Room and Meier House, followed by joint sessions in the Conference Room.

SESSION 1: Presentations of Teams 1 and 2

Team 1: N. Marko, Cancer Research UK, Cambridge Research Institute, United Kingdom; **S. Maurer,** D.E. Shaw & Co., New York.

Team 2: L. Xie, Hunter College, New York; **J. Brody,** Thomas Jefferson University, Philadelphia, Pennsylvania; **A. Tito Fojo,** National Cancer Institute, Bethesda, Maryland.

SESSION 2: Presentations of Teams 3 and 4

Team 3: D. Ruderman, University of Southern California, Los Angeles; J. Schnitzer, Proteogenomics Research Institute for Systems Medicine, San Diego, California.

Team 4: D. Levitin, McGill University, Montreal, Quebec, Canada; **D. Agus,** University of Southern California, Beverly Hills.

S. Hingorani

P. Mallick

SESSION 3: Presentations of Teams 5 and 6

Team 5: A. Lo, Massachusetts Institute of Technology, Cambridge; **L. Nagahara,** National Cancer Institute, Bethesda, Maryland.

Team 6: M. Gross, University of Southern California, Westside Cancer Center, Beverly Hills; **E. Gradman,** Eric Gradman, Inc., Los Angeles, California.

SESSION 4: Presentations of Teams 7–9

Team 7: M. Meyer, University of Utah, Salt Lake City; **P. Mallick,** Stanford School of Medicine, California; **S. Hingorani,** Fred Hutchinson Cancer Research Center, Seattle, Washington.

Team 8: A. Sharif, Daedalus Software, Inc., Cambridge, Massachusetts; **M. Vander Heiden,** Massachusetts Institute of Technology, Cambridge; **D. Byrd,** Seattle Cancer Care Alliance, Washington.

Team 9: B. Chaudhry, Healthcare Analytics IBM Research, Washington, DC; **A. Minn,** University of Pennsylvania, Philadelphia; **S. Hanlon,** National Cancer Institute, NIH, Bethesda, Maryland.

SESSION 5: Presentations of Teams 1–4

Team 1: N. Marko, Cancer Research UK, Cambridge Research Institute, United Kingdom; **S. Maurer,** D.E. Shaw & Co., New York

Team 2: L. Xie, Hunter College, New York; **J. Brody,** Thomas Jefferson University, Philadelphia, Pennsylvania; **A. Tito Fojo,** National Cancer Institute, Bethesda, Maryland.

Team 3: D. Ruderman, University of Southern California, Los Angeles; **J. Schnitzer,** Proteogenomics Research Institute for Systems Medicine, San Diego, California.

Team 4: D. Levitin, McGill University, Montreal, Quebec, Canada; **D. Agus,** University of Southern California, Beverly Hills.

SESSION 6: Presentations of Teams 5–9

Team 5: A. Lo, Massachusetts Institute of Technology, Cambridge; **L. Nagahara,** National Cancer Institute, Bethesda, Maryland.

Team 6: M. Gross, University of Southern California, Westside Cancer Center, Beverly Hills; **E. Gradman,** Eric Gradman, Inc., Los Angeles, California.

Team 7: M. Meyer, University of Utah, Salt Lake City; **P. Mallick,** Stanford School of Medicine, California; **S. Hingorani,** Fred Hutchinson Cancer Research Center, Seattle, Washington.

Team 8: A. Sharif, Daedalus Software, Inc., Cambridge, Massachusetts; **M. Vander Heiden,** Massachusetts Institute of Technology, Cambridge; **D. Byrd,** Seattle Cancer Care Alliance, Washington.

Team 9: B. Chaudhry, Healthcare Analytics IBM Research, Washington, DC; **A. Minn**, University of Pennsylvania, Philadelphia; **S. Hanlon**, National Cancer Institute, NIH, Bethesda, Maryland.

General Discussion and Wrap-Up

E. Gradman

Systems Biology of Autism: From Basic Science to Therapeutic Strategies

September 9–12

FUNDED BY **Marie Robertson Research Fund** and **Roberston Research Fund,** Cold Spring Harbor Laboratory, New York; **Pfizer Inc.,** Memphis, Tennessee; **Simons Foundation,** New York, New York; **Certerra, Inc.,** Cold Spring Harbor, New York

ARRANGED BY **P. Osten,** Cold Spring Harbor Laboratory
M. Sur, Massachusetts Institute of Technology, Cambridge

Genetic analyses of autism have identified a confusingly large number of genes associated with autism. One way of trying to bring some order to the field is to try to group these genes based on common pathways. A combination of different systems biology methods (mouse and other animal models, human iPS cells, genetics, and bioinformatics) could result in a powerful research synergy and lead to formulation of generalizable hypotheses about neurodevelopmental changes in autism. This meeting explored whether this was feasible. Ultimately, the goal of such research must be to develop therapies, and this requires deciding on what experimental endophenotypes will provide the most useful platforms for drug discovery. Participants included scientists studying autism genes in animal models and using human iPS cells for screening of cellular functions; geneticists and bioinformaticists; representatives from pharmaceutical companies interested in autism drug development; and systems neuroscientists.

Introduction: **P. Osten,** Cold Spring Harbor Laboratory

SESSION 1: Genetics and Gene Networks

Theme: There are many genes implicated in autism. How to guide the selection of the best candidates?

Chairperson: M. Wigler, Cold Spring Harbor Laboratory

M. Wigler, Cold Spring Harbor Laboratory: What to do with candidate gene data?

I. Iossifov, Cold Spring Harbor Laboratory: Role of de novo and rare variants in the genetics of autism.

J. Darnell

D. Geschwind, G. Dawson

D. Vitkup, Columbia University, New York: Discovering gene networks associated with ASD.
J. Darnell, the Rockefeller University, New York: Genome-wide identification of mRNA targets of translational repression by the fragile-X mental retardation protein, FMRP.
D. Geschwind, University of California, Los Angeles: Pathway convergence in autism: What might it mean?
J. Gillis, Cold Spring Harbor Laboratory: Assessing bias in network analysis of gene function.

SESSION 2: Genetics and Epigenetics in Cell and Animal Models

Continued Theme: There are many genes implicated in autism. How to guide the selection of the best candidates?
Chairperson: L. Kadiri, Certerra, Inc., Cold Spring Harbor

H. Song, Johns Hopkins University, Baltimore, Maryland: Systems biology of epigenetic mechanisms in autism.
P. Jin, Emory University School of Medicine, Atlanta, Georgia: New DNA modification(s) in neurodevelopment and autism.
R. Dolmetsch, Stanford University, California: Using stem cells and mice to study genetic forms of autism.
F. Vaccarino, Yale University, New Haven, Connecticut: Induced pluripotent stem cells to study autism: Promise and challenges.
R. Greenspan, University of California, San Diego: Exploring the broad network of behavioral gene interactions.

SESSION 3: Animal Models: Genes, Brain Circuits, and Synapses

Theme: Mouse models of selected autism genes are beginning to show a broad range of circuit and synaptic phenotypes. How to interpret the results? How to address the differences between the animal and human brain?

Chairperson: G. Fischbach, Simons Foundation, New York

Z.J. Huang, Cold Spring Harbor Laboratory: Altered maturation of GABAergic interneurons, critical period plasticity, and visual perception in a mouse model of Rett syndrome.
A. Mills, Cold Spring Harbor Laboratory: Engineered mouse models of 16p11.2CNVs.
D. Page, the Scripps Research Institute, Jupiter, Florida: Toward a mechanistic understanding of autism-relevant phenotypes in Pten haploinsufficient mice.
R. Tsien, New York University, New York: Deciphering underpinnings of autism and oxytocin enhancement of circuit signal to noise. K. Huber, University of Texas Southwestern Medical Center, Dallas: Destabilized mGluR5 synaptic scaffolds, fragile X, and autism.

SESSION 4: Animal Models: Behavioral Studies and Translational Opportunities

Theme: Can animal behavior and other animal assays be used as biomarkers in preclinical drug discovery?
Chairperson: C. Schutt, Princeton University, the Nancy Lurie Marks Family Foundation, New Jersey

H. Sive, Whitehead Institute, Cambridge, Massachusetts: Lessons from zebra fish.
J. Crawley, University of California, Davis, Sacramento: Pharmacological reversal of social deficits and repetitive behaviors in mouse models of autism.
A. Kepecs, Cold Spring Harbor Laboratory: Sniff and decide: Quantitative assessments of social behavior in mice.
M. Sur, Massachusetts Institute of Technology, Cambridge: Signals that affect synapses in autism: Insights from Rett syndrome.
P. Osten, Cold Spring Harbor Laboratory: Mapping brain circuits in genetic mouse models of autism and preclinical drug discovery.

L. Young, Emory University, Atlanta, Georgia: Oxytocin and social cognition: Implications for novel therapies for autism.

SESSION 5: Therapeutic Work and Clinical Trials

Theme: How basic research can help and guide clinical work and starting clinical trials?
Chairperson: W. Chung, Columbia University, New York

J. Spiro, Simons Foundation, New York and W. Chung, Columbia University, New York: Studying ASD through the context of an identified recurrent genetic event (16p11.2).

A. Healy, Seaside Therapeutics, Cambridge, Massachusetts: Clinical trials in FXS: Effects on neurobehavioral function and strategies to develop molecular biomarkers

E. Anagnostou, Bloorview Research Institute, Toronto, Canada: Oxytocin and social cognition/function: Early clinical studies.

G. Dawson, University of North Carolina, Autism Speaks, Chapel Hill: Autism spectrum disorders: Challenges in bridging the gap from preclinical research to clinical trials.

G. Lyon, Cold Spring Harbor Laboratory: Clinical progress in autism genetics and treatment.

Review and Summary

C. Schutt, R. Greenspan

Inspire2Live

September 14–16

FUNDED BY **Inspire2Live,** Utrecht, Netherlands

ARRANGED BY **Inspire2Live Discovery Network Team,** Amsterdam, Cambridge, Oxford, Paris, New York, Boston, Seattle

The Inspire2Live Foundation was created with the aim of motivating as many people as possible to constantly challenge and expand their boundaries and to raise funds to fight cancer by organizing fund-raising events. The Foundation has shifted its funding strategy toward mobilizing a team of committed sponsors. To this end, the presidents of the Alp d'HuZes fund-raiser and the Dutch Cancer Foundation KWF came to Banbury Center together with senior members of the cancer research community. The meeting began with updates on the scientific research of the Foundation's program, followed by presentations on the clinical aspects of the program. Discussion then turned to the Foundation's funding plans, followed by a detailed focus on setting up the organizational necessities needed in 2012 in preparation for 2013.

Welcoming Remarks: J.A. Witkowski, Director, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Update on the Scientific Parts

Moderator: A. Eggermont, President, Institut Gustave Roussey, Paris, France

M. Stratton, Sanger Institute, Cambridge: Overall program need and impact.

H. Clevers, the Hebrecht Institute, the Netherlands: Organoids.

M. Stratton, the Sanger Institute, Cambridge, United Kingdom: Screening.

M. Meyerson, Dana-Farber Cancer Institute, Boston, Massachusetts: Sequencing.

S. Friend, Sage Bionetworks, Seattle, Washington: Model sharing.

SESSION 2: Update on the Clinical Parts

J. Baselga, Massachusetts General Hospital, Boston: Plan of a breast program and team incorporating organoids, other models, screening, sequencing, and trials.

D. Tuveson, Cold Spring Harbor Laboratory: Plan of the pancreas program and team incorporating organoids, other models, screening, sequencing, and trials.

C. Sawyers, Memorial Sloan-Kettering Cancer Center, New York: Plan of the prostate program and team incorporating organoids, other models, screening, sequencing, and trials.

E. Coen van Veenendaal/Rob Snelders, Inspire2Live Foundation: Overall program approach and funding requests.

The Science Team met in the conference room and focused on setting up the organization and planning for the program.

Moderators: H. Clevers, S. Friend, P. Kapitein

The Funders Team discussed the necessary steps during 2012 to get the program up and running in 2013 and beyond.

Moderators: A. Eggermont, E. Coen van Veenendaal, R. Snelders

Feedback of Funders Team and Science Team Moderators on Their Approach

General Wrap-Up Discussion

J. Watson, E. Coen van Veenendaal, A. Trounson

B. Conley

Plant–Environment Interactions

September 18–21

FUNDED BY **CSHL/DuPont Pioneer Joint Collaborative**

ARRANGED BY **M. Timmermans,** Cold Spring Harbor Laboratory
M. Komatsu, DuPont Pioneer, Wilmington, Delaware
R. Martienssen, Cold Spring Harbor Laboratory
S. Tingey, DuPont Pioneer, Wilmington, Delaware

The goals of this meeting were to review the latest advances in our understanding of plants' responses to abiotic environmental cues and pathogens, and during the establishment of symbiotic associations. These reviews were expected to drive discussions on current research addressing natural variation and adaptation to biotic and abiotic stresses. The meeting included, in addition to members of the CSHL/DuPont Pioneer Joint Collaborative, speakers from outside the collaboration.

Welcoming Remarks: **J.A. Witkowski,** Director, Banbury Center, Cold Spring Harbor

Introduction: **M. Komatsu,** DuPont Pioneer, Wilmington, Delaware

SESSION 1: Inflorescence Development

Chairperson: M. Komatsu, DuPont Pioneer, Wilmington, Delaware

Z. Lippman, Cold Spring Harbor Laboratory: A surprising link between meristem maintenance and pollen development in tomato and *Arabidopsis*.

B. Il Je, Cold Spring Harbor Laboratory: Fasciated ear3, a potential new CLAVATA receptor.

O. Danilevskaya, DuPont Pioneer, Johnston, Iowa: Maize ear development under drought stress.

SESSION 2: Small RNAs and Cell Fate Specification

Chairperson: D. Ware, Cold Spring Harbor Laboratory

F. Van Ex, Cold Spring Harbor Laboratory: Argonautes, small RNA, and germ cell fate.
M. Dotto, Cold Spring Harbor Laboratory: Update on collaborative projects: tasiR-RNA pathways in maize and new players in leaf polarity.
M. Timmermans, Cold Spring Harbor Laboratory: Small RNAs as instructive signals in development.

SESSION 3: Regulation of Yield

Chairperson: B. Williams, DuPont Pioneer, Wilmington, Delaware

D. Jackson, Cold Spring Harbor Laboratory: Maize meristem signaling and yield.
A. Mohanty, DuPont Pioneer, Hyderabad, India: Mapping of novel genetic loci in rice for improvement of hybrid producibility and defensive traits.
R. Williams, DuPont Pioneer, Wilmington, Delaware: Drought lead characterization: Using *Arabidopsis* as a model to support Ag traits.
K. Jiang, Cold Spring Harbor Laboratory: Molecular dynamics of dosage in tomato single gene heterosis.

SESSION 4: Epigenetic Gene Regulation

Chairperson: Z. Lippman, Cold Spring Harbor Laboratory

J. Reinders, DuPont Pioneer, Wilmington, Delaware: Maize methylome project: Pilot study review and update on epigenetic variation induced by drought stress.
A. Olson, Cold Spring Harbor Laboratory: Building and classifying coding and noncoding gene models with transcriptome and methylome sequencing data.
R. Martienssen, Cold Spring Harbor Laboratory: The maize methylome.

SESSION 5: Responses to Abiotic Environmental Cues

Chairperson: M. Timmermans, Cold Spring Harbor Laboratory

C. Fankhauser, University of Lausanne, Switzerland: Light regulation of plant growth and development.
J. Dinneny, Stanford University, California: Spatiotemporal control of environmental response.
J. Schroeder, University of California, San Diego: Drought/abscisic acid signaling and chemical genetic dissection of immune: ABA interference mechanisms.
T. Mitchell-Olds, Duke University, Durham, North Carolina: Complex traits in complex environments.
D. Ware, Cold Spring Harbor Laboratory: Understanding stress-related traits through root-specific gene networks.

SESSION 6: Symbiosis and Biotic Stress Responses

Chairperson: M. Komatsu, DuPont Pioneer, Wilmington, Delaware

U. Paszkowski, University of Lausanne, Switzerland: *Arbuscular mycorrhizal* symbiosis in cereals.
H. Bouwmeester, Wageningen University, the Netherlands: The role of strigolactones in plant–environment interaction.

M. Timmermans, H. Bouwmeester

M. Albertsen, Z. Lippman

G. Oldroyd, John Innes Center, BBSRC, Norwich, United Kingdom: Nutrient acquisition by plants through symbiotic associations.

D. Bulgarelli, Max-Planck Institute, Koln, Germany: Structure and functional significance of the root-inhabiting bacterial microbiota.

P. Wolters, DuPont Pioneer, Wilmington, Delaware: Disease resistance in maize: Identification of genes involved in resistance to *Colletotrichum graminicola* and their use in maize breeding.

G. Rairdan, DuPont Pioneer, Wilmington, Delaware: Exploiting the plant-pathogen "arms race" to engineer resistance to Asian soybean rust.

H. Bouwmeester, P. Wolters

SESSION 7: Molecular Analysis of Developmental Progressions

Chairperson: P. Wolters, DuPont Pioneer, Wilmington, Delaware

S.-J. Park, Cold Spring Harbor Laboratory: Genetic and molecular dissection of tomato shoot architecture.

M. Javelle, Cold Spring Harbor Laboratory: An expression atlas of functional domains in the shoot apical meristem.

J. Calarco, Cold Spring Harbor Laboratory: The pollen methylome.

A. Eveland, Cold Spring Harbor Laboratory: Unraveling the developmental networks controlling determinacy and inflorescence architecture in maize.

General Discussion: S. Tingey and R. Martienssen

O. Danilevskaya, D. Jackson

Decoding Clinical Trials to Improve Treatment of ME/CFS

September 30–October 3

FUNDED BY **CFIDS Association of America,** Charlotte, North Carolina, **Centers for Disease Control and Prevention**, Atlanta, Georgia

ARRANGED BY **S. Vernon,** CFIDS Association of America, Charlotte, North Carolina
R. Silverman, Cleveland Clinic Lerner Research Institute, Ohio
E. Unger, Centers for Disease Control and Prevention, Atlanta, Georgia
J.A. Witkowski, Cold Spring Harbor Laboratory

The focus of this meeting was on chronic fatigue syndrome treatment trials. Chronic fatigue syndrome, now referred to as ME/CFS (myalgic encephalomyelitis/chronic fatigue syndrome), is a severe and debilitating illness characterized by a constellation of nonspecific symptoms including fatigue, cognitive impairment, muscle pain, joint pain, disturbed sleep, and general weakness. The objective of this workshop was to review the science behind the efficacy or lack of efficacy of treatment trials in ME/CFS. This was to be accomplished by bringing investigators who have conducted ME/CFS pharmacological randomized controlled trials (RCTs) that have a high level of evidence together with experts in clinical methodology, pharmacology, molecular biology, and ME/CFS. The output of this meeting is to be a peer-reviewed publication describing the workshop findings and proposing a set of guidelines that will help optimize study design and clinical methodology of future RCTs for ME/CFS.

SESSION 1: Background and Clinical Impressions

Chairpersons: R. Dodd, American Red Cross, Rockville, Maryland, and **A. Waring** (rapporteur), University of California, Los Angeles, Torrance

Guidelines for Session 1: Chairpersons captured key points from each presentation to use these during the breakout sessions. Speakers included relevance of topic to CFS characteristics of CFS populations and cohorts being discussed.

J.A. Witkowski, Director, Banbury Center, Cold Spring Harbor Laboratory: Welcoming remarks.

V. Racaniello, Columbia University, New York: Viruses and chronic disease.
K.K. McCleary, CFIDS Association, Charlotte, North Carolina: Evidence for and against microbial pathogens in CFS.
J.-M. Lin, Centers for Disease Control and Prevention, Atlanta, Georgia: Healthcare utilization in CFS.
M. Cooperstock, University of Missouri Health Care, Columbia: Clinical perspectives on pediatric postinfection CFS.
F. Maldarelli, National Cancer Institute, Bethesda, Maryland: Clinical perspectives on an adult CFS population selected to study XMRV.
D. Cook, University of Wisconsin, Madison: The metabolic profile of PEM.

SESSION 2: Lessons Learned from CFS Treatment Trials

Chairpersons: V. Racaniello, Columbia University, New York, and **K.K. McCleary** (rapporteur), CFIDS Association of America, Charlotte, North Carolina

Guidelines for Session 2: Chairpersons captured key points from each presentation to use these during the breakout sessions. Speakers described study design (e.g., case/control), selection of study subjects (e.g., volunteer and advertisement), inclusion/exclusion criteria, trial setting, and outcome measures and characteristics of CFS populations/cohort being discussed.

P. Rowe, Johns Hopkins University, Baltimore, Maryland: Lessons from the florinef trial.
C.-G. Gottfries, Gottfries Clinic, Moindal, Sweden: The use of dopaminergic stabilizer OSU6162 in treatment of fatigue.
K. Rowe, the Royal Children's Hospital, Victoria, Australia: Lessons from gamma globulin treatment trial.
J. G. Montoya, Palo Alto Medical Foundation, California: Viral and immune profiles of responders to valcyte.
L. Bateman, the Fatigue Consultation Clinic, Salt Lake City, Utah: Lessons from treatment of CFS patients with ampligen.
N. Klimas, University of Miami School of Medicine, Florida: Can response to drugs help us reverse-engineer CFS?

SESSION 3: Considerations for CFS Treatment Trial Design

Chairpersons: R. Bromley, TRAC Consulting, Redwood City, California, and **K. Morren**, ServiceSource, Denver, Colorado

Guidelines for Session 3: Chairpersons captured key points from each presentation to use these during the breakout sessions. Speakers described aspects of the clinical trial study design impacted by their presentation topic.

Ø. Fluge, Department of Oncology and Medical Physics, Haukeland University Hospital, Bergen, Norway: Benefit from B-cell depletion in CFS.
E. Crawley, University of Bristol, United Kingdom: Considerations when planning trials in children with ME/CFS.
J. Jones, Center for Disease Control and Prevention, Atlanta, Georgia: Choice of control subjects in CFS clinical trials.
L. Chang, David Geffen School of Medicine at UCLA, Los Angeles, California: Comorbid conditions and impact of CFS treatment trials.

M. Cooperstock, S. Vernon, L. Bateman, K. Rowe

E. Unger, Centers for Disease Control and Prevention, Atlanta, Georgia: Deconstructing CFS: Search for endophenotypes reflecting pathogenesis.
M. Demitrack, Neuronetics, Inc., Malvern, Pennsylvania: Treatment resistance: Real or just not the right drug?

SESSION 4: Plausible Therapeutic Targets

Chairpersons: S. Deftereos, Biovista, Inc., Charlottesville, Virginia, and **A Persidis** (rapporteur), Biovista Inc., Charlottesville, Virginia

Guidelines for Session 4: Chairpersons captured key points from each presentation to use these during the breakout sessions. Speakers described where in the biomarker and clinical trial pipeline the data in their presentation "fits" and what is required to move it to the next phase.

I. Biaggioni, Vanderbilt University School of Medicine, Nashville, Tennessee: Is sympathetic tone a target in CFS?
G. Broderick, University of Alberta, Canada: Network analysis for druggable target identification.
S. Shukla, Marshfield Clinic Research Foundation, Marshfield, Wisconsin: The "CFS" microbiome as a possible therapeutic target.
M. Medow, New York Medical College, Hawthorne, New York: Brain blood flow and brain fog in CFS.
P. McGowan, University of Toronto, Scarborough, Canada: Targeting methylation as a CFS treatment.
S. Deftereos, Biovista Inc., Charlottesville, Virginia: Preliminary drug repurposing findings in CFS.
B. Munos, InnoThink Center for Research in Biomedical Innovation, Indianapolis, Indiana: How to energize drug innovation for CFS.

SESSION 5: Advancing the Science of Medicine for CFS

Three breakout groups met from 9 am to 10 am to brainstorm questions and issues generated by the session chairpersons. Each group produced an outline that was presented to the workshop participants following the break.

Breakout Group 1: Subtypes vs. symptoms: What is the best strategy for CFS treatment trials? (Sessions 1 and 3)
Led By: I. Biaggioni and L. Bateman
Group 1 Participants: M. Cooperstock, E. Crawley, C.-G. Gottfries, M. Medow, K. Rowe, P. Rowe, E. Unger, A. Waring

Breakout Group 2: Moving treatment forward now: How and who are the players? (Session 2)
Led By: B. Munos and N. Klimas
Group 2 Participants: M. Demitrack, S. Deftereos, R. Dodd, J. Jones, N. Klimas, F. Maldarelli, K.K. McCleary, J. Montoya, S. Vernon

Breakout Group 3: Strategies for data analysis to identify therapeutic targets. (Session 4)
Led By: R. Bromley and A. Persidis
Group 3 Participants: G. Broderick, D. Cook, J.-M. Lin, P. McGowan, K. Morren, V. Racaniello, S. Shukla, R. Silverman, A. Waring

Presentation and Discussion of Group 1 Report: L. Bateman
Presentation and Discussion of Group 2 Report: N. Klimas
Presentation and Discussion of Group 3 Report: A. Persidis

Review of Workshop Outcome(s): S. Vernon

S. Vernon, S. Shukla

K.K. McCleary

Grand Challenges in Organismal Biology

November 8–11

FUNDED BY **National Science Foundation through a grant to Stony Brook University**

ARRANGED BY **D. Padilla,** Stony Brook University, New York
B. Swalla, University of Washington, Seattle
B. Tsukimura, California State University, Fresno

The National Research Foundation has established Research Coordination Networks (RCNs) in various fields, intended to "…advance a field or create new directions in research or education by supporting groups of investigators to communicate and coordinate their research, training, and educational activities across disciplinary, organizational, geographic, and international boundaries." Investigators came to Banbury to consider the potential value of an RCN for metazoan organismal biology, specifically the issue "How organisms walk the tightrope between stability and change" and, more broadly, "What will be needed as far as infrastructure to solve complex problems and interactions across scales?" One of the goals of the meeting was to plan a full-scale workshop on the topic.

Welcoming Remarks: J.A. Witkowski, Director, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Goals of Meeting, Introductions, and Approaches to Answering Big Questions

D. Padilla, Stony Brook University, New York: Background and goals; Introductions–Who are we?

B. Swalla, University of Washington, Seattle: What is an RCN and how can they work? EDEN–the Evo-Devo-Eco Network RCN.

D. Padilla, Stony Brook University, New York: Synthesis Centers.

B. Tsukimura, California State University, Fresno: iPlant.
D. Manahan, University of Southern California, Los Angeles: Thinking integratively in organismic biology: Lessons from decade-long NSF graduate training programs.
T. Daniel, University of Washington, Seattle: New approaches needed to address big questions.

Group Discussion: Think big—"Blue sky" ideas about needs associated with specific plans of attack on the GCOB.

SESSION 2: Breakout Groups

Priorities for making progress on this GCOB (more data, more cross disciplinary collaboration, a new generation of thinkers?), and best paths to getting there (cyberinfrastructure, synthesis of existing data, new mathematical models?).

Wrap-Up Discussion

SESSION 3: Agenda for the Next Workshop

General Discussion: Setting agenda for the big workshop, types of participants, and scientific expertise needed.

Breakout Groups: Recommendations, narrowing scope.
Reports and Discussion: Agenda for workshop, homework for workshop participants, recommended areas of expertise, and action plan for Steering Committee.
Breakout Groups: Additional elements that need to be included.

Group Discussion
Breakout Groups: Agenda for workshop and homework for workshop participants.

Report Out and Group Discussion

SESSION 4: Finalize Action Plans

For agenda, homework, and types of scientists to include in next workshop.
For Steering Committee.

Wrap-Up Discussion

Final Thoughts: Set dates for the workshop, nominating participants/research areas

C. Hayashi

T. Daniel, W. Zamer

Cell Plasticity in Cancer Evolution

November 11–14

FUNDED BY **Astellas-OSI Oncology, Farmingdale, New York**

ARRANGED BY **J. Condeelis,** Albert Einstein College of Medicine, Bronx, New York
D. Epstein, Astellas-OSI Oncology, Farmingdale, New York
J. Haley, Astellas-OSI Oncology, Farmingdale, New York

Banbury Center has been the location for several meetings on the epithelial-mesenchymal transition (EMT) in cancer. This is the most studied form of cellular plasticity and is characterized by the combined loss of epithelial cell junction proteins and cell polarity and the gain of mesenchymal markers. More recently, EMTs have been characterized in which the interconversion of vessels and fibroblastic elements can contribute to cancer pathogenesis and fibrosis. These findings have important implications for cancer treatment and prevention. For example, cellular sensitivity to multiple targeted therapies, chemotherapy, and radiotherapy was shown to be governed by the extent to which cells have undergone an EMT-like transition. Resistance associated with cellular plasticity and heterogeneity has been observed in multiple systems derived from adenocarcinomas and squamous carcinomas. The aim of this conference was to explore the molecular and pathobiological significance of cellular plasticity in carcinomas and to further elucidate the signaling pathways that promote plasticity.

Welcoming Remarks: J.A. Witkowski, Director, Banbury Center, Cold Spring Harbor Laboratory

Introduction: R. Kalluri, Harvard Medical School, Boston, Massachusetts

SESSION 1: Epithelial, Endothelial, and Neuroendocrine Plasticity

R. Kalluri, Harvard Medical School, Boston, Massachusetts: Cell plasticity and its energy requirements.

D. Lyden, Weill Cornell Medical College, New York: Tumor-derived exosomes promote plasticity at the premetastatic niche.

J. Condeelis, Albert Einstein College of Medicine, Bronx, New York: EMT and *trans*-endothelial migration during breast tumor cell dissemination.

W. Lowry, University of California, Los Angeles: Molecular mechanisms of stem-cell-initiated carcinoma.

T. Brabletz, University of Freiburg Medical Center, Germany: MicroRNAS, EMT, and cancer stem cells.

General Discussion

SESSION 2: Cell Plasticity in Model Systems

B. Stanger, University of Pennsylvania, Philadelphia: Analysis of cellular plasticity in an autochthonous model of pancreatic cancer.

A. Biddle, University of London, England: Developing an in vitro model for characterization of heterogeneous and plastic cancer stem cell phenotypes, and for therapeutic development.

W. Guo, Albert Einstein College of Medicine, Bronx, New York: Plasticity of epithelial cell hierarchy: induction of the stem-cell state in the breast.

A. Patsialou, Albert Einstein College of Medicine, Bronx, New York: Cell plasticity in breast tumor invasion: The cell's decision to go or grow.

General Discussion

SESSION 3: Signaling Networks in EMT and CSCs

A. Cano, Instituto de Investigaciones Biomedicas, Madrid, Spain: Regulation of cellular plasticity and the tumor microenvironment by lysyl oxidases (LOX, LOXL1-4).

D. McClay, Duke University, Durham, North Carolina: Transcriptional control of EMT-subcircuits control each cell biological component.

R. Carstens, University of Pennsylvania, Philadelphia: The role of alternative splicing in EMT and cancer.

H. Nakagawa, University of Pennsylvania, Philadelphia: Notch regulation in squamous cancer cell plasticity and mitochondrial functions.

P. Keely, University of Wisconsin, Madison: Matrix stiffness in regulating the proliferation and metabolic plasticity of cancer cells.

General Discussion

SESSION 4: Epigenetics and Cellular Conversions

S. Baylin, Johns Hopkins University, Baltimore, Maryland: DNA methylation, cancer stem cells and implications for cell plasticity.

C. Chaffer, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Cellular plasticity: The role of epigenetics in generating CSCs from non-CSCs.

C. Kleer, University of Michigan Medical School, Ann Arbor: Role of the epigenetic regulator EZH2 in breast cancer initiation and progression.

R. Thompson, St. Vincents Hospital, Melbourne, Australia: Epithelial Mesenchymal Plasticity (EMP) in Breast Cancer Dissemination–What to target and when?

G. Van der Pluijm, Leiden University Medical Centre, the Netherlands: BMP7, epithelial plasticity, and metastasis.

General Discussion

SESSION 5: Impacts of Cell Conversion on Cancer Therapy

J. Engelman, Massachusetts General Hospital, Charlestown: Evolution of cancers through tyrosine kinase inhibitors in lung cancer.

R. Sordella, Cold Spring Harbor Laboratory: An epigenetic switch modulates cancer cell-state plasticity by regulating Mir-335 expression.

J. Rosen, Baylor College of Medicine, Houston, Texas: EMT programs, therapeutic resistance, and cancer stem cells.

J. Haley, Astellas-OSI Oncology, Farmingdale, New York: Cell plasticity and drug resistance.

S. Alford, Massachusetts Institute of Technology Center for Cancer Research, Cambridge: Dysregulation of EGFR signaling during invasion and metastasis.

J. Condeelis, Albert Einstein College of Medicine, Bronx, New York:

Wrap-Up: Specific Issues and Priorities

Inflammation, Cancer, and Metabolism

November 26–29

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **D. Green,** St. Jude Children's Hospital, Memphis, Tennessee
L. O'Neill, Trinity College, Dublin, Ireland

Otto Warburg showed that tumor cells performed aerobic glycolysis and proposed that this metabolic change was fundamental for pathogenesis of cancer. Mutations in important metabolic enzymes have been shown to be important for tumorigenesis, and more recently, molecular insights into the basis of the Warburg effect have emerged, including the roles of the transcription factor HIF-1α and also enzymes such as PKM2. Immunologists have also recently turned their attention to changes in immune cell function, and the Warburg effect is now known to occur in activated macrophages and certain T-cell lineages. Other metabolic processes, including those involving AMP kinase and mTOR, are also now seen as important for the immune and inflammatory processes. A number of questions arise. Why are these metabolic changes occurring and what is their mechanistic basis? Might changes in metabolism during cancer and inflammation be critical for disease development? Might these metabolic changes provide an explanation for the link between inflammation and cancer? Is there a prospect here that new treatments might emerge from these insights?

Welcoming Remarks: **J.A. Witkowski,** Director, Banbury Center, Cold Spring Harbor

Introduction and Meeting Goals: **L. O'Neill,** Trinity College, Dublin, Ireland

SESSION 1: Cancer

E. Gottlieb, Beatson Institute for Cancer Research, Glasgow, United Kingdom: Metabolomics approaches in cancer research.

R. DeBerardinis, University of Texas Southwestern Medical Center, Dallas, Texas: Diversity of core metabolic pathways in human cancer cells.

M. VanderHeiden, Massachusetts Institute of Technology, Cambridge: Regulation of anabolic metabolism.
G. Kroemer, Institut Gustave Roussy, Villejuif, France: Immunosurveillance induced by conventional anticancer therapies: Metabolism matters.

General Discussion

SESSION 2: Macrophages/TLRs

L. O'Neill, Trinity College, Dublin, Ireland: Metabolic regulation of inflammatory signaling by Toll-like receptors and inflammasomes.
D. Underhill, Cedars-Sinai Medical Center, Los Angeles, California: Linking phagocytosis and microbial degradation to inflammatory signaling.
D. Green, St Jude Children's Research Hospital, Memphis, Tennessee: Noncanonical autophagy in innate immunity.
M. Netea, Radboud University, Nijmegen, the Netherlands: Trained immunity: Metabolic pathways involved in innate monocyte reprogramming.

General Discussion

SESSION 3: Microbiome, Inflammation, and Obesity

M. Murphy, University of Cambridge, United Kingdom: How mitochondrial ROS can modulate metabolism.
J. Ayres, Salk Institute for Biological Studies, San Diego, California: Host–microbiota interactions in health and disease.
M. Saleh, McGill University, Montreal, Canada: Innate detection mechanisms and inflammation in obesity and type-2 diabetes.
V. Stambolic, University of Toronto, Princess Margaret Hospital, Ontario, Canada: The relationship between obesity and cancer: The insulin connection.

General Discussion

SESSION 4: T Cells

R. Xavier, Massachusetts General Hospital, Boston: Metabolic pathways in immunity.
D. Cantrell, University of Dundee, Scotland: Metabolism, migration, and memory in cytotoxic T cells.
N. Chandel, Northwestern University, Chicago, Illinois: Mitochondrial ROS regulate T cells.
R. Siegel, National Institute of Arthritis and Musculoskeletal and Skin diseases, NIH, Bethesda, Maryland: Mitochondrial reactive oxygen species and autoinflammatory disease.
G. Matarese, University of Salerno, Italy: Oscillatory intracellular metabolic pathways control immune tolerance.

General Discussion

SESSION 5: T Cells

H. Chi, St Jude Children's Research Hospital, Memphis, Tennessee: mTOR and metabolic pathways in T-cell fate decisions.
J. Powell, Johns Hopkins University, Baltimore, Maryland: mTOR: Master integrator of T-cell metabolism, differentiation, and function.
J. Rathmell, Duke University Medical Center, Durham, North Carolina: Glucose uptake in lymphocyte activation and subsets.
E. Pearce, Washington University School of Medicine, St Louis, Missouri: Posttranscriptional control of T-cell function by Warburg metabolism.
M. Karin, University of California, San Diego: Virchow explained: The origin of tumor elicited inflammation and its significance.

General Discussion

M. Saleh

R. Xavier, M. Murphy

DNA LEARNING CENTER

DNA LEARNING CENTER EXECUTIVE DIRECTOR'S REPORT

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Mary Lamont
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Many science educators search for ways to scale student research from local, individual projects to distributed, class-based experiments that involve many students working simultaneously on aspects of the same problem. DNA barcoding fulfills the promise of modern, Internet-enabled biology—allowing students to work with the same data, with the same tools, at the same time as high-level researchers. Just as the unique pattern of bars in a universal product code (UPC) identifies each consumer product, a short "DNA barcode" (~600 nucleotides in length) is a unique pattern of DNA sequence that can potentially identify any living thing. DNA barcoding projects can stimulate independent student thinking across different levels of biological organization, linking molecular genetics to ecology and evolution, with the potential to contribute new scientific knowledge about biodiversity, conservation biology, and human effects on the environment. DNA barcoding also integrates different methods of scientific investigation, from in vivo observations to in vitro biochemistry to in silico bioinformatics.

DNA barcoding provides a practical way to bring open-ended experimentation into biology classes. Projects can operate at various scales, from working with other students to investigate a local ecosystem, museum collection, or conservation issue, to joining an International Barcode of Life "campaign" to explore an entire taxonomic group or global biome. Projects may also take on a forensic slant, when students attempt to identify product fraud (such as mislabeled food items) or to identify the sources of commercial products (such as plants or animals used in traditional medicines). The core lab and phylogenetic analysis can be mastered in a relatively short time, allowing students to reach a satisfying research endpoint within a single academic term. Using DNA barcoding as the common method across a range of projects decreases the need for intensive, expert preparation and mentoring, thus providing a practical means to engage large numbers of students in meaningful research.

To help realize this promise, during the past 2 years, we have devoted ourselves to developing an integrated biochemical and bioinformatics (B&B) workflow for DNA barcode analysis. The biochemistry uses noncaustic reagents to isolate DNA from plant, animal, or fungal organisms. The barcode region is amplified by polymerase chain reaction (PCR) and visualized by agarose gel electrophoresis. The barcode amplicons are mailed to GENEWIZ Inc., a company that provides inexpensive sequencing—$3.00 per forward and reverse read. Within 48 hours, the finished barcode sequences are automatically uploaded to *DNA Subway*, the DNALC's intuitive bioinformatics workflow for education developed under the National Science Foundation (NSF) *iPlant Collaborative* (described below).

The Blue Line of *DNA Subway* includes all tools needed to visualize and edit barcode sequences, search GenBank (www.ncbi.nlm.nih.gov/genbank) for matches, align sequences, and construct phylogenetic trees. The Blue Line features web applications that heretofore could only be used as stand-alone applications, including an electropherogram viewer/editor and a "zoomable" sequence aligner/barcode viewer. An export feature simplifies barcode sequence submissions to GenBank, automatically providing sequence files, associated metadata, and sequence annotations in the required National Center for Biotechnology Information (NCBI) format.

The barcode experiment, including extensive teacher prep and planning for both lab and bioinformatics components, is available in three formats: the online lab notebook at www.dnabarcoding101.org, the lab-text *Genome Science: A Practical and Conceptual Introduction to Molecular Genetic Analysis in Eukaryotes* (Cold Spring Harbor Laboratory Press), and a stand-alone kit marketed by our collaborator, Carolina Biological Supply Company (CBSC).

Urban Barcode Project

With a grant from the Alfred P. Sloan Foundation, we tested the B&B workflow in the context of the *Urban Barcode Project (UBP)*, a high school science competition spanning the five boroughs of New York City (NYC). A dedicated Internet site (www.urbanbarcodeproject.org) supported all aspects of the project, including vodcasts on barcoding and student projects, protocols, guidelines for proposal preparation, and database management tools for tracking student projects and metadata. Participating during the 2011–2012 academic year were more than 100 teams comprising more than 300 students (37% Asian, 32% White, and 31% Latino or African-American) from 31 public and nine private high schools. Students worked on their

A *UBP* student works on her project during an *Open Lab* at *Harlem DNA Lab*.

Following the *UBP* Symposium award ceremony at AMNH, the 10 finalist teams display their prizes with *UBP* staff and judges.

projects in the schools—often using freely available DNALC footlockers—or at 70 *Open Lab* sessions at *Harlem DNA Lab*, and Genspace, a nonprofit citizen science center in Brooklyn. DNALC staff also made more than 40 school visits to assist with experiments and phylogenetic analyses. Teams collected and processed over 1000 samples for DNA sequencing, with over 2500 single sequences provided by GENEWIZ.

Seventy-five teams—comprising 218 students from 30 high schools—completed their projects in time to present their results at two poster sessions held at the American Museum of Natural History (AMNH) on May 31 and June 1. The projects spanned five categories: (1) wildlife in parks and public spaces, (2) commercial products and trade in endangered species, (3) food mislabeling, (4) public health and disease vectors, and (5) exotic and invasive species. A jury of 34 experts in biodiversity, conservation biology, DNA barcoding, and education selected the top 10 teams, who gave oral presentations at the AMNH on June 6. The Grand Prize team from Hostos Academy of Sciences in the Bronx won $10,000 for their finding that many herbal Ginkgo products contained little to no *Ginkgo biloba* DNA. Other finalists shared $10,000 in prizes for projects including the first DNA barcoding census of fungi in Central Park, mapping clades of Atlantic silversides and killifish in New York coastal waters, identifying species of bed bugs in Brooklyn through bacterial DNA, comparing morphological identification versus DNA barcoding of ants in the South Bronx, and improving the identification of insects seized by U.S. Customs and Border Protection at Newark International Airport.

Importantly, *UBP* students produced 65 novel DNA sequences that did not match existing GenBank data. These barcodes included new sequence differences (polymorphisms), as well as new records of species for which no barcode had been previously reported. Using the simplified export feature of *DNA Subway*, these sequences were submitted to GenBank with student and teacher authors. The published sequences represented animals, plants, and fungi collected in a range of student projects: 36 from wild organisms collected in parks or natural areas; 13 from products purchased in markets and shops around the city; seven from exotic or invasive species; six from invertebrate disease vectors; and three from investigations of food mislabeling.

Surveys of participating teachers suggested that the *UBP* will potentially improve science instruction for many students beyond those who actually did barcoding projects. A majority said they plan to implement barcoding concepts, independent research, and bioinformatics exercises into a range of general biology, *AP Biology*, and biology electives. Surveys and structured interviews overwhelmingly showed that students appreciated the ownership of their projects and the sense of "doing real science." For most, it was their first experience with open-ended research.

Compared to previous experiences with science fairs or competitions, the vast majority of students thought the *UBP* had provided "much more" experience in learning science, doing independent inquiry, understanding the scientific process, developing critical thinking skills, and increasing interest in science careers. Thus, in the context of the nation's largest school system, we have demonstrated that DNA barcoding projects can be scaled to introduce large numbers of students to authentic research.

Barcode Long Island

David Liittschwager (*above,* in hat) works with barcoding students collecting specimens at CSHL and photographing specimens (*below*) at the DNALC.

During the summer, we explored a different format for student DNA barcoding—a sustained "campaign" to explore the biodiversity of Cold Spring Harbor. The diversity of biological niches found at the interface of land and water was the impetus for founding the Biological Laboratory at Cold Spring Harbor in 1890 as a field station for the study of evolution in the natural world. For several decades, naturalists came for summer courses to study organisms of the intertidal zone, salt marshes, sand spit, and open waters of the harbor and Long Island Sound. Their sustained and detailed work formed the basis for the modern field of ecology. When Cold Spring Harbor Laboratory's (CSHL) research shifted toward genetics, several generations of local school students carried on the ecological tradition during summer *Nature Study Courses* conducted from the 1940s through to the mid 2000s.

The *Barcoding Biodiversity* student workshops marked a return to CSHL's roots. Naturalist photographer David Liittschwager joined us for the first workshop. David's stunning photographs of life in 1 cu ft samples from habitats in Costa Rica, French Polynesia, South Africa, Tennessee, and New York appeared in the February 2010 issue of *National Geographic*. David instructed students in how to tease out all of the visible plants and animals (1 mm or larger) in a "biocube" and trained DNALC staff how to take publication-quality photographs.

Ninety-seven students, including 20 students from Beijing No. 166 High School, participated in six workshops. The students collected and processed more than 300 samples, obtaining 165 high-quality DNA barcodes. Most DNA barcodes came from aquatic invertebrates such as bryozoans, hydrozoans, gastropods, amphipods, isopods, and crustaceans. We published 30 novel sequences to GenBank with students as primary authors. Among the organisms for which there were published barcode sequences was a still unidentified "mini" nudibranch (a 3-mm snail without a shell) and an inch-long mud crab (*Eurypanopeus depressus*). Students identified three sequence polymorphisms in the mud crab, indicating a great deal of genetic diversity and suggesting that the southern members of this group may be a different species.

An unidentified nudibranch specimen.

New Experiments in Epigenetics

We continued a collaboration with CSHL professor Rob Martienssen and North Carolina State researcher Bill Thompson on their NSF-funded project "Epigenome Dynamics during DNA Replication." Epigenetics describes heritable phenomena that affect gene expression without changing

the DNA sequence. Methylation—the addition of methyl groups (CH_3) to DNA—is one type of epigenetic effect. In the simplest case, methyl groups attached to a promoter region block binding of transcription factors needed to express a gene. The pattern of DNA methylation is stably inherited through at least several generations.

Although epigenetics is an increasingly important area of genome research, it is not widely taught in classes. Thus, during the year, we completed development of two labs that demonstrate epigenetic effects in model plants: maize and *Arabidopsis thaliana*. The experiments will be released as kits in 2013 from CBSC.

A simple observational experiment looks at epigenetic inheritance of the *R* gene, which controls the expression of a dark red pigment in maize kernels. The *R* allele is dominant over the *r* allele, which produces no pigment (yellow kernels). At first glance, kernels from a cross between two heterozygous plants (*Rr*) conform to the expected 3:1 phenotypic ratio of red pigmented to unpigmented kernels. However, close inspection reveals that one-third of pigmented kernels are actually yellow-speckled. Methylation at the *R* promoter (*R**) disrupts pigment production in some cells in the developing kernels and the kernels show a yellow-speckled pattern. Extending molecular analysis to the maize *R* locus is difficult, because the imprinted region has not yet been precisely mapped. The methylated *R** is inherited from the male pollen, making this an example of imprinting.

An advanced experiment examines the molecular basis of epigenetics in plants by relating flowering time to DNA methylation. In *Arabidopsis,* methylation of the *FWA-1* promoter region in DNA silences the gene in wild-type adults, whereas loss of methylation causes late flowering in *FWA-1* mutants. To study this phenomenon, students isolate DNA from wild-type and *FWA-1* mutant plants. Digestion with the restriction enzyme *McrBc*, which recognizes methylated regions, cleaves the methylated wild-type promoter but leaves the unmethylated *FWA-1* promoter intact. Subsequent PCRs amplify a product from the intact *FWA-1* DNA, but none from the wild-type DNA.

Genome Science Published

At year's end, we received the first copies of *Genome Science*: *A Practical and Conceptual Introduction to Molecular Genetic Analysis in Eukaryotes*. The work is the culmination of 15 years of development by co-authors Dave Micklos, Bruce Nash, and Uwe Hilgert, with artwork by Susan Lauter and former DNALC designer Stephen Blue and help from many staff, students, teachers, and granting organizations. *Genome Science* follows the DNALC's original lab text *DNA Science: A First Course in Recombinant DNA Technology*. Now in its second edition, *DNA Science* has sold more than 90,000 copies and is credited with helping catalyze the movement to bring hands-on experiments with DNA into high school and beginning college classrooms. Two experiments are now found in the *AP Biology* curriculum, giving them a nationwide audience. Associated kits from CBSC reach well over 100,000 students per year.

Like its predecessor, *Genome Science* aims to help beginners use modern tools to explore the unseen world of genes. However, the new book aims to take students to a higher level of biological and technological integration to study the function of eukaryotic genes and genomes. Nineteen laboratories focus on four revolutionary technologies—PCR, DNA sequencing, RNA interference (RNAi), and bioinformatics—across three eukaryotic systems—humans, plants, and the

roundworm *Caenorhabditis elegans*. The work includes our newest labs on DNA barcoding and the *FWA-1* epigenetic switch in *Arabidopsis*. All labs stress the modern synthesis of molecular biology and computation, integrating in vitro experimentation with in silico bioinformatics. In addition to well-tested biochemical methods, *Genome Science* introduces *DNA Subway* to make easy work of gene and genome analysis.

The four major technologies are organized into chapters with extensive text introductions that place related labs into a common historical and conceptual framework. This modular approach provides easy options to integrate sets of labs into existing courses, provide the basis for new courses, or serve as the foundation for student research projects. *Genome Science* borrows many user-friendly features from its predecessor, including flow charts, marginal notes, reagent recipes, and extensive instructor information. To ease implementation, most labs are available as CBSC kits.

iPlant Collaborative

In contemplating the cosmos in 1927, the great mathematical geneticist J.B.S. Haldane famously said, "My own suspicion is that the universe is not queerer than we suppose, but queerer than we can suppose." Had he been alive today, Haldane would almost certainly have the same suspicion about the genomes of higher organisms. In this sense, genome scientists are the new cosmologists of biology, uncovering the strange and beautiful structure of the genetic material that runs through all life. Like cosmology, genome science is based on the collaborative analysis of massive data sets that exceed the capacity of desktop computing and sometimes even outstrip the available resources of an entire institution.

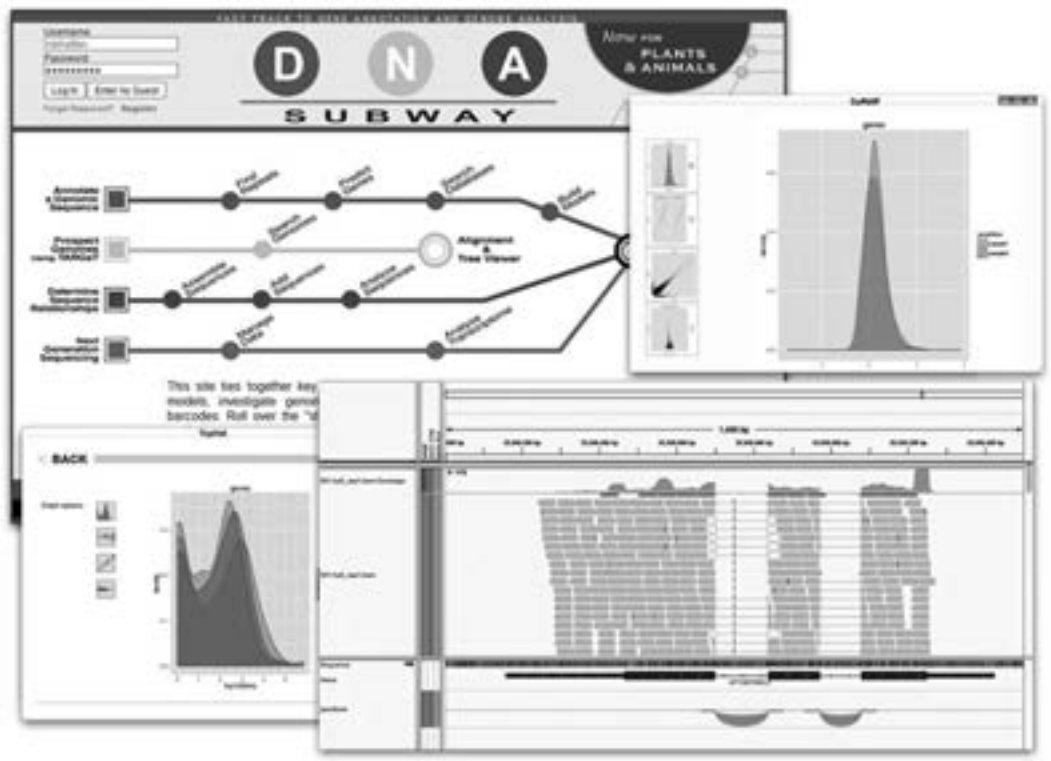
The *iPlant Collaborative* is an NSF-funded project to develop a national computer infrastructure (CI) to support biologists working within the new paradigm of large-scale genomic, phenomic, ecological, and phylogenetic data. The primary components of the *iPlant* CI include the *Data Store*, *Atmosphere* cloud computing, and the *Discovery Environment* analysis platform. The DNALC takes the lead in Education, Outreach, and Training (EOT) among consortium partners at the University of Arizona, the University of Texas, and CSHL. The EOT group has developed a sophisticated computational and Internet design group that occupies a unique niche in science education. EOT leverages scientific knowledge, teaching pedagogy, and bioinformatics expertise to translate the *iPlant* CI into workflows that are accessible across the continuum of research and education from "entry-level" and education-oriented users to sophisticated bioinformaticians and computational scientists.

We continued to evolve *iPlant's* educational discovery environment, *DNA Subway*. In keeping with the increased use of the *iPlant* infrastructure across the diversity of life, the Red Line (for gene annotation) and Blue Line (for barcode and phylogenetic analysis) were upgraded with appropriate algorithms to analyze mammals, birds, fish, insects, and fungi. The Blue Line became the analysis workhorse for the *Urban Barcode* and *Barcode Long Island* Projects described above, helping to double registered accounts to 4254 and increasing visitation 40% (to 33,376) over the previous year. We devoted substantial resources to develop the Green Line, which aims to make next-generation sequence (NGS) analysis of eukaryotic genomes accessible to a broad audience of first-time users. The Green Line integrates the Tuxedo Protocol, a workflow incorporating open source components for all steps of RNA sequence (RNA-Seq) data analysis from the processing of raw data from major sequencing platforms through to publication-quality results. The Green Line will be integrated with the Red Line, where assembled transcripts can provide evidence for community (or class) annotation of sequenced genomes. The Green Line articulates directly with

high-performance clusters at the Texas Advanced Computing Center, providing what we believe to be the first easy biological on-ramp to the national supercomputing highway.

We worked very hard to get the word out about *iPlant*, holding 24 2-day training workshops in 2012, in addition to dozens of seminars and talks at professional meetings and universities. From Arkansas to Alaska, 340 researchers and 201 educators attended *Tools and Services* and *Genomics in Education* workshops, respectively, introducing them to advanced computational resources for science and teaching. This year, we provided additional travel awards for 40 minority faculty and faculty who reach large numbers of Hispanic or African American students, minorities that are underrepresented in science study and careers (awardees reported an average of 47% underrepresented students in their classes).

In 2012, we assumed responsibility for internal *iPlant* project evaluation and began to coordinate with East Main Educational Consulting, LLC, to complement its existing program of external evaluation. Of the respondents to a *Genomics in Education* workshop follow-up survey ($n = 241$), 80% had used the workshop materials, reaching more than 1600 students. The materials were used in a range of courses, including general biology (34%), genetics/genomics (30%), molecular biology (19%), and bioinformatics (13%). Materials were used for background information (59%), class resources (37%), and laboratory protocols (23%). One in five (21%) had developed a new topic, wet lab, or bioinformatics lab. Participants also shared materials with colleagues (20%) and provided training in bioinformatics (15%). Of 60 respondents to a follow-up survey of the *Tools and Services* workshop, 57% had used at least one *iPlant* tool, with 57% using *Data Store* and 31% using *Atmosphere* or the *Discovery Environment*. Respondents used the tools to analyze their own data (54%), a colleague's data (30%), or to share data (27%). Encouragingly, 68% of respondents introduced *iPlant* tools and services to colleagues, and 41% provided training.



The *DNA Subway* Green Line includes visualizations of RNA-sequence reads and graphs detailing differences in gene expression.

We distributed a "community" survey at the American Society of Plant Biologists meeting. Of 51 respondents, 88% said they currently used large data sets or expected to in the next 1–5 years. This suggests that a large majority of plant researchers are potential users of the *iPlant* CI. Structured interviews with 15 attendees revealed major challenges in extracting, storing, and sharing data; keeping abreast of current tools; collating tools into one workflow; and learning how to perform analyses.

Nationwide Faculty Training

The DNALC continued its tradition of providing cutting-edge biochemical and bioinformatics training for educators at sites nationwide. In 2012, 743 educators and scientists participated in workshops conducted at 30 sites across the United States. Overall, underrepresented minorities in science composed 19% of educators trained at 37 workshops of 1–10 days. An additional 466 educators and scientists attended seminars, short workshops, and presentations at professional meetings, including the American Society of Plant Biologists, Botanical Society of America, CSHL President's Council, International Barcode of Life, National Association of Biology Teachers, National Science Teachers Association, International Plant & Animal Genome Conference, and the Purdue Statistics Symposium. (For a complete list of training activities and host sites, see "2012 Workshops, Meetings, and Collaborations" at the end of this report.)

During the last 2 years, with funding from the NSF, we have collaborated with community college faculty and the National Advanced Technological Education (ATE) Center for Biotechnology (Bio-Link) to develop and disseminate *Genomic Approaches in BioSciences*. This workshop introduces the four key technologies included in *Genome Science*—PCR, DNA sequencing, RNAi, and bioinformatics—which provide a scientific foundation for biotechnology careers in the genome age. The first seven workshops were held in 2011 and 2012, reaching 155 faculty (of 272 applicants). Forty-three percent of participants taught at 2-year colleges, 31% at 4-year colleges or universities, and 26% at high school. One-fourth of participants were minorities that are underrepresented in science. Summer workshops were conducted at Austin Community College (Austin, Texas), Bluegrass Community and Technical College (Lexington, Kentucky), and Southern Maine Community College (South Portland, Maine). Follow-up activities were conducted at 2011 workshop sites: Gwinnett Technical College (Lawrenceville, Georgia), Madison Area Technical College (Madison, Wisconsin), Shoreline Community College (Shoreline, Washington), and Universidad del Turabo (Gurabo, Puerto Rico). We are carefully tracking the impact of the *Genomic Approaches in BioSciences* Workshops. Pre-workshop ($n = 145$) and post-workshop ($n = 142$) surveys showed notable increases in faculty knowledge and confidence. Before the workshop, only 12% of participants knew "a lot" about the key genomic concepts, compared with 41% afterward. There was a marked increase in participants who felt confident in teaching the genomics labs (17% to 41%) and bioinformatics (8% to 34%). Follow-up surveys ($n = 70$) found strong classroom implementation of the four genomic technologies—DNA barcoding (41%), PCR (55%), RNAi (22%), and bioinformatics (42%)—as well as career modules (75%). These faculty reported 9029 student exposures to labs, 4796 to bioinformatics, and 2605 to biotech careers, with an average of 46% underrepresented minority students.

New York City Faculty Training

In 2012, we concluded the Howard Hughes Medical Institute (HHMI) teacher-training program, which we administered in collaboration with the NYC Department of Education (DOE). This program was designed to develop a strong base of 8–12th grade science teachers who can competently introduce six experiments in genetics and biotechnology at identified points in state-required science courses. During the 4-year term of the grant, 835 NYC teachers completed

an average of 15 hours of training each, accounting for 1642 lab exposures and 9852 workshop hours. In 2012, 101 teachers took one or more workshop sessions, and 45 completed a 24-hour sequence of certificate training. Eighty-five percent of workshop participants taught 8th–10th grades, our target audience, including 38% African or Hispanic American teachers. Preworkshop and postworkshop surveys showed significant knowledge gains, and 13 faculty also participated in advanced leadership training.


Melissa Lee (*far left*), *Harlem DNA Lab* Manager, works with NYC teachers.

To ease classroom implementation, in 2010 funding from the Richard Lounsbery Foundation allowed us to develop 15 biotechnology Footlocker Kits for use by teachers trained in the HHMI program. Each Footlocker contains equipment and expendable supplies sufficient for three to four classes; a modest restocking fee of $50 per class of 32 students is less than half the cost of a commercial reagent kit. During a 2-year period, Footlockers were used to do experiments with 5708 students, 68% of whom were underrepresented minorities. Footlocker rental fees were waived for 75% of schools with high proportions of disadvantaged students, based on Title I status. An experimental study compared outcomes for students in classes that implemented the HHMI labs versus classes that did not. We focused on restriction analysis, because a paper-based lab simulation is a mandatory component of the NYS Regents biology curriculum, *Living Environment*. To control for teacher proficiency, we recruited study participants from the pool of HHMI-trained teachers, who taught the mandated *Living Environment* curriculum. To control for student variance we chose 9th or 10th grade general studies classes. Student learning was assessed using a standardized quiz, which incorporated Regents exam questions on DNA structure and function, restriction enzymes, gel electrophoresis, and applications of restriction analysis.

Teachers who had completed an HHMI workshop on restriction analysis were in the experimental arm of the study and implemented the wet lab using a Footlocker. Teachers who had completed a different HHMI workshop were in the control arm and used the mandated paper-based activity. Twelve teachers and 294 students (115 experimental, 179 control) participated in the study. Results showed that doing the hands-on DNALC biotechnology lab improved student learning by almost a full letter grade over the pen-and-paper equivalent (68% versus 60% quiz scores, $p < 0.001$). The wet lab also impacted students' interest in science more than the paper-based lab, with 46% versus 35% of students saying they were now more interested in science.

Student Lab Programs

On October 29, Hurricane Sandy made an appearance along the northeastern seaboard, leaving parts of New York and New Jersey completely immobilized. The DNALC and most school systems were without power for at least a week. School closings and adjustments in school calendars to make up for lost days resulted in 25 cancelled lab field trips. Despite this unanticipated setback, 19,327 students attended lab field trips at our three facilities: Dolan DNALC, DNALC *West*, and *Harlem DNA Lab*. An additional 7221 students received in-school instruction from DNALC staff members.

Summer camp enrollment reached an all-time high in 2012, with 1069 student participants in 55 weeks of camps conducted at eight locations on Long Island and NYC: Dolan DNALC,

DNALC *West,* Stony Brook University (SBU), Brookhaven National Laboratory, Mulligan Elementary School, the Chapin School, and Trinity School. Among the campers were 20 students from Beijing No. 166 High School, the first specialized school of life sciences in the Chinese capital. This was the second cohort of students from Beijing to participate in a 3-week immersion program that included classical genetics, human and plant genomics, and independent research using DNA barcoding.

We focused considerable resources on underserved youth. Grants from the Dana Foundation and the William Townsend Porter Foundation provided scholarships totaling $51,000 for 2209 students to attend lab field trips at the *Harlem DNA Lab*. Three-quarters of the students receiving scholarships were underrepresented minorities from Title I schools. We also provided intensive enrichment to students from two schools housed with the *Harlem DNA Lab* in the John S. Roberts Educational Complex. Students from the Coalition School for Social Justice participated in three biotechnology laboratories, and 6th–8th graders from MS 45 enjoyed seven introductory genetics laboratories. A $15,000 grant from the National Grid Foundation supported in-school instruction for 996 5th–7th graders from Central Islip School District. Students used microscopes to view plant and animal cells, extracted DNA from living cells, and observed mutations in fruit flies. Twelve 5th–6th graders received scholarships to participate in a *Fun with DNA* summer camp at Central Islip's Mulligan Elementary School.

During the spring, fall, and winter, 249 students (10 years and older) and parents participated in monthly *Saturday DNA!* sessions. DNALC staff members provided hands-on investigations of a variety of topics, including family genealogy, unicellular organisms, dinosaur evolution, the Romanov family mystery, DNA extraction, protein purification, gene regulation, the agricultural revolution, and the role of carbon dioxide in our lives.

We continued to work intensively with Trinity School and the Chapin School, who belong to our Charter Membership program for independent schools in the New York metro area. The program includes field trips to the DNALC and in-school instruction by DNALC instructors, as well as exclusive, on-site summer camps for students. In September, Convent of the Sacred Heart School in Greenwich, Connecticut, became the third Charter Member school. Together, we planned an extensive program of in-school visits for their environmental science, biology, *AP Biology*, and chemistry across 5th through 8th grade classes. With a strong research program already in place, they wasted no time organizing workshops using *C. elegans* as a model to study gene function and launching several student projects with roundworms.

Amanda McBrien, DNALC Assistant Director for Education, teaches pipetting techniques to *Green Genes* summer camp students.

Bob Stallone, James Watson, and Dave Micklos at the DNALC *West* celebration.

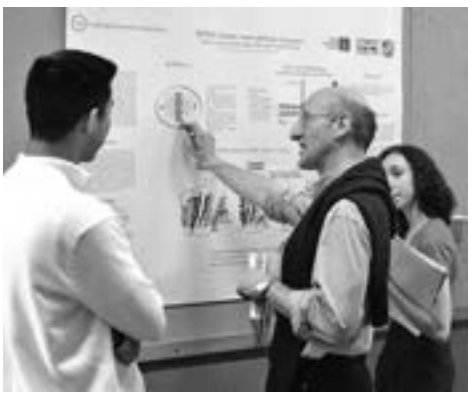
In May, we celebrated the 10th anniversary of DNALC *West*, the first licensed DNALC affiliate. Developed in collaboration with the North Shore–Long Island Jewish (NS-LIJ) Health System, DNALC *West* opened in 2002 in space adjacent the NS-LIJ Clinical Core Laboratory in Lake Success. During its first decade, DNALC *West* provided lab field trips for 28,163 students, and intensive summer camps were attended by 1919 students. With strong support from CSHL Trustee Arthur Spiro, DNALC *West* was originally supported by a core grant from NS-LIJ's Feinstein Institute for Medical Research. When Feinstein funding lapsed in 2011 and there was no ready source of funds during the recession, Bob Stallone, Vice President of NS-LIJ Laboratories, stepped up to continue funding for DNALC *West*. Thus, we were doubly thankful when Bob hosted a cocktail reception to honor the long collaboration—with guest of honor, CSHL Chancellor Emeritus James Watson and NS-LIJ leaders, including Dr. James Crawford, Senior Vice President for Laboratory Services and Chair of Pathology, and Dr. Christine Ginocchio, Senior Director of Infectious Disease Diagnostics.

By year's end, the Dolan DNALC had received a mini-facelift. A larger lunchroom was created within former exhibit space and the former lunchroom was redesigned as our fourth teaching lab. Although our exhibit space shrank, we maintained most of the popular elements of the "Genes We Share" exhibit. The room on genetic origins and evolution, affectionately called "the Cave," was recreated in the main exhibit area. New Lab #4 is our smallest teaching space; at 525 square feet, it demonstrates the most compact floor plan that can accommodate 24 students. It is also our most beautiful lab. A wall of windows, with mullions designed as a visual metaphor for the DNA bands in an electrophoresis gel, looks serenely out onto a patio and landscaped hillside. The Dolan DNALC prep labs, carved out of the crusty basement in 1989 and not updated since, were remodeled and reorganized to better support a range of activities, including inorganic reagent preparation, bacterial and *C. elegans* culture, microscopy, student research, and new experiment development. We also reorganized the storeroom as a center to restock the Footlockers that support in-school instruction by DNALC staff and are used independently by classroom teachers. These Footlockers were used by 8937 students in 2012.

Graduate Training and Student Research

We continued to support the graduate training of the CSHL Watson School of Biological Sciences (WSBS) students. From January through March, the students work under the watchful eye of experienced DNALC instructors to learn effective teaching techniques that prepare them to be better science communicators. During the first phase of training, each student team observed a DNALC instructor performing a restriction analysis experiment with a class of local high school students. Phase two, co-instruction, involved preparing a lesson plan that integrated their own perspectives, then delivering the practical portion of the lab to high school students. DNALC instructors provided constructive critique to help the WSBS students prepare for the third phase of training: team teaching the entire experiment to a high school class. After additional reflection, this learning cycle was repeated with a DNA extraction lab for middle school students, which required increased pacing. Upon completion of both high school and middle school teaching rotations, each WSBS student team delivered three additional lessons to demonstrate mastery of instructional and classroom management skills.

We continued our collaboration with the New York Academy of Sciences, training young scientists to help improve science literacy among high-needs students throughout NYC. DNALC instructors worked with 67 graduate students and postdoctoral researchers participating in the Academy's after-school mentoring program. Each mentor received 6 hours of training in effective strategies to provide hands-on laboratories to middle school students.



CSH high school students discuss their project poster on the MAD2 protein with a CSHL "Cell Cycle" meeting participant.

Our partnership with Cold Spring Harbor High School (CSHHS) to offer a college-level laboratory course entitled *Molecular and Genomic Biology* continued. Co-instructed by DNALC staff members and CSHHS faculty member Jaak Raudsepp, the year-long course occupies the last two periods of the day and alternates instruction between CSHHS and the Dolan DNALC. The 2011–2012 cohort, our seventh class, focused on hands-on experimentation and independent projects across a range of biological systems. Highlights of the year included units presented by WSBS graduate students Colleen Carlston, Katie Liberatore, and Eugene Plavskin. Colleen assisted in setting up a genetic screen in the roundworm *C. elegans* that identified three new genes that affect RNAi function. Katie taught a unit, "Cell Signaling: Communication, Development and Disease." Eugene guided experiments on environmental factors that affect fitness and natural selection in bacteria. Joan Kiely, of SBU's Biotechnology Teaching Center, followed up with a unit on modeling proteins involved in cell signaling. Student teams selected proteins, developed computer files describing their structure, and then used a 3D printer to output small-scale models. The students presented their work during a poster session of the CSHL "Cell Cycle" meeting on May 18.

Building on the success of the CSHHS partnership, we offered a similar course for students from St. Dominic High School in Oyster Bay. The course capitalized on St. Dominic's new science building, made possible through a major gift from a parishioner. In collaboration with science teacher Teresa Kuehhas, DNALC staff introduced students to experimental methods for future independent projects: species diversity using DNA barcoding, gene function using RNAi in *C. elegans*, and human variation using PCR.

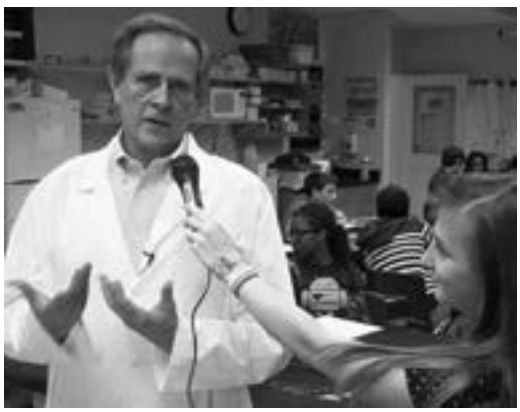
The annual *Great Moments in DNA Science* seminar series attracted 185 top Long Island high school students for presentations on current biological research at CSHL. Michael Feigin explained how cells generate and maintain shape and how defects in these processes can lead to human disease. Jonathan Ipsaro discussed new scientific methods to study the

micro-scale orchestration of DNA and proteins that sustain life. Shane McCarthy discussed advances in genome technology that provide insights into the neurobiology and treatment of schizophrenia.

BioMedia Projects and Visitation

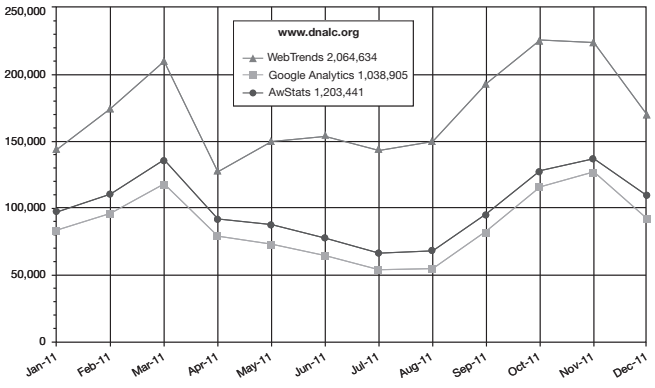
In spring 2012, we launched the *Weed to Wonder* e-book and enhanced website, developed as outreach for an NSF research project with CSHL's Marja Timmermans and Mike Scanlon at Cornell University. *Weed to Wonder* shows the continuity of research on corn—from Native American agriculturalists to agricultural breeders, corn geneticists, plant physiologists, and molecular biologists—that culminated in the Maize Genome Sequencing Project. The interactive e-book is available as a website, tablet app, or printable PDF. Six chapters tell the story of the development of modern maize, including domestication and hybrid vigor, genome sequencing and transposon complexity, and genetic modification and biofortification. The e-book revolves around video footage from Mexico, interviews with prominent scientists, and animations of different approaches to sequencing the maize genome. The history of maize research is brought to life through reconstructions of George Shull and Barbara McClintock's work at CSHL, rare photographs, and links to original publications and artifacts. The e-book also includes a time-lapse video of growing maize plants, from germination, through growth spurts, to pollination, senescence, and harvest.

We also completed the *Lab Center* microsite (http://labcenter.dnalc.org/harlem), which supports the six genetics and biotechnology labs in the HHMI-funded program. The final task was the production of *New York Stories*, a series of minidocumentaries about past or current work of notable scientists based at New York institutions. Students from NYC high schools contributed significantly to writing and production of the last three videos in the set. Teams of 9th and 11th grade students from Trinity School worked on productions about DNA fingerprinting and restriction enzyme analysis, while a team of 11th and 12th graders from Brooklyn International High School worked on a production about Thomas Hunt Morgan's "Fly Room" at Columbia University. Each video features a scientist "explainer": Larry Kobilinsky of John Jay College of Criminal Justice on the use of DNA fingerprinting in forensics; former CSHL scientist Scott Lowe, now of Memorial Sloan-Kettering Cancer Center, on the use of restriction analysis techniques in cancer research; and CSHL researcher Glenn Turner on the use of "Morgan's fly," *Drosophila melanogaster,* as a model in neuroscience research. The videos are featured on the *Lab Center* website and on YouTube (www.youtube.com/dnalearningcenter). To date, the set has received more than 5000 views.



Trinity School students interview their teacher (*above*) and classmates for *New York Stories*.

During the year, we continued our effort to disaggregate and redistribute content across different multimedia platforms. The majority of *BioMedia* content is now available in an original content site or app, as well as through our searchable database on www.dnalc.org and our YouTube channel. We also feature and cross-link resources in our social media accounts (Facebook and Twitter), blogs, presentations, and publications. A good example of multitasking is the Punnett square interactive originally developed for our iPhone app,



Gene Screen. It was reformatted for iPad, and as a website for personal use or on a classroom Smartboard.

Counting visitors to the DNALC family of 21 websites is important, but complicated. *Webtrends* was the dominant analytic software at the inception of many of our websites, and we used it faithfully over the years to track long-term trends in visitation to all of our websites. So it was with some trepidation that in 2012, we transitioned all of our websites to the *Google Analytics* package, which has become the de facto standard for measuring web visitation.

To ensure that we can analyze trends retrospectively, we compared 2011 visitation data for the DNALC homepage (www.dnalc.org) using *Webtrends*, *Google Analytics*, and a third tool, *AWStats*. As shown above, the three tools use different rules to analyze the same web logs, resulting in different numbers of visitors. This is primarily because *Google Analytics* defines one visit as up to 60 minutes of viewing versus 20 minutes for *Webtrends*. (For example, if a visitor spends 60 minutes at a site, *Google Analytics* counts one visit whereas *Webtrends* counts three.) There are also other differences in how the tools count views of images, videos, and Flash animation files.

Although the overall trends are similar—with March and November peaks, and a July trough—the results from *Webtrends* and *Google Analytics* cannot be accurately scaled to compare year-to-year changes in visitation across all of our websites. We used *Google Analytics* to compare 2011 and 2012 data for the DNALC homepage, revealing a 42% increase to 1.48 million visitors. Overall, *Google Analytics* counted 4,213,086 visits to our suite of 21 DNALC websites in 2012. Our *YouTube* videos received 865,899 views, and the *3D Brain*, *Weed to Wonder*, and *Gene Screen* apps were downloaded 568,879 times. Therefore, total multimedia visitation—websites, YouTube, and smartphone/tablet apps—was 5.65 million. This will become the new baseline against which we measure future visitation.

Staff and Interns

In 2012, the DNALC bid farewell to two staff members: Tedhar (Tedi) Setton, middle and high school educator, and Dr. Alexandra Manaia, Ph.D., *UBP* outreach team member. Tedi left the DNALC to begin a master's program in human genetics at Tulane University, where she will pursue a degree in medicine. She joined our educational team in 2008, following studies in biological science at Wellesley College and forensic pathology at the Suffolk County Medical Examiner's Office. Tedi brought spirit and high energy to her teaching, helping us establish a new summer camp in forensic biology for middle school students.

Dr. Alexandra Manaia returned to her native Portugal to coordinate the Ph.D. program at Lisbon Medical School. Alexandra's internship at the DNALC, including a key role in establishing the *UBP,* fulfilled the final phase of her Fulbright Fellowship and master's degree in International Education Development at Columbia University. Alexandra met Dave Micklos in 2003 at a kickoff meeting of the European Learning Laboratory for the Life Sciences, an education and outreach facility at the European Molecular Biological Laboratory in Heidelberg, where she was then a science officer.

In December 2012, we welcomed Brooke Roeper and Katie McAuley to our DNALC teaching staff. Raised in northeastern Ohio, high school educator Brooke Roeper received a bachelor's degree in biology from Kenyon College. After doing research on West Nile virus at Case Western Reserve University, she earned a master's in science at Tulane University's School of Public Health

and Tropical Medicine and a master's in ecology at Colorado State University. She went on to work as a middle school science teacher in Wyoming, a ranger at Wind Cave National Park, and, most recently, outreach coordinator at Teddy Roosevelt Sanctuary in Oyster Bay.

Katie McAuley, a native Long Islander and graduate of Harborfields High School, has come full circle to the DNALC in the role of middle school educator. Solving the "Mystery of Anastasia" on a class field trip to the DNALC inspired her to pursue science in college. As an undergraduate at the University of Rhode Island, Katie added education to biology for a double major. Her life experiences included studying abroad at Macquarie University in Sydney, Australia, and the University of the South Pacific, Fiji, and helping students build robots in Lego's *Bricks 4 Kidz* program.

Brooke Roeper (*left*) and Katie McAuley.

Our year-round internship program continued to draw some of Long Island's most talented high school and college students, engaging them in science research and providing practical laboratory experience. We were pleased to accept new high school interns Matthew Angeliadis, Rachel Bosco, Brittany Coscio, Pauline McGlone, and Robert Scott (all from Huntington); Anne Bode (St. Anthony's); Magdalene Economou (Syosset); Nathaniel Hogg (Friends Academy); Jack Manzi (Kings Park); Anant Mehrotra (Oyster Bay); Sophie Podhurst (Walt Whitman); and Daliah Ross (Harborfields). The new hires joined veterans Katie Bellissimo (Walt Whitman) and Anna Saum (home school).

We bid farewell to a number of high school interns as they left for their freshman year at college: Cyril Danielcutty (Stony Brook University), Alexa DeAngelis (Georgetown), Jack Greenfield (Massachusetts Institute of Technology), Devika Gupta (Lehigh), Frieda Haerter (Renssalear Institute of Technology), Julie Hemphill (University of Pittsburgh), Paras Patel (Hofstra), and Gianna Torre (Hunter).

Our busy summer season of camps requires additional help. Returning interns Becky Hirsch (Friends Academy) and Paul Donat (SUNY Geneseo) were joined at our Dolan facility by newbies Sabrina Gallego (Half Hollow Hills), William Harlow (Trinity School), Grant Murphy (Friends Academy), and Steven Kunis (Seton Hall Prep). Veteran Juliet Jacobson (Hebrew Academy of Nassau County) returned to help at our DNALC *West* location, with newbies Arvind Rajabhathor and Shenika Shah (both Herricks), Kalliopi Chatzis (Bayside), and Angell Xiang (Great Neck).

College interns mentor younger interns and fulfill requests for DNA sequencing and RNAi targeting vectors from faculty nationwide. Returning in 2012 were David Dopfel and Lina-Mari Varghese (both Stony Brook University), Arielle Scardino (City College of New York), Katherine Villalon (John Jay College of Criminal Justice), and Sulaiman Usman (New York Institute of Technology). Arielle and Sulaiman also had key roles in the *UBP* in Manhattan.

Some high school interns compete in research competitions, including the Siemens Science Competition and the New York and Long Island Science and Engineering Fairs. Matt Angeliadis (Huntington) is studying the effects of ultraviolet radiation on the population growth of yeast, while Robert Scott (also of Huntington) is exploring a method to counteract toxins released by a species of garlic mustard.

2012 Workshops, Meetings, and Collaborations

January 4	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
January 7	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
January 10	Site visit by Kelley Remole, Columbia University, *Harlem DNA Lab*
January 11	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
January 12	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
	Urban Barcode Project presentation at the Vienna Open Lab, Vienna, Austria
January 13	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
January 14	*Saturday DNA!* "When Genes are Switched Off," Dolan DNALC
January 14–18	International Plant & Animal Genome XX Conference 2012, "*DNA Subway* Places Students on the Fast Track to Genome Analysis," Town and Country Convention Center, San Diego, California
January 17	*Urban Barcode Project* Open Lab, Genspace, Brooklyn, New York
January 18	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
January 19	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
January 20	HHMI Professional Development Workshop, "PCR and Human DNA Variation, Part 2," *Harlem DNA Lab*
January 21	HHMI Professional Development Workshop, "PCR and Human DNA Variation, Part 2," *Harlem DNA Lab*
January 25	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
January 26	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
January 28	STEM Mentor Training, New York Academy of Sciences, New York
January 30	STEM Mentor Training, New York Academy of Sciences, New York
February 2	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
February 4	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
February 7	*Urban Barcode Project* Open Lab, Genspace, Brooklyn, New York
February 8	*Urban Barcode Project* Open Lab, Genspace, Brooklyn, New York
	Site visit by Larry Kingsley, Pall Corporation, Port Washington, New York
February 10	HHMI Professional Development Workshop, "DNA Structure and Isolation," *Harlem DNA Lab*
February 11	HHMI Professional Development Workshop, "DNA Structure and Isolation," *Harlem DNA Lab*
	Saturday DNA! "Selection Detection," Dolan DNALC
February 13	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
February 14	*Urban Barcode Project* Open Lab, Genspace, Brooklyn, New York
February 15	*Urban Barcode Project* Open Lab, Genspace, Brooklyn, New York
	Urban Barcode Project Open Lab, *Harlem DNA Lab*
	Ecology and Evolutionary Biology Graduate Programs Presentation, "Bushmeat Trade and Diseases," Columbia University, New York
	Urban Barcode Project Open Lab, Genspace, Brooklyn, New York
February 16	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
	Urban Barcode Project Open Lab, Genspace, Brooklyn, New York
February 20	New York City Department of Education Professional Development Workshop, "DNA Barcoding," American Museum of Natural History, New York
February 21	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
	Urban Barcode Project Open Lab, Genspace, Brooklyn, New York
February 22	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
	New York City Department of Education Professional Development Workshop, "DNA Barcoding," American Museum of Natural History, New York
February 23	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
	Urban Barcode Project Open Lab, Genspace, Brooklyn, New York
February 24	City College of New York Teacher Academy Professional Development, "DNA Extraction," *Harlem DNA Lab*
	New York City Department of Education Professional Development Workshop, "DNA Barcoding," American Museum of Natural History, New York
February 27–28	HHMI CUREnet Meeting, Howard Hughes Medical Institute, Chevy Chase, Maryland
February 28	*Urban Barcode Project* Open Lab, Genspace, Brooklyn, New York
February 29	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
	Urban Barcode Project Open Lab, Genspace, Brooklyn, New York
March 2	HHMI Professional Development Workshop, "Variability and Inheritance," *Harlem DNA Lab*
March 3	NSF ATE Professional Development *Genomic Approaches in BioSciences* Follow-up Workshop, Universidad del Turabo, Gurabo, Puerto Rico
	HHMI *New York Stories*, "Restriction Analysis," filming Ileana Rios and the Trinity School 11th Grade Laboratory Class, New York
March 5	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*

March 7	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
	Urban Barcode Project Open Lab, Genspace, Brooklyn, New York
March 8	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
	Urban Barcode Project Open Lab, Genspace, Brooklyn, New York
March 10	*Saturday DNA!* "Genetics of HIV Resistance," Dolan DNALC
March 12–13	NSF *iPlant Collaborative* Professional Development, *Tools and Services* Workshop, University of California, Davis
March 13	*Urban Barcode Project* Open Lab, Genspace, Brooklyn, New York
March 14	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
March 15	*Urban Barcode Project* Open Lab, Genspace, Brooklyn, New York
	HHMI Professional Development Workshop, "Bacterial Transformation," *Harlem DNA Lab*
March 17	HHMI Professional Development Workshop, "Bacterial Transformation," *Harlem DNA Lab*
March 19	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
March 21	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
	Urban Barcode Project Open Lab, Genspace, Brooklyn, New York
March 22	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
	Urban Barcode Project Open Lab, Genspace, Brooklyn, New York
March 24	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
March 27	*Urban Barcode Project* Open Lab, Genspace, Brooklyn, New York
March 27–28	NSF *iPlant Collaborative* Professional Development *Genomics in Education* Workshop, Purdue University, West Lafayette, Indiana
March 28	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
March 29	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
March 29–April 1	National Science Teachers Association 2012 National Conference on Science Education, "Exploring Genetics with *C. elegans*," "Silencing Genomes," "DNA Subway," "DNA Barcoding in Your Classroom," Indianapolis, Indiana
March 31	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
April 2	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
April 4	Presentation for Master's Course in Conservation Medicine, "Parasites and Bushmeat: Molecular Identification of Parasites Found in Wildlife Traded in Markets," Columbia University, New York
April 11	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
	New York City Department of Education Professional Development Workshop, "DNA Barcoding," American Museum of Natural History, New York
April 13	New York City Department of Education Professional Development Workshop, "DNA Barcoding," American Museum of Natural History, New York
April 14	NSF ATE Professional Development *Genomic Approaches in BioSciences* Follow-up Workshop, Gwinnett Technical College, Lawrenceville, Georgia
April 17–18	2012 Student Research Conference, "Eugenics in 20th Century America: The Kirksville Connection," Truman State University, Kirksville, Missouri
April 19	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
April 20	HHMI *New York Stories,* "DNA Fingerprinting," filming Bob Hipkens, Ileana Rios, and the Trinity School 9th Grade Laboratory Class, New York
	HHMI Professional Development Workshop, "DNA Analysis and Forensics," *Harlem DNA Lab*
	New York City Department of Education STEM Expo/Fair, "DNA Extraction," The Armory Track and Field Center, New York
April 21	HHMI Professional Development Workshop, "DNA Analysis and Forensics," *Harlem DNA Lab*
	Saturday DNA! "The Extraction Attraction," Dolan DNALC
	NSF ATE Professional Development *Genomic Approaches in BioSciences* Follow-up Workshop, Shoreline Community College, Shoreline, Washington
April 23	HHMI *New York Stories,* "Fly Lab," interview with Glenn Turner, CSHL
April 24	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
April 25	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
	BioCube Project Planning Meeting, Smithsonian National Museum of Natural History, Washington, D.C.
April 26	*Great Moments in DNA Science* Honors Seminar, "Can Cell Shape Influence Human Cancer?" Dr. Michael Feigin, CSHL, Dolan DNALC
	Urban Barcode Project Open Lab, *Harlem DNA Lab*
	HHMI *New York Stories,* "DNA Fingerprinting," interview with Larry Kobilinsky, John Jay College of Criminal Justice, New York
April 26–27	NSF *iPlant Collaborative* Professional Development *Genomics in Education* Workshop, Southern University at New Orleans, New Orleans, Louisiana

April 28	NSF ATE Professional Development *Genomic Approaches in BioSciences* Follow-up Workshop, Madison Area Technical College, Madison, Wisconsin
April 28–29	USA Science and Engineering Festival, Walter E. Washington Convention Center, Washington, D.C.
April 30	Site visit by Richard Snyder and Melanie Jardim, GENEWIZ Inc., South Plainfield, New Jersey
May 1	*Great Moments in DNA Science* Honors Seminar, "Seeing the Invisible: Atomic Pictures of DNA in Action," Dr. Jonathan Ipsaro, CSHL, Dolan DNALC
May 2	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
May 3	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
May 4	HHMI Professional Development Workshop, "PCR and Human DNA Variation, Part 1," *Harlem DNA Lab*
May 8	10-Year Anniversary Celebration, DNALC *West*
May 9	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
May 10	*Urban Barcode Project* Open Lab, *Harlem DNA Lab* *Great Moments in DNA Science* Honors Seminar, "Next-Generation Schizophrenia Genetics: Finding Needles in the Haystack with Help from Your Family," Dr. Shane McCarthy, CSHL, Dolan DNALC
May 11	Site visit by Vence Bonham and Belen Hurle, National Human Genome Research Institute, Bethesda, Maryland *Urban Barcode Project* Open Lab, DNA Learning Center *West*
May 12	*Saturday DNA!* "Micro RNAs: Tiny RNAs to Turn Off Genes," Dolan DNALC
May 14	Site visit by Science, Education, and Governmental Delegation, Kuala Lumpur, Malaysia
May 17–18	NSF *iPlant Collaborative* Professional Development *Tools and Services* Workshop, University of Arkansas, Little Rock HHMI Professional Development Workshop, "PCR and Human DNA Variation, Part 2," *Harlem DNA Lab*
May 21–22	NSF *iPlant Collaborative* Professional Development *Tools and Services* Workshop, Purdue University, Indianapolis, Indiana
May 21–25	NSF ATE Professional Development *Genomic Approaches in BioSciences* Workshop, Austin Community College, Austin, Texas
May 23	*Urban Barcode Project* Evaluators Workshop, American Museum of Natural History, New York
May 29	Site visit by Association Relative à la Télévision Européenne (ARTE) for "Naturopolis" documentary filming, Strasbourg Cedex, France, *Harlem DNA Lab*
May 31	*Urban Barcode Project* Symposium–Poster Session 1, American Museum of Natural History, New York NSF *iPlant Collaborative* Professional Development *Tools and Services* Workshop, North Carolina State University, Raleigh
June 1	*Urban Barcode Project* Symposium–Poster Session 2, American Museum of Natural History, New York
June 3	*Urban Barcode Project* presentation, World Science Festival, New York
June 6	*Urban Barcode Project* Symposium Award Ceremony, American Museum of Natural History, New York Site visit by Phil Ferralli, Carolina Biological Supply Company, Burlington, North Carolina Site visit by Mardi Matheson, Mill Neck, New York
June 7	New York City Department of Education Professional Development Workshop, P.S. 69, Brooklyn, New York
June 8	Site visit by Kim Jasmin, JP Morgan Foundation, New York
June 9	*Saturday DNA!* "Food for Thought," Dolan DNALC
June 11	Site visit by Indra Nooyi and Mehmood Khan, PepsiCo, Inc., Purchase, New York
June 12	19th Annual Golf Outing, Piping Rock Club, Locust Valley, New York
June 13	HHMI *New York Stories,* "Restriction Analysis," interview with Scott Lowe, Memorial Sloan-Kettering Cancer Center, New York
June 16	New York City Department of Education Professional Development Workshop, "DNA Barcoding," American Museum of Natural History, New York
June 18	Site visit by Her Serene Excellency, Dr. Zsana Kawar, Royal Family of Jordan
June 18–22	*DNA Science* Workshop, The Chapin School, New York *Fun with DNA* Workshop, The Chapin School, New York *World of Enzymes* Workshop, The Chapin School, New York *World of Enzymes* Workshop, Trinity School, New York
June 20–21	NSF *iPlant Collaborative* Professional Development *Genomics in Education* Workshop, Tulsa Community College, Tulsa, Oklahoma
June 21	NSF *iPlant Collaborative* Professional Development *Tools and Services* Workshop, 8th International Purdue Statistics Symposium, Purdue University, Indianapolis, Indiana
June 25–29	*DNA Science* Workshop, Dolan DNALC *Fun with DNA* Workshop, Dolan DNALC *World of Enzymes* Workshop, Dolan DNALC *Fun with DNA* Workshop, DNALC *West* *Forensics* Workshop, The Chapin School, New York

June 26–28	*NSF iPlant Collaborative* Professional Development *RNA-Sequencing with the iPlant Collaborative* Workshop, and "The iPlant Collaborative: Bringing Together High Performance Computing and Biology," The International Symposium on Genetics in Aquaculture XI, Auburn University, Auburn, Alabama
June 28	New York City Department of Education Professional Development Workshop, "Barcoding," American Museum of Natural History, New York
June 29–30	NSF *iPlant Collaborative* Professional Development Workshop *Genomics in Education*, North Dakota State University, Fargo
	NSF *iPlant Collaborative* Professional Development *Tools and Services* Workshop, North Dakota State University, Fargo
July 2–6	HHMI Professional Development Workshop, "Living Environment," *Harlem DNA Lab*
	DNA Barcoding: The Biocube Workshop, Dolan DNALC
	Forensic Detectives Workshop, Dolan DNALC
	Green Genes Workshop, Dolan DNALC
	Plant Genomics Workshop, Dolan DNALC
	World of Enzymes Workshop, DNALC *West*
July 2–13	Site visit by David Liittschwager, National Geographic Society, Washington, D.C.
July 8–10	Botanical Society of America Conference, NSF *iPlant Collaborative* Professional Development Workshop, *Tools and Services* and *DNA Subway*, Columbus, Ohio
July 9–12	HHMI Professional Development Workshop, "AP Biology," *Harlem DNA Lab*
July 9–13	NSF ATE Professional Development *Genomic Approaches in BioSciences* Workshop, Bluegrass Community and Technical College, Lexington, Kentucky
	DNA Barcoding: The Biocube Workshop, Dolan DNALC
	DNA Science Workshop, Dolan DNALC
	Human Genomics Workshop, Dolan DNALC
	World of Enzymes Workshop, Dolan DNALC
	Green Genes Workshop, DNALC *West*
July 12	Site visit by Ethan Berman, Roxbury Latin School, West Roxbury, Massachusetts
July 16–20	*DNA Science* Workshop, DNALC
	Fun with DNA Workshop, DNALC
	Silencing Genomes Workshop, DNALC
	Fun with DNA Workshop, Central Islip
	DNA Science Workshop, DNA Learning Center *West*
July 16–27	HHMI New York City Leadership Symposium, *Harlem DNA Lab*
July 19–20	Woodrow Wilson Teaching Fellows Conference, "DNA Barcoding in the Classroom," Columbus Hilton, Columbus, Ohio
July 20–24	American Association of Plant Biologists, "E-Books," "Educational Genome Interfaces," and "Distributed Student Research: Model Outreach Methods for Plant Research Collaborations," Austin, Texas
July 23–27	*Barcoding Biodiversity* Workshop, Dolan DNALC
	Forensic Detectives Workshop, Dolan DNALC
	Fun with DNA Workshop, Dolan DNALC
	Genomics Workshop, Dolan DNALC
	Fun with DNA Workshop, DNALC *West*
July 25–26	NSF *iPlant Collaborative* Professional Development *Tools and Services* Workshop, U.S. Department of Agriculture Arid-Land Agriculture Research Center, University of Arizona Maricopa Agricultural Center, Maricopa
July 27	NSF *iPlant Collaborative* Professional Development *RNA Sequencing with iPlant Collaborative* Workshop, University of Arizona, Tucson
July 30–August 2	HHMI Professional Development Workshop, "Living Environment," *Harlem DNA Lab*
July 30–August 3	*DNA Barcoding* Workshop, Dolan DNALC
	DNA Science Workshop, Dolan DNALC
	World of Enzymes Workshop, Dolan DNALC
	DNA Science Workshop, DNALC *West*
	DNA Science Workshop, Stony Brook University, Stony Brook, New York
August 6–9	HHMI Professional Development Workshop, "AP Biology," *Harlem DNA Lab*
August 6–10	NSF ATE Professional Development *Genomic Approaches in BioSciences* Workshop, Southern Maine Community College, South Portland
	Barcoding Biodiversity Workshop, Dolan DNALC
	Fun with DNA Workshop, Dolan DNALC
	Green Genes Workshop, Dolan DNALC

	Human Genomics Workshop, Dolan DNALC
	World of Enzymes Workshop, DNALC *West*
August 13–14	NSF *iPlant Collaborative* Professional Development *Genomics in Education* Workshop, Montana State University, Bozeman
August 13–17	*DNA Science* Workshop, Dolan DNALC
	Fun with DNA Workshop, Dolan DNALC
	Green Genes Workshop, Dolan DNALC
	World of Enzymes Workshop, Dolan DNALC
	Human Genomics Workshop, DNALC *West*
August 16–17	NSF *iPlant Collaborative* Professional Development *Genomics in Education* Workshop, Brigham Young University, Provo, Utah
August 20–21	NSF *iPlant Collaborative* Professional Development *Tools and Services* Workshop, University of Wisconsin, Madison
August 20–24	*DNA Science* Workshop, Dolan DNALC
	Fun with DNA Workshop, Dolan DNALC
	Green Genes Workshop, Dolan DNALC
	Silencing Genomes Workshop, Dolan DNALC
	Barcoding Biodiversity Workshop, DNALC *West*
August 22	Site visit by Dr. Pola Rosen, Education Update, New York
August 22–23	NSF *iPlant Collaborative* Professional Development *Genomics in Education* Workshop, University of Wisconsin, Madison
August 27–31	*Barcoding Biodiversity* Workshop, Dolan DNALC
	Forensic Detectives Workshop, Dolan DNALC
	World of Enzymes Workshop, Dolan DNALC
August 28	NSF *iPlant Collaborative* Professional Development *Using the Generic Synteny Browser* Workshop, Genetic Model Organism Database Project, The National Evolutionary Synthesis Center, Durham, North Carolina
September 12–14	NSF *iPlant Collaborative* Professional Development *Tools and Services* Workshop, and "The iPlant Collaborative: Bringing Together High-Performance Computing and Biology," U.S. Department of Agriculture Livestock Cyberinfrastructure, Hinxton, England
September 13	Interviews with Caroline Dean, John Innes Centre, Norfolk, United Kingdom and Ali Shilatifard, Stowers Institute for Medical Research, Kansas City, Missouri, CSHL
September 14	STEM Mentor Training, New York Academy of Sciences, New York
September 15	STEM Mentor Training, New York Academy of Sciences, New York
September 17–18	Site visit by Steve Goff, *iPlant Collaborative*, University of Arizona, Tucson
September 20–21	Site visit by Julia Willingale-Theune, Wellcome Trust Sanger Institute, Hinxton, Cambridge, England
September 27	Interview with Edith Herd, Institut Curie, Paris, France, CSHL
October 5	Site visit by Katarina Nordqvist, Nobel Museum, Stockholm, Sweden
October 10	NSF *iPlant Collaborative* Professional Development *DNA Subway* Workshop, University of Washington, Seattle
October 13	*Saturday DNA!* "Captivating Carbon Dioxide," Dolan DNALC
	CSHL Presidents Council, "Engineering Society: The American Eugenics Movement," CSHL
October 22–23	Site visit by Winship Herr, Severine Trouilloud, and Vincent Parisy, University of Lausanne Eprouvette, Lausanne, Switzerland
October 26–27	NSF *iPlant Collaborative* Professional Development *Genomics in Education* Workshop, Schoolcraft College, Livonia, Michigan
October 29	Bay Area Biotechnology Education Consortium (BABEC), "DNA Barcoding and DNA Subway: Integrating B&B Workflows," San Mateo, California
October 30–31	NSF *iPlant Collaborative* Professional Development *Genomics in Education* Workshop, University of Alaska, Anchorage
November 1–3	National Association of Biology Teachers Annual Meeting, "Sense in Molecules," "What DNA Says about Our Human Family," "Silencing Genomes," "DNA Subway in the Classroom," Dallas, Texas
November 6	Center for Educational Innovation–Public Education Association (CEI-PEA) Professional Development Workshop, "DNA Structure and Isolation," *Harlem DNA Lab*
November 10	*Saturday DNA!* "Protein Explosion!" Dolan DNALC
December 1	KidCreate Art, Music, and Science Fair, "DNA Extraction," O'Shea Educational Complex, New York
December 8	*Saturday DNA!* "Single Cell-ebration!" Dolan DNALC
December 10–11	NSF *iPlant Collaborative* Professional Development *Tools and Services* Workshop, University of Hawaii at Manoa, Honolulu
December 13–14	NSF *iPlant Collaborative* Professional Development *Genomics in Education* Workshop, University of Hawaii at Manoa, Honolulu

Sites of Major Faculty Workshops 1985–2012

Program Key: *Middle School* High School **College**

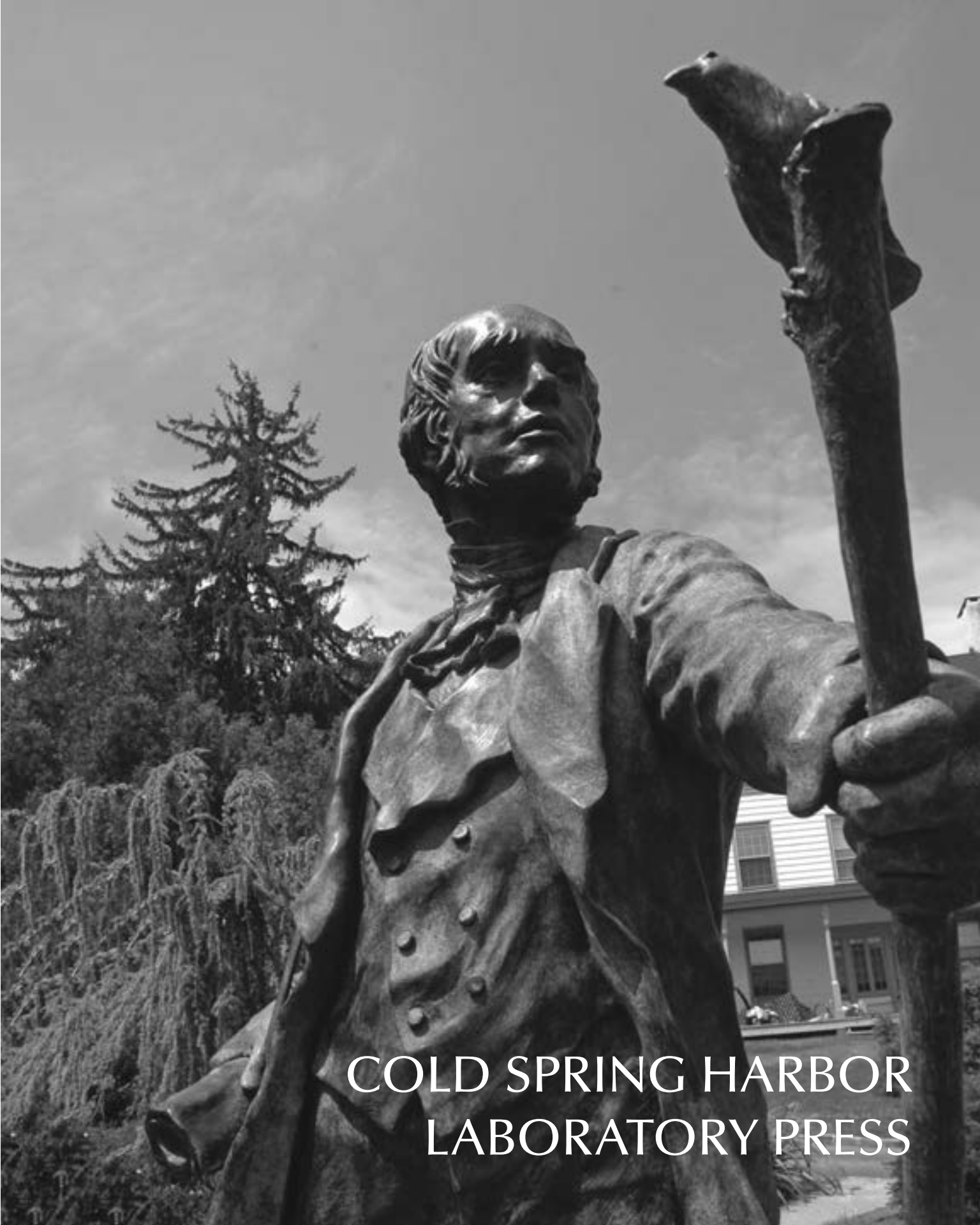
ALABAMA	University of Alabama, Tuscaloosa	1987–1990
ALASKA	**University of Alaska, Anchorage**	**2012**
	University of Alaska, Fairbanks	1996
ARIZONA	Arizona State University, Tempe	2009
	Tuba City High School	1988
	University of Arizona, Tucson	**2011**
	United States Department of Agriculture, Maricopa	**2012**
ARKANSAS	Henderson State University, Arkadelphia	1992
	University of Arkansas, Little Rock	**2012**
CALIFORNIA	**California State University, Dominguez Hills**	**2009**
	California State University, Fullerton	**2000**
	California Institute of Technology, Pasadena	**2007**
	Canada College, Redwood City	1997
	City College of San Francisco	2006
	City College of San Francisco	**2011**
	Contra Costa County Office of Education, Pleasant Hill	2002, 2009
	Foothill College, Los Altos Hills	**1997**
	Harbor-UCLA Research & Education Institute, Torrance	2003
	Los Angeles Biomedical Research Institute (LA Biomed), Torrance	**2006**
	Laney College, Oakland	1999
	Lutheran University, Thousand Oaks	1999
	Oxnard Community College, Oxnard	2009
	Pasadena City College	**2010**
	Pierce College, Los Angeles	**1998**
	Salk Institute for Biological Studies, La Jolla	2001, 2008
	San Francisco State University	1991
	San Diego State University	**2012**
	San Jose State University	**2005**
	Santa Clara University, Santa Clara	2010
	Stanford University, Palo Alto	**2012**
	University of California, Berkeley	**2010, 2012**
	University of California, Davis	1986
	University of California, Davis	**2012**
	University of California, Northridge	**1993**
	University of California, Riverside	2011
	University of California, Riverside	**2012**
COLORADO	Aspen Science Center	2006
	Colorado College, Colorado Springs	1994, 2007
	United States Air Force Academy, Colorado Springs	**1995**
	University of Colorado, Denver	1998, 2009–2010
CONNECTICUT	Choate Rosemary Hall, Wallingford	1987
DISTRICT OF COLUMBIA	**Howard University, Washington**	**1992, 1996, 2009–2010**
FLORIDA	Armwood Senior High School, Tampa	1991
	Florida Agricultural & Mechanical University, Tallahassee	2007–2008
	Florida Agricultural & Mechanical University, Tallahassee	**2011**
	North Miami Beach Senior High School	1991
	University of Miami School of Medicine	2000
	University of Western Florida, Pensacola	1991
GEORGIA	Fernbank Science Center, Atlanta	1989, 2007
	Gwinnett Technical College, Lawrenceville	**2011, 2012**
	Morehouse College, Atlanta	**1991, 1996–1997**
	Spelman College, Atlanta	**2010**
HAWAII	Kamehameha Secondary School, Honolulu	1990
	University of Hawaii at Manoa	**2012**
ILLINOIS	Argonne National Laboratory	1986–1987
	iBIO Institute/Harold Washington College, Chicago	2010
	Illinois Institute of Technology, Chicago	2009

	University of Chicago	**1992, 1997, 2010**
INDIANA	Butler University, Indianapolis	1987
	Purdue University, West Lafayette	**2012**
IDAHO	University of Idaho, Moscow	1994
IOWA	Drake University, Des Moines	1987
KANSAS	University of Kansas, Lawrence	1995
KENTUCKY	**Bluegrass Community & Technical College, Lexington**	**2012**
	Murray State University	1988
	University of Kentucky, Lexington	1992
	Western Kentucky University, Bowling Green	1992
LOUISIANA	Bossier Parish Community College	2009
	Jefferson Parish Public Schools, Harvey	1990
	John McDonogh High School, New Orleans	1993
	Southern University at New Orleans	**2012**
MAINE	Bates College, Lewiston	1995
	Southern Maine Community College	**2012**
	Foundation for Blood Research, Scarborough	2002
MARYLAND	Annapolis Senior High School	1989
	Bowie State University	**2011**
	Frederick Cancer Research Center	1995
	McDonogh School, Baltimore	1988
	Montgomery County Public Schools	1990–1992
	National Center for Biotechnology Information, Bethesda	2002
	St. John's College, Annapolis	*1991*
	University of Maryland, School of Medicine, Baltimore	**1999**
MASSACHUSETTS	**Arnold Arboretum of Harvard University, Roslindale**	**2011**
	Beverly High School	1986
	Biogen Idec, Cambridge	2002, 2010
	Boston University	**1994, 1996**
	CityLab, Boston University School of Medicine	1997
	Dover-Sherborn High School, Dover	1989
	Randolph High School	1988
	The Winsor School, Boston	1987
	Whitehead Institute for Biomedical Research, Cambridge	2002
MICHIGAN	Athens High School, Troy	1989
	Schoolcraft College, Livonia	**2012**
MINNESOTA	Minneapolis Community and Technical College	2009
	University of Minnesota, St. Paul	2005
	University of Minnesota, St. Paul	**2010**
MISSISSIPPI	Mississippi School for Math & Science, Columbus	1990–1991
	Rust College, Holly Springs	**2006–2008, 2010**
MISSOURI	**St. Louis Science Center**	**2008–2010**
	Stowers Institute for Medical Research, Kansas City	2002, 2008
	University of Missouri, Columbia	**2012**
	Washington University, St. Louis	**1989, 1997, 2011**
MONTANA	**Montana State University, Bozeman**	**2012**
NEVADA	University of Nevada, Reno	1992
NEW HAMPSHIRE	Great Bay Community College, Portsmouth	2009
	New Hampshire Community Technical College, Portsmouth	**1999**
	St. Paul's School, Concord	1986–1987
NEW JERSEY	Coriell Institute for Medical Research, Camden	2003
	Raritan Valley Community College, Somerville	2009
NEW MEXICO	Biolink Southwest Regional Meeting, Albuquerque	2008
NEW YORK	Albany High School	1987
	American Museum of Natural History, New York	2007
	Bronx High School of Science	1987
	Canisius College, Buffalo	2007
	Canisius College, Buffalo	**2011**
	City College of New York	2012
	Cold Spring Harbor High School	1985, 1987

	Columbia University, New York	**1993**
	Cornell University, Ithaca	**2005**
	DeWitt Middle School, Ithaca	*1991, 1993*
	Dolan DNA Learning Center	1988–1995, 2001–2004, 2006–2009
	Dolan DNA Learning Center	**1990, 1992, 1995, 2000–2011**
	Dolan DNA Learning Center	*1990–1992*
	DNA Learning Center *West*	2005
	Fostertown School, Newburgh	*1991*
	Harlem DNA Lab, East Harlem	2008–2009, 2011, 2012
	Huntington High School	1986
	Irvington High School	1986
	John Jay College of Criminal Justice	2009
	Junior High School 263, Brooklyn	*1991*
	Lindenhurst Junior High School	*1991*
	Mount Sinai School of Medicine, New York	1997
	New York City Department of Education	2007, 2012
	New York Institute of Technology, New York	2006
	New York Institute of Technology, New York	**2006**
	Orchard Park Junior High School	*1991*
	Plainview-Old Bethpage Middle School	*1991*
	State University of New York, Purchase	1989
	State University of New York, Stony Brook	1987–1990
	Stuyvesant High School, New York	1998–1999
	The Rockefeller University, New York	2003
	The Rockefeller University, New York	**2010**
	Titusville Middle School, Poughkeepsie	*1991, 1993*
	Trudeau Institute, Saranac Lake	2001
	Union College, Schenectady	2004
	United States Military Academy, West Point	**1996**
	Wheatley School, Old Westbury	1985
NORTH CAROLINA	CIIT Center for Health Research, Triangle Park	2003
	North Carolina Agricultural & Technical State University, Greensboro	**2006–2007, 2009–2011**
	North Carolina School of Science, Durham	1987
	North Carolina State University, Raleigh	**2012**
NORTH DAKOTA	**North Dakota State University, Fargo**	**2012**
OHIO	Case Western Reserve University, Cleveland	1990
	Cleveland Clinic	1987
	Langston University, Langston	2008
	North Westerville High School	1990
OKLAHOMA	**Oklahoma City Community College**	**2000**
	Oklahoma City Community College	2006–2007, 2010
	Oklahoma Medical Research Foundation, Oklahoma City	2001
	Oklahoma School of Science and Math, Oklahoma City	1994
	Tulsa Community College	2009
	Tulsa Community College	**2012**
OREGON	Kaiser Permanente-Center for Health Research, Portland	2003
PENNSYLVANIA	Duquesne University, Pittsburgh	1988
	Germantown Academy	1988
	Kimmel Cancer Center, Philadelphia	2008
RHODE ISLAND	Botanical Society of America, Providence	2010
SOUTH CAROLINA	**Clemson University**	**2004**
	Medical University of South Carolina, Charleston	1988
	University of South Carolina, Columbia	**1988**
TENNESSEE	NABT Professional Development Conference, Memphis	2008
TEXAS	Austin Community College–Rio Grande Campus	2000
	Austin Community College–Eastview Campus–Roundrock Campus	**2007–2009**

	Austin Community College–Roundrock Campus	**2012**
	Houston Community College Northwest	**2009–2010**
	J.J. Pearce High School, Richardson	1990
	Langham Creek High School, Houston	1991
	University of Lone Star College, Kingwood	**2011**
	Midland College	2008
	Southwest Foundation for Biomedical Research, San Antonio	2002
	Taft High School, San Antonio	1991
	Texas A&M, AG Research and Extension Center, Weslaco	2007
	Trinity University, San Antonio	**1994**
	University of Texas, Austin	**1999, 2004, 2010, 2012**
	University of Texas, Brownsville	**2010**
UTAH	**Brigham Young University, Provo**	**2012**
	University of Utah, Salt Lake City	1993
	University of Utah, Salt Lake City	**1998, 2000**
	Utah Valley State College, Orem	**2007**
VERMONT	University of Vermont, Burlington	1989
VIRGINIA	Eastern Mennonite University, Harrisonburg	1996
	Jefferson School of Science, Alexandria	1987
	Mathematics and Science Center, Richmond	1990
	Mills Godwin Specialty Center, Richmond	1998
	Virginia Polytechnic Institute and State University, Blacksburg	**2005, 2008–2009**
WASHINGTON	Fred Hutchinson Cancer Research Center, Seattle	1999, 2001, 2008
	Shoreline Community College	2011, 2012
	University of Washington, Seattle	**1993, 1998, 2010**
WEST VIRGINIA	Bethany College	1989
WISCONSIN	Blood Center of Southeastern Wisconsin, Milwaukee	2003
	Madison Area Technical College	**1999, 2009, 2011, 2012**
	Marquette University, Milwaukee	1986–1987
	University of Wisconsin, Madison	1988–1989
	University of Wisconsin, Madison	**2004, 2012**
WYOMING	University of Wyoming, Laramie	1991
PUERTO RICO	**Universidad del Turabo, Gurabo**	**2011, 2012**
	University of Puerto Rico, Mayaguez	1992
	University of Puerto Rico, Mayaguez	**1992**
	University of Puerto Rico, Rio Piedras	**1993**
	University of Puerto Rico, Rio Piedras	1994

AUSTRALIA	Walter and Eliza Hall Institute and University of Melbourne	1996
AUSTRIA	**Vienna Open Lab**	**2007, 2012**
CANADA	Red River Community College, Winnipeg, Manitoba	1989
CHINA	**Ho Yu College, Hong Kong**	**2009**
GERMANY	Urania Science Center, Berlin	2008
ITALY	International Institute of Genetics and Biophysics, Naples	1996
	Porto Conte Research and Training Laboratories, Alghero	1993
MEXICO	ASPB Plant Biology, Merida	2008
PANAMA	**University of Panama, Panama City**	**1994**
RUSSIA	Shemyakin Institute of Bioorganic Chemistry, Moscow	1991
SINGAPORE	National Institute of Education	2001–2005
SWEDEN	Kristineberg Marine Research Station, Fiskebackskil	1995
	Uppsala University	2000
THE NETHERLANDS	**International Chromosome Conference, Amsterdam**	**2007**
UNITED KINGDOM	**Wellcome Trust Conference Center, Hinxton, UK**	**2012**



COLD SPRING HARBOR
LABORATORY PRESS

2012 PRESS PUBLICATIONS

Serials

Genes & Development, Vol. 26 (www.genesdev.org)

Genome Research, Vol. 22 (www.genome.org)

Learning & Memory, Vol. 19 (www.learnmem.org)

RNA, Vol. 18 (www.rnajournal.org)

Cold Spring Harbor Symposia in Quantitative Biology, Vol. 76: *Metabolism and Disease,* edited by Terri Grodzicker, Bruce Stillman, and David Stewart

Cold Spring Harbor Protocols (www.cshprotocols.org)

Cold Spring Harbor Perspectives in Biology (www.cshperspectives.org)

Cold Spring Harbor Perspectives in Medicine (www.perspectivesinmedicine.org)

Laboratory Manuals

Molecular Cloning: A Laboratory Manual, Fourth Edition, by Michael R. Green and Joseph Sambrook

Handbooks

Lab Dynamics: Management and Leadership Skills for Scientists, Second Edition, by Carl M. Cohen and Suzanne L. Cohen

Basic Stereology for Biologists and Neuroscientists, by Mark J. West

Next-Generation DNA Sequencing Informatics, edited by Stuart M. Brown

Textbooks

Genome Science, by David Micklos, Bruce Nash, and Uwe Hilgert

Monographs (Topic Collections from *Perspectives in Biology* and *Perspectives in Medicine*)

The Synapse, edited by Morgan Sheng, Bernardo L. Sabtini, and Thomas C. Südhof

Type 1 Diabetes, edited by Jeffrey A. Bluestone, Mark A. Atkinson, and Peter Arvan

Protein Synthesis and Translational Control, edited by John W.B. Hershey, Nahum Sonenberg, and Michael B. Mathews

Parkinson's Disease, edited by Serge Przedborski

Wnt Signaling, edited by Roel Nusse, Xi He, and Renée van Amerongen

Addiction, edited by R. Christopher Pierce and Paul J. Kenny

History

The Annotated and Illustrated Double Helix, by James D. Watson, edited by Alexander Gann and Jan Witkowski

Other

At the Bench: A Laboratory Navigator, Updated Edition, by Kathy Barker

At the Helm: Leading Your Laboratory, Second Edition, by Kathy Barker

CSHL Annual Report 2011, Yearbook Edition

CSHL Annual Report 2011, Executive Summary

Banbury Center Annual Report 2011

Watson School of Biological Sciences Annual Report 2011

E-books (Kindle editions)

RNA: Life's Indispensable Molecule, by James Darnell

Incurable: A Life After Diagnosis, by Charles Harris

Lab Dynamics: Management and Leadership Skills for Scientists, Second Edition, by Carl M. Cohen and Suzanne L. Cohen

Websites

Cold Spring Harbor Monographs Archive Online (www.cshmonographs.org)

Cold Spring Harbor Symposium on Quantitative Biology Archive (symposium.cshlp.org)

COLD SPRING HARBOR LABORATORY PRESS EXECUTIVE DIRECTOR'S REPORT

Cold Spring Harbor Laboratory has a unique presence and an admired reputation in the international scientific community, and its publications have contributed much to that status. The research and review journals and books published by the Laboratory's Press are a vital part of how the institution connects with scientists worldwide. What began in 1933 as one printed symposium proceedings volume each year is now a digitally based publishing program with seven online journals and more than 170 print and electronic books. In 2012, CSHL journal articles were electronically downloaded a remarkable 10 million times, a 12% increase year-on-year, and 38,000 print books were shipped to 150 countries. In this way, the Press continues to fulfill its mission of helping scientists succeed while promoting the Laboratory's reputation for excellence and supporting its work financially.

The journals once again had an exceptional year. Subscriptions to every title increased, a significant result in an increasingly challenging library market. There was revenue growth of 6% even in the four long-established research journals and, gratifyingly, the review journals—more recent in origin and creatively straddling the boundaries of book and journal content—grew by more than 15%. Despite their maturity, *Genes & Development* (celebrating its 25th anniversary) and *Genome Research* continued to attract new subscribers, particularly in emerging scientific communities abroad. Although the impact factor is an increasingly embattled metric of a journal's quality or value—justifiably, given its willfully distorted application to the worth of individuals in academic circles—it remains important to those who must decide which journals to purchase for institutions and also to scientists seeking a vehicle for the publication of work that will enhance their careers. Judged by impact factor, *Genome Research* and *Genes & Development* remained within the top 1% of all published journals. They are two of the top three research journals in genetics, continuing to publish important work that has received careful and fair peer review.

Learning & Memory was founded as a specialized journal within the broad sweep of neuroscience, but in the past 2 years, it has expanded its scope into the advancing field of molecular cognition. *RNA*, published on behalf of the RNA Society, maintained its position as the leading journal of RNA biology, enhancing both the reputation and the financial health of the Society.

Our research journals retain a subscription basis. However, the Press was among the first publishers to embrace three practices intended to widen access to the published scientific record: deposition of research articles for indexing and access at the government-funded database PubMedCentral; release of all journal content online for access without charge 6 or 12 months after publication; and the opportunity for individual scientists to make their papers freely accessible immediately on publication in return for a fee. In 2012, 19% of the articles published by the Press research journals became immediately available on publication, the largest proportion yet recorded. To enhance the re-usability of content in *Genome Research*, a new license to publish was adopted. Papers released for free access 6 months after publication now have a Creative Commons BY-NC license that permits their noncommercial re-use and adaptation by the academic community.

The editorial excellence of the review journal *Cold Spring Harbor Perspectives in Biology* in its first 3 years was rewarded with a substantial first-impact factor. As planned, a sister publication, *Cold Spring Harbor Perspectives in Medicine*, was launched this year. Both these journals have an innovative print plus online publishing model that strongly appeals to both authors and readers. Their important and well-received collections of articles gained audience and scholarly recognition, with widely ranging themes that included diabetes, addiction, and Parkinson's disease, as

well as protein synthesis and signal transduction. These journals have taken the place of print-only monographs as a means of providing authoritative, in-depth surveys of specific research fields—but in a more immediately discoverable way online. Our first foray into the journal-based delivery of information formerly published in books was the methods journal *Cold Spring Harbor Laboratory Protocols.* Launched 6 years ago, this online-only publication is now well-established financially, with an expanding circulation and a record number of downloads.

Twelve new print books and five new e-books were added to the Press list in 2012. Most print volumes were themed collections of articles derived from the *Perspectives* journals. The additional titles included *Genome Science*, a new collection of laboratory exercises by the teaching staff at the Dolan DNA Learning Center. Most notably, a new edition of the laboratory manual *Molecular Cloning* was published in June. Long established as the gold standard for molecular biology techniques, and by far the most successful book ever published by the Press, with more than 200,000 copies sold, this new edition was a complete revision by founding author Joe Sambrook and his new coauthor Michael Green, assisted by a dozen expert contributors. Another classic, of a different sort, was copublished by the Press and Simon & Schuster: an annotated and illustrated edition of James Watson's 1968 autobiographical account of the discovery of the structure of DNA, *The Double Helix*. Laboratory Professors Alex Gann and Jan Witkowski expanded the text with footnotes, photos, correspondence, and other documents that placed this most famous account of scientific discovery in its social, intellectual, and historical context.

In 2012, there were two further organizational changes. After more than 20 years of owning systems for book warehousing and order processing, these functions were contracted out to a large-scale, technologically sophisticated and more cost-efficient facility run by Oxford University Press in North Carolina. In addition, the marketing operations for books and journals were merged, and a sales agreement with Oxford was reached that will improve our market reach and relationships with book resellers. For more than a decade, advancing digital technologies and changing reader inclinations have transformed the learning landscape in which the Press operates. As methods of content delivery have continued to evolve, so has the Press, revising its approaches, restructuring its operations, and adjusting its business models. The process must continue, but 2012 provided very satisfactory evidence of success.

Staff

During the year, we said farewell to Richard Abreu, Matthew D'Amico, Pauline Henick, Lauren Schmidt, and Hashanthi Wijayatilaki, and also to Jacqueline Matura and Sharon Story, both of whom dedicated long years of excellent service to our customer service and fulfillment operations. We also welcomed Christina Kary to the editorial staff of *Genes & Development.* And we congratulated Alex Gann as he assumed the role of Dean of the Watson School of Biological Studies at the end of December, retaining a part-time editorial role at the Press. A full list of staff members of the Press at December 31, 2012, is listed elsewhere in this volume. An effective publishing team must combine people with a variety of skills. The Laboratory is fortunate to have skilled and dedicated individuals in all of the operations of the Press. But I am particularly grateful for the quiet and cheerful efficiency of my assistant Mala Mazzullo and to the talented and hard-working individuals who have important leadership roles in the activities of the Press: Jan Argentine, Terri Grodzicker, Wayne Manos, Stephen Nussbaum, Richard Sever, Marcie Siconolfi, Hillary Sussman, Linda Sussman, and Denise Weiss.

John Inglis
Executive Director
and Publisher

FINANCE

FINANCIAL STATEMENTS

CONSOLIDATED BALANCE SHEET
December 31, 2012
(with comparative financial information as of December 31, 2011)

		2012	2011
Assets:			
Cash and cash equivalents	$	75,471,404	58,055,329
Grants receivable		10,012,401	5,733,104
Contributions receivable, net		145,289,626	163,144,645
Publications inventory		2,767,427	3,292,898
Investments		296,611,329	269,786,326
Restricted use assets		3,597,846	2,882,590
Other assets		15,891,942	15,027,787
Land, buildings, and equipment, net		240,625,332	241,828,796
Total assets	$	790,267,307	759,751,475
Liabilities and net assets:			
Liabilities:			
Accounts payable and accrued expenses	$	10,456,412	11,135,584
Deferred revenue		5,258,525	5,467,566
Interest rate swap		35,556,347	37,726,697
Bonds payable		97,200,000	97,200,000
Total liabilities		148,471,284	151,529,847
Net assets:			
Unrestricted		243,281,390	205,967,407
Temporarily restricted		293,464,158	298,351,339
Permanently restricted		105,050,475	103,902,882
Total net assets		641,796,023	608,221,628
Total liabilities and net assets	$	790,267,307	759,751,475

CONSOLIDATED STATEMENT OF ACTIVITIES
Year ended December 31, 2012
(with summarized financial information for the year ended December 31, 2011)

	Unrestricted	*Temporarily Restricted*	*Permanently Restricted*	*2012 Total*	*2011 Total*
Revenue and other support:					
Public support–contributions and nonfederal grant awards	$ 19,618,621	40,079,983	1,147,593	60,846,197	113,684,017
Federal grant awards	28,711,510	–	–	28,711,510	34,490,267
Indirect cost allowances	24,445,726	–	–	24,445,726	25,623,148
Investment return utilized	23,396,825	–	–	23,396,825	22,585,474
Program fees	8,049,856	–	–	8,049,856	6,963,110
Publications sales	10,957,863	–	–	10,957,863	9,848,446
Dining services	4,614,691	–	–	4,614,691	4,309,694
Rooms and apartments	3,770,473	–	–	3,770,473	3,345,273
Miscellaneous	2,527,870	–	–	2,527,870	3,745,958
Net assets released from restrictions	58,423,282	(58,423,282)	–	–	–
Total revenue and other support	184,516,717	(18,343,299)	1,147,593	167,321,011	224,595,387
Expenses:					
Research	88,821,283	–	–	88,821,283	91,848,784
Educational programs	17,815,388	–	–	17,815,388	16,117,941
Publications	10,092,372	–	–	10,092,372	9,323,927
Banbury Center conferences	1,389,048	–	–	1,389,048	1,409,384
DNA Learning Center programs	1,578,211	–	–	1,578,211	1,857,078
Watson School of Biological Sciences programs	3,356,033	–	–	3,356,033	3,584,716
General and administrative	16,860,891	–	–	16,860,891	15,596,163
Dining services	5,642,788	–	–	5,642,788	5,717,278
Total expenses	145,556,014	–	–	145,556,014	145,455,271
Excess (deficiency) of revenue and other support over (under) expenses	38,960,703	(18,343,299)	1,147,593	21,764,997	79,140,116
Other changes in net assets:					
Investment (loss) return excluding amount utilized	(3,817,070)	13,456,118	–	9,639,048	(22,610,255)
Change in fair value of interest rate swap	2,170,350	–	–	2,170,350	(21,287,538)
Increase (decrease) in net assets	37,313,983	(4,887,181)	1,147,593	33,574,395	35,242,323
Net assets at beginning of year	205,967,407	298,351,339	103,902,882	608,221,628	572,979,305
Net assets at end of year	$ 243,281,390	293,464,158	105,050,475	641,796,023	608,221,628

CONSOLIDATED STATEMENT OF CASH FLOWS
Year ended December 31, 2012
(with comparative financial information for the year ended December 31, 2011)

	2012	2011
Cash flows from operating activities:		
Increase in net assets	$ 33,574,395	35,242,324
Adjustments to reconcile increase in net assets to net cash provided by operating activities:		
Change in fair value of interest rate swap	(2,170,350)	21,287,538
Depreciation and amortization	15,422,891	14,697,263
Net (appreciation) depreciation in fair value of investments	(29,322,335)	4,173,189
Contributions restricted for long-term investment	(10,527,878)	(13,475,213)
Changes in assets and liabilities:		
Grants receivable	(4,279,297)	1,992,555
Contributions receivable, net of financing activities	25,176,987	(53,517,765)
Publications inventory	525,471	262,295
Other assets	(912,934)	(5,008,296)
Restricted use assets	(715,256)	(302,119)
Accounts payable and accrued expenses, net of financing activities	(139,990)	1,835,064
Deferred revenue	(209,041)	(2,743,102)
Net cash provided by operating activities	26,422,663	4,443,733
Cash flows from investing activities:		
Capital expenditures	(14,219,427)	(17,748,180)
Proceeds from sales and maturities of investments	73,005,504	33,325,960
Purchases of investments	(70,508,172)	(30,860,745)
Net change in investment in employee residences	48,779	(97,394)
Net cash used in investing activities	(11,673,316)	(15,380,359)
Cash flows from financing activities:		
Contributions restricted for long-term investment	1,147,593	1,600,563
Contributions restricted for investment in capital	9,380,285	11,874,650
Increase in contributions receivable	(7,321,968)	(5,354,289)
(Decrease) increase in accounts payable relating to capital expenditures	(539,182)	104,669
Net cash provided by financing activities	2,666,728	8,225,593
Net increase (decrease) in cash and cash equivalents	17,416,075	(2,711,033)
Cash and cash equivalents at beginning of year	58,055,329	60,766,362
Cash and cash equivalents at end of year	$ 75,471,404	58,055,329
Supplemental disclosure:		
Interest paid	$ 3,683,436	3,614,370
Noncash investing and financing activity:		
Contributed property	$ 50,000	1,095,000

FINANCIAL SUPPORT OF THE LABORATORY

Cold Spring Harbor Laboratory, Banbury Center, and the Dolan DNA Learning Center receive a substantial portion of their funding through grants from the federal government and through grants, capital gifts, and annual contributions from New York State, private foundations, corporations, and individuals. The following section summarizes funding that occurred during 2012.

GRANTS January 1–December 31, 2012

COLD SPRING HARBOR LABORATORY

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2012 Funding*[1]	
FEDERAL GRANTS					
NATIONAL INSTITUTES OF HEALTH					
Program Project and Center Support	Drs. Hannon/Krainer/Spector/Stillman	05/25/12	12/31/16	$ 4,486,042	*
	Dr. Stillman, Cancer Center Core	08/17/11	07/31/16	4,346,720	
Cooperative Research Agreement Support[2]	Dr. Gingeras	09/21/12	07/31/16	1,975,096	*
	Drs. Lowe/Hannon/Hicks/Powers	09/01/09	08/31/14	564,230	
	Drs. Powers/Hannon/Krasnitz/Sordella	05/01/12	04/30/17	1,013,392	*
Equipment Support	Dr. Hannon	04/01/12	03/31/13	519,814	*
Research Support	Dr. Churchland	09/01/10	08/31/13	247,403	
	Dr. Dubnau	09/15/09	08/31/14	466,125	
	Drs. Enikolopov/Koulakov	09/15/11	08/31/16	411,276	
	Dr. Furukawa	03/01/10	02/28/15	457,875	
	Dr. Hannon	09/01/09	08/31/13	378,401	
	Dr. Huang	07/01/11	03/31/16	560,565	
	Drs. Kepecs/Huang	07/01/11	05/31/16	411,250	
	Drs. Koulakov/Enikolopov	07/15/10	02/28/14	356,621	
	Dr. Krainer	04/01/12	03/31/17	728,529	*
	Dr. Krainer	09/01/12	08/31/14	235,416	*
	Dr. Li	07/01/10	03/31/15	560,038	
	Dr. Martienssen	09/15/11	08/31/15	344,865	
	Dr. Martienssen	01/20/12	11/30/15	737,743	*
	Dr. McCombie	07/23/10	02/28/15	1,069,587	
	Dr. Mills	12/26/07	11/30/12	37,605	
	Dr. Mitra	09/30/09	03/31/14	975,957	
	Dr. Mitra	08/01/10	05/31/13	406,574	
	Dr. Muthuswamy	02/01/09	12/31/13	405,930	
	Dr. Osten	04/01/12	03/31/17	485,438	*
	Dr. D. Spector	04/01/11	03/31/15	740,360	
	Dr. Stenlund	12/01/06	06/30/13	470,000	
	Dr. Stillman	06/01/12	05/31/16	696,400	*
	Dr. Tonks	07/01/10	04/30/15	761,643	
	Dr. Tonks	01/11/10	12/31/13	434,648	
	Dr. Trotman	04/01/10	01/31/14	301,376	
	Dr. Turner	07/15/10	06/30/15	454,960	

[1]Includes direct and indirect costs

[2]Cooperative research agreement funding amounts include only CSHL portion of award

*New or competing renewal grants awarded in 2012

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2012 Funding*[1]	
	Dr. Van Aelst	07/01/08	03/31/14	$ 417,658	
	Dr. Van Aelst	01/01/09	12/31/13	376,384	
	Dr. Zador	09/18/08	07/31/13	354,339	
	Dr. Zador	09/27/10	05/31/15	464,475	
	Dr. Zador	09/01/12	08/31/17	400,209	*
	Dr. Zhong	06/01/10	05/31/13	368,154	
Research Subcontracts					
NIH/Allen Institute for Brain Science Consortium Agreement	Dr. Mitra	09/15/09	08/31/13	186,076	
NIH/Cornell University Consortium Agreement	Dr. Mitra	04/03/08	01/31/13	21,091	
NIH/Georgia Institute of Technology Consortium Agreement	Dr. D. Spector	09/30/06	07/31/15	290,000	
NIH/The Johns Hopkins University Consortium Agreement	Dr. Schatz	09/21/11	08/31/14	161,013	
NIH/The Scripps Research Institute Consortium Agreement	Dr. Osten	09/30/12	08/31/13	75,600	*
NIH/University of California - San Francisco Consortium Agreement	Dr. Egeblad	09/01/09	08/31/14	174,905	
NIH/University of Southern California Consortium Agreement	Dr. Hannon	07/01/10	06/30/14	117,450	
NIH/University of Texas Consortium Agreement	Dr. Krainer	08/01/10	03/31/13	44,732	
NIH/University of Texas Consortium Agreement	Dr. Furukawa	09/30/11	05/31/15	50,514	
Fellowship Support	Dr. Ipsaro	09/01/11	08/31/13	52,190	
	Dr. Jansen	01/01/11	07/31/14	47,756	
	J. Tucciarone	09/16/12	09/15/15	32,582	*
Institutional Training Program Support	Dr. Joshua-Tor, Watson School of Biological Sciences	07/01/12	06/30/17	267,927	*
	Dr. Mills	09/01/11	08/31/16	181,564	
Course Support	Advanced Immunocytochemistry: In Situ Hybridization and Live Cell Imaging	09/01/10	08/31/15	100,323	
	Advanced Sequencing Technologies and Applications	04/10/12	03/31/15	52,885	*
	Cell and Developmental Biology of *Xenopus*	04/01/09	03/31/14	63,345	
	Cellular Biology of Addiction	03/01/11	02/29/16	80,694	
	Computational and Comparative Genomics	08/01/08	07/31/13	51,792	
	Drosophila Neurobiology: Genes, Circuits, and Behavior	07/15/12	06/30/17	35,000	*
	Eukaryotic Gene Expression	04/01/12	03/31/17	114,306	*
	Mouse Development, Stem Cells, and Cancer	04/01/12	03/31/17	110,288	*
	Programming for Biology	09/01/09	08/31/14	63,654	
	Protein Purification and Characterization	04/01/12	03/31/17	95,019	*
	Proteomics	08/01/12	04/30/17	105,068	*
	X-Ray Methods in Structural Biology	09/01/12	08/31/17	81,540	*
	Yeast Genetics and Genomics	09/18/10	06/30/13	53,462	

[1]Includes direct and indirect costs

*New or competing renewal grants awarded in 2012

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2012 Funding*[1]	
Meeting Support	Automated Imaging and High-Throughput Phenotyping	04/01/12	03/31/13	$ 5,000	*
	Axon Guidance, Synapse Formation, and Regeneration	07/01/12	06/30/13	10,000	*
	Bacteria, Archaea, and Phages	05/15/12	04/30/13	6,000	*
	The Biology of Genomes	04/01/08	03/31/13	39,339	
	Evolution of *Caenorhabditis* and Other Nematodes	04/01/12	03/31/13	15,000	*
	Gene Expression and Signaling in the Immune System	04/01/08	03/31/13	10,000	
	Germ Cells	07/01/08	06/30/13	6,000	
	Mechanisms and Models of Cancer	08/01/08	07/31/13	8,500	
	Molecular Chaperones and Stress Responses	05/01/10	04/30/13	20,602	
	Molecular Genetics of Aging	03/01/08	02/28/13	31,600	
	Neurodegenerative Diseases: Biology and Therapeutics	09/26/12	09/25/13	23,000	*
	Neuronal Circuits	03/01/12	02/28/13	15,000	*
	Nuclear Receptors and Disease	09/21/12	08/31/13	20,025	*
	Pharmacogenomics and Personalized Therapy	09/30/10	08/31/15	10,000	
	PTEN Pathways and Targets	09/30/11	08/31/12	3,000	
	Retroviruses	03/15/12	02/28/17	36,645	*
	Systems Biology: Global Regulation of Gene Expression	03/01/10	02/28/15	5,000	
	Vertebrate Organogenesis	04/01/11	03/31/15	6,000	
NATIONAL SCIENCE FOUNDATION					
Multiple Project Award Support	Dr. Jackson	10/01/10	09/30/15	1,115,265	
	Drs. Lippman/Schatz	11/01/12	10/31/15	854,233	*
	Dr. Ware	06/01/12	05/31/17	1,762,326	*
Research Support	Dr. Churchland	08/01/11	07/31/14	43,156	
	Dr. Jackson	09/01/10	08/31/13	150,000	
	Dr. Timmermans	08/15/10	07/31/13	150,000	
	Drs. Timmermans/Hammell	05/01/12	04/30/16	181,550	*
Research Subcontracts					
NSF/Cornell University Consortium Agreement	Dr. Timmermans	09/01/08	08/31/12	16,000	
NSF/New York University Consortium Agreement	Drs. McCombie/Martienssen	08/01/10	07/31/14	407,632	
NSF/North Carolina State University Consortium Agreement	Dr. Martienssen	03/01/11	02/29/16	510,779	
NSF/University of Arizona Consortium Agreement	Dr. Ware	08/15/10	07/31/14	583,136	
NSF/University of Arizona Consortium Agreement	Drs. Ware/Micklos/Schatz/Stein	02/01/12	01/31/13	1,863,578	*
NSF/University of California - Berkeley Consortium Agreement	Dr. Jackson	08/01/06	07/31/12	269,815	
NSF/University of Maryland Consortium Agreement	Dr. Schatz	02/01/12	03/31/13	60,000	*

[1]Includes direct and indirect costs

*New or competing renewal grants awarded in 2012

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2012 Funding*[1]	
NSF/Washington University Consortium Agreement	Dr. Ware	03/01/10	02/28/13	$ 92,150	
Fellowship Support	Dr. Liberatore	06/01/10	05/31/13	43,500	
	Dr. Carlston	06/01/10	05/31/13	42,000	*
Undergraduate Training Program Support	Dr. Lippman	04/01/12	03/31/15	115,000	*
Course Support	Advanced Bacterial Genetics	07/01/09	06/30/14	95,634	
	Computational Cell Biology	08/01/12	07/31/15	70,000	*
	Frontiers and Techniques in Plant Science	05/15/12	04/30/15	97,675	*
	Neurobiology of *Drosophila*	08/01/12	07/31/14	20,000	*
UNITED STATES DEPARTMENT OF AGRICULTURE					
Research Support	Dr. Jackson	01/15/11	01/14/15	120,963	
	Dr. McCombie	09/11/12	09/10/13	224,106	*
UNITED STATES DEPARTMENT OF THE ARMY					
Research Support	Dr. Hannon	09/15/12	09/14/17	1,069,481	*
	Dr. Hannon	09/01/08	08/31/13	709,165	
	Drs. Hicks/Trotman	09/30/12	09/29/14	276,672	*
	Dr. Zhong	06/15/10	06/14/13	302,572	
Research Subcontracts					
US Army/University of Pittsburgh Consortium Agreement	Dr. Hicks	09/01/12	08/31/13	152,921	*
Fellowship Support	E. Nakasone	01/01/11	03/13/12	10,800	
	Dr. Park	04/01/12	03/31/15	187,500	*
	Dr. Sheppard	09/01/12	08/31/15	38,927	*
UNITED STATES DEPARTMENT OF ENERGY					
Research Subcontracts					
DOE/Brookhaven National Laboratory Consortium Agreement	Drs. Ware/Schatz	11/29/11	09/30/16	1,076,238	
MISCELLANEOUS SOURCES OF FUNDING					
Equipment Support					
Academy for Medical Development and Collaboration (AMDeC)	Dr. Stillman	01/01/12	12/31/12	60,000	*
Edward P. Evans Foundation	Dr. Vakoc	08/01/12	07/31/13	120,000	*
Memorial Sloan-Kettering Cancer Center/Helena Rubinstein Foundation	Dr. Wigler	03/30/12	03/29/13	300,000	*
New York State Urban Development Corporation	Dr. Stillman	05/01/12	04/30/13	2,000,000	*
Edith and Alan Seligson	Dr. Wigler	06/01/11	05/31/14	250,000	

[1]Includes direct and indirect costs

*New or competing renewal grants awarded in 2012

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2012 Funding*[1]
Program Project Support				
Pfizer Inc.	Dr. Stillman	01/01/12	12/31/13	$ 2,050,000 *
Pioneer Hi-Bred International, Inc.	Drs. Jackson/Lippman/Martienssen/ Timmermans/Ware	07/01/12	06/30/17	1,600,000 *
The Simons Foundation/Autism	Dr. Wigler	01/01/12	12/31/15	6,557,422 *
The Simons Foundation/Center for Quantitative Biology	Dr. Wigler	09/01/08	08/31/15	1,500,000
Theodore R. and Vada S. Stanley	Drs. Watson/McCombie	06/01/11	05/31/14	5,000,000
Research Support				
Aboff's Inc.	Dr. Tonks	12/19/12	12/18/13	10,318 *
Paul G. Allen Family Foundation	Dr. Zador	10/01/10	09/30/13	575,200
Rita Allen Foundation	Dr. Hammell	09/01/12	08/31/17	110,000 *
American Association for Cancer Research	Dr. Egeblad	07/01/12	06/30/14	100,000 *
American Cancer Society	Dr. Wigler	01/01/12	08/31/12	10,000 *
	Dr. Wigler	01/01/11	08/31/13	47,000
American Legion Auxiliary	Dr. Egeblad	11/30/12	11/29/13	250 *
Anonymous	Drs. Mitra/Huang	02/01/12	01/31/14	300,000 *
Banfi Vineyards	Dr. Martienssen	01/01/12	12/31/12	5,679 *
The Breast Cancer Research Foundation	Drs. Wigler/Hicks	10/01/12	09/30/13	240,000 *
Burroughs Wellcome Fund	Dr. Vakoc	09/01/11	08/31/16	151,250
Caring for Carcinoid Foundation	Dr. Tuveson	07/01/12	06/30/14	150,000 *
The H.A. and Mary K. Chapman Foundation	Dr. Churchland	12/27/12	12/26/13	170,000 *
The Mary K. Chapman Foundation	Dr. Osten	12/31/11	12/30/13	150,000
Coferon Inc.	Dr. Vakoc	09/17/12	09/16/13	115,316 *
Dart Neuroscience LLC	Dr. Dubnau	12/01/12	11/30/13	250,000 *
Dysautonomia Foundation Inc.	Dr. Krainer	07/01/12	06/30/14	90,000 *
Lee MacCormick Edwards, Ph.D.	Dr. Stillman	12/01/12	11/30/13	5,000 *
The Ellison Medical Foundation	Dr. Enikolopov	09/09/09	09/08/13	252,000
Entertainment Industry Foundation	Dr. Hannon	11/01/12	10/31/13	100,000 *
Edward P. Evans Foundation	Dr. Vakoc	11/01/12	10/31/13	500,000 *
Charitable Lead Annuity Trust under the Will of Louis Feil	Drs. Albeanu/Kepecs/Li/Osten	12/15/12	12/14/13	1,000,000 *
Find a Cure Today Long Island Foundation	Dr. Egeblad	03/01/12	02/28/13	5,000 *
The Joni Gladowsky Breast Cancer Foundation	Dr. Tonks	05/01/12	04/30/13	55,000 *
Glen Cove C.A.R.E.S., Inc.	Dr. Egeblad	02/01/12	01/31/13	10,000 *
The Irving A. Hansen Memorial Foundation	Dr. Tonks	07/01/12	06/30/13	25,000 *
Jo-Ellen and Ira Hazan	Drs. Osten/Tonks/Krainer	12/01/12	11/30/13	200,000 *
Hearts for Cancer	Dr. Egeblad	04/01/12	03/31/13	28,456 *
The Hope Foundation	Dr. Egeblad	07/01/12	06/30/14	125,000 *
Howard Hughes Medical Institute–Gordon and Betty Moore Foundation	Dr. Martienssen	12/01/11	11/30/16	333,333
Isis Pharmaceuticals, Inc.	Dr. Krainer	11/01/11	10/26/13	150,000
Japan Science and Technology Agency	Dr. Taniguchi	03/01/12	07/31/12	54,799 *
Sidney Kimmel Foundation for Cancer Research	Dr. Zheng	07/01/11	06/30/13	100,000

[1]Includes direct and indirect costs

*New or competing renewal grants awarded in 2012

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2012 Funding*[1]	
F.M. Kirby Foundation, Inc.	Dr. Vakoc	12/15/12	12/14/13	$ 150,000	*
The Susan G. Komen Breast Cancer Foundation, Inc.	Dr. Egeblad	04/13/10	04/12/13	150,000	
Mara and Thomas Lehrman Charitable Fund	Drs. Hammell/Schatz	03/01/12	02/28/13	65,000	*
The Lehrman Institute	Dr. Hammell	03/01/12	02/28/13	200,000	*
Long Island Cruzin' For a Cure Inc.	Drs. Hicks/Trotman	08/01/12	07/31/13	20,000	*
The Long Island 2-Day Walk to Fight Breast Cancer	Dr. Egeblad	08/15/12	08/14/13	33,000	*
The Lustgarten Foundation	Dr. Hannon	01/01/10	12/31/12	835,531	
	Dr. Tuveson	06/15/12	06/14/17	50,000	*
	Drs. Tuveson/Pappin	09/01/12	08/30/17	1,000,000	*
The Manhasset Women's Coalition Against Breast Cancer, Inc.	Dr. Egeblad	01/01/11	12/31/12	50,744	
Carol Marcincuk Fund	Dr. Tonks	01/01/12	12/31/12	6,800	*
In Honor of Carissa Maringo	Dr. Egeblad	10/01/12	09/30/13	5,310	*
The G. Harold and Leila Y. Mathers Charitable Foundation	Dr. Mitra	09/01/10	08/31/13	203,920	
Breast Cancer Awareness Day in Memory of Elizabeth McFarland	Dr. Wigler	01/01/12	12/31/13	64,805	*
The McKnight Endowment Fund for Neuroscience	Dr. Churchland	07/01/12	06/30/15	75,000	*
The Melanoma Research Alliance	Drs. Hannon/Hammell/Vakoc	08/01/11	07/31/14	200,000	
The John Merck Fund	Dr. Churchland	06/01/11	05/31/15	75,000	
	Dr. Kepecs	06/01/10	05/31/14	75,000	
The Don Monti Memorial Research Foundation	Dr. Stillman	03/01/12	02/28/13	400,000	*
Louis Morin Charitable Trust	Dr. Stillman	12/01/12	11/30/13	75,000	*
National Alliance for Research on Schizophrenia and Depression (NARSAD)	Dr. Li	07/15/11	07/14/13	30,000	
Pancreatic Cancer UK	Dr. Tuveson	10/23/12	10/22/15	65,000	*
Panera Bread/Doherty Breads, LLC	Dr. Tonks	12/15/12	12/14/13	8,466	*
The Pew Charitable Trusts	Dr. Albeanu	08/01/12	07/31/17	60,000	*
	Dr. Hannon	01/01/11	12/31/15	349,452	
Pioneer Hi-Bred International, Inc.	Dr. Ware	03/15/12	06/30/15	65,385	*
The Hazen Polsky Foundation	Dr. Vakoc	01/01/12	12/31/14	50,000	*
The Prostate Cancer Foundation	Dr. Vakoc	01/20/12	01/19/14	150,000	*
Christina Renna Foundation Inc.	Dr. Van Aelst	05/01/12	04/30/13	20,300	*
The Research Foundation for the State University of New York	Dr. Furukawa	10/10/12	03/31/14	25,000	*
	Dr. Jackson	10/10/12	03/31/14	22,500	*
Marie Robertson Memorial Fund	CSHL Neuroscience Program Support	01/01/12	12/31/12	137,500	*
Damon Runyon Cancer Research Foundation	Dr. Sordella	01/01/10	12/31/12	150,000	
Diane Emdin Sachs Memorial Fund	Dr. Sordella	09/01/12	08/31/13	8,461	*
Eleanor Schwartz Charitable Foundation	Dr. Churchland	07/15/11	07/14/14	200,000	
The Seraph Foundation	Dr. Vakoc	12/21/12	12/20/13	125,000	*
Ms. Constance Silveri	Dr. Tonks	12/27/12	12/26/13	15,000	*
The Simons Foundation	Dr. Mills	07/01/10	06/30/13	350,000	
	Drs. Mitra/Huang	12/01/11	11/30/14	300,000	

[1]Includes direct and indirect costs

*New or competing renewal grants awarded in 2012

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2012 Funding*[1]	
	Dr. Osten	10/01/11	09/30/13	$ 175,000	
	Dr. Osten	07/01/12	06/30/13	60,000	*
Mary F. Smith Family Foundation	Dr. Tonks	12/01/12	11/30/13	5,000	*
Smithtown Steakhouse LLC D/B/A Insignia Prime Steak: Sushi	Dr. Sordella	01/01/12	12/31/13	75,000	*
Starr Cancer Consortium	Dr. Hannon	08/01/11	07/31/13	216,000	
	Dr. Hannon	08/01/11	07/31/13	270,000	
	Drs. Hicks/Wigler	08/01/11	07/31/13	320,760	
	Dr. Sordella	08/01/11	07/31/13	216,000	
	Drs. Sordella/Pappin	08/01/11	07/31/13	221,988	
	Dr. Vakoc	08/01/11	07/31/13	270,000	
Swim Across America	Dr. Sordella	12/15/11	12/14/14	75,000	
U.S.–Israel Binational Agricultural Research and Development Fund	Dr. Krainer	10/01/10	09/30/14	10,000	
	Dr. Lippman	06/01/10	05/31/13	42,000	
	Dr. Zador	10/01/10	09/30/14	5,750	
University Health Network	Dr. Muthuswamy	01/01/11	12/31/12	28,339	
University of Pennsylvania	Dr. Krainer	08/22/12	08/31/14	150,000	*
The V Foundation	Dr. Vakoc	11/01/12	10/31/14	100,000	*
	Dr. Zheng	11/01/11	10/31/13	100,000	
Whitehall Foundation, Inc.	Dr. Albeanu	01/01/12	12/31/14	75,000	*
	Dr. Shea	09/01/11	08/31/15	75,000	
West Islip Breast Cancer Coalition for Long Island Inc.	Dr. Tonks	11/01/12	10/31/13	10,000	*
Elisabeth R. Woods Foundation Inc.	Dr. Sordella	08/16/12	08/15/13	27,000	*
Yale University/Gilead Sciences Inc.	Dr. Sordella	07/16/12	07/15/14	410,058	*
The Bradley Zankel Foundation, Inc.	Dr. Zheng	02/22/12	02/21/13	15,000	*
Fellowship Support					
American Cancer Society	Dr. Feigin	01/01/11	12/31/12	52,000	
	Dr. Preall	10/01/11	09/30/14	50,000	
Cashin Family Fund	Watson School of Biological Sciences	09/01/10	08/31/12	80,000	
CSHL Association Fellowship	Dr. Tuveson	01/01/12	12/31/12	280,000	*
Genentech Foundation	Watson School of Biological Sciences	10/01/12	09/30/15	58,751	*
German Academic Exchange Service	Dr. Bergmann	09/01/11	08/31/13	38,000	
Lola A. Goldring	Dr. Stillman	09/01/12	08/31/13	80,000	*
Hope Funds for Cancer Research	Dr. Knott	07/01/12	06/30/15	45,500	*
Human Frontier Science Program	Dr. Benkovics	05/01/12	04/30/13	39,400	*
Annette Kade Charitable Trust	Watson School of Biological Sciences	09/01/12	08/31/13	25,000	*
Karp Foundation Inc.	Dr. Penzo	08/01/12	07/31/13	50,000	*
Estate of Gale Kavaliuskas	Watson School of Biological Sciences	09/01/12	08/31/13	293,849	*
The Esther A and Joseph Klingenstein Fund Inc.	Dr. Shea	07/01/10	06/30/13	50,000	
Life Sciences Research Foundation	Dr. MacAlister	08/01/11	07/31/14	56,000	
Muscular Dystrophy Association	Dr. Sahashi	02/01/12	01/31/15	60,000	*
National Alliance for Research on Schizophrenia and Depression (NARSAD)	Dr. Mirrione	07/15/11	07/14/13	30,000	
National Agriculture and Food Research Organization (Japan)	Dr. Hiraga	09/17/12	09/16/13	14,642	*
Mr. and Mrs. John C. Phelan	Watson School of Biological Sciences	09/01/10	08/31/15	100,000	
Damon Runyon Cancer Research Foundation	Dr. Sabin	01/01/12	12/31/14	52,000	*

[1]Includes direct and indirect costs

*New or competing renewal grants awarded in 2012

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2012 Funding*[1]	
Sass Foundation for Medical Research Inc.	Dr. Vakoc	11/01/10	10/31/12	$ 8,752	
Lauri Strauss Leukemia Foundation	Dr. Kawaoka	09/01/12	08/31/13	10,000	*
The Swartz Foundation	Dr. Zador	01/01/12	12/31/12	75,000	*
	Drs. Koulakov/Wei	01/01/12	12/31/12	55,000	*
	Drs. Kepecs/Hangya	01/01/12	12/31/12	55,000	*
	Dr. Zador	01/01/12	12/31/12	15,000	*
The Swedish Society of Medicine	Dr. Ohlund	10/08/12	12/31/12	8,300	*
Training Support					
Clare College	Undergraduate Research Program	06/01/12	05/31/13	5,645	*
The Lita Annenberg Hazen Foundation	Undergraduate Research Program	05/01/08	04/30/18	10,000	
Howard Hughes Medical Institute	Undergraduate Research Program	06/01/12	05/31/13	8,250	*
William Townsend Porter Foundation	Undergraduate Research Program	04/01/12	03/31/13	12,000	*
Steamboat Foundation	Undergraduate Research Program	05/01/12	04/30/13	12,000	*
Trinity College	Undergraduate Research Program	06/01/12	05/31/13	3,970	*
Course Support					
Alzheimer's Drug Discovery Foundation	Workshop on Cognitive Aging	11/01/11	10/31/12	2,500	
American Brain Tumor Association	Brain Tumors	02/01/12	01/31/13	45,000	*
American Express Foundation	Leadership in Bioscience	11/01/11	10/31/14	65,000	
American Federation for Aging Research	Workshop on Cognitive Aging	01/01/12	12/31/12	2,500	*
Carl Zeiss Microscopy, LLC	Imaging Structure and Function in the Nervous System	06/15/12	06/14/13	5,000	*
The Ellison Medical Foundation	Workshop on Cognitive Aging	05/01/12	04/30/13	15,000	*
Howard Hughes Medical Institute	Course Support	01/01/07	12/31/12	600,000	
Nancy Lurie Marks Family Foundation	Workshop on Biology of Social Cognition	07/01/12	06/30/16	25,000	*
Roche Diagnostics Corporation	Advanced Sequencing Technologies and Applications	02/01/12	01/31/13	30,000	*
Society for Neuroscience/International Brain Research Organization	Summer Neuroscience Course	07/01/12	06/30/13	17,210	*
Meeting Support					
Abbott Laboratories	Gene Expression and Signaling in the Immune System	04/01/12	03/31/13	3,000	*
Amgen, USA	Molecular Pathways in Organ Development and Disease	09/01/11	08/31/12	5,000	
	Gene Expression and Signaling in the Immune System	04/01/12	03/31/13	5,000	*
Art Guild, Inc.	Corporate Exhibitors	03/01/12	02/28/13	5,000	*
Clontech Laboratories, Inc.	Single-Cell Analysis	11/01/12	10/31/13	5,000	*
The Company of Biologists LTD	Molecular Pathways in Organ Development and Disease	09/01/11	08/31/12	7,898	
Complete Genomics, Inc.	Corporate Exhibitors	03/01/12	02/28/13	14,000	*
Constellation Pharmaceuticals	Gene Expression and Signaling in the Immune System	04/01/12	03/31/13	2,000	*
Enzo Life Sciences	Molecular Chaperones and Stress Responses	04/01/12	03/31/13	2,500	*
Exiqon, Inc.	Corporate Exhibitors	03/01/12	02/28/13	500	*
Forest Research Institute Inc.	Blood Brain Barrier	12/01/12	11/30/13	25,000	*

[1]Includes direct and indirect costs

*New or competing renewal grants awarded in 2012

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2012 Funding*[1]	
The Gatsby Charitable Foundation	77th CSHL Symposium: The Biology of Plants	02/01/12	01/31/13	$ 3,150	*
Gilead Sciences Inc.	Retroviruses	05/01/12	04/30/13	12,500	*
Illumina, Inc.	Corporate Exhibitors	03/01/12	02/28/13	19,000	*
Life Technologies Corporation	Personal Genomes and Medical Genomics	12/15/12	12/14/13	10,000	*
March of Dimes Foundation	Molecular Pathways in Organ Development and Disease	04/01/12	09/30/12	5,000	*
MedImmune, LLC	Molecular Genetics of Bacteria and Phage	11/07/11	11/06/12	5,000	
Merck Sharp and Dohme Corporation	Molecular Pathways in Organ Development and Disease	03/01/12	02/28/13	15,000	*
Molecular Devices Corporation	Corporate Exhibitors	03/01/12	02/28/13	5,000	*
Gordon and Betty Moore Foundation	77th CSHL Symposium: The Biology of Plants	02/01/12	01/31/13	10,000	*
Nature America Inc.	Molecular Genetics of Bacteria and Phage	11/07/11	11/06/12	750	
Novartis Pharmaceuticals Corp.	PTEN Pathways and Targets	11/18/11	03/16/13	25,000	
Christine O'Neil	Corporate Exhibitors	03/01/12	02/28/13	6,000	*
Opgen, Inc.	Corporate Exhibitors	03/01/12	02/28/13	7,000	*
Phytotechnology Laboratories	Corporate Exhibitors	03/01/12	02/28/13	500	*
Promega Corporation	Molecular Genetics of Bacteria and Phage	11/07/11	11/06/12	750	
Martin Reese	Corporate Exhibitors	03/01/12	02/28/13	4,000	*
Rutgers, the State University of New Jersey	Protein Data Bank 40th Anniversary	12/01/10	11/30/12	2,300	
Society for Developmental Biology	Molecular Pathways in Organ Development and Disease	09/01/11	08/31/12	4,000	
Lemna Tec	Corporate Exhibitors	03/01/12	02/28/13	2,500	*
Library Support					
The Ellen Brenner Memorial Fund		12/15/12	12/14/13	2,000	*
The New York State Education Department		07/01/10	06/30/12	3,497	
The New York State Education Department		07/01/11	06/30/12	16,584	
Wellcome Trust		08/01/11	07/31/13	302,364	
Library Meeting Support					
Alfred P. Sloan Foundation		04/01/12	05/01/12	20,000	*
The Rockefeller University		04/01/12	03/31/13	15,000	*

[1]Includes direct and indirect costs

*New or competing renewal grants awarded in 2012

DNA LEARNING CENTER GRANTS

Grantor	*Program*	*Duration of Grant*	*2012 Funding*[†]
FEDERAL GRANTS			
National Science Foundation, University of Arizona	Educational Outreach for *iPlant Collaborative*: A Cyberinfrastructure for Plant Sciences	2/08–1/13	860,267
National Science Foundation, North Carolina State University	GEPR: Epigenome Dynamics during DNA Replication	3/11–2/13	73,108
National Science Foundation	Advanced Technology Education (ATE) Program: *Genomic Approaches in BioSciences*	4/11–3/14	157,894
NONFEDERAL GRANTS			
Victor Centers for Prevention of Jewish Genetic Diseases, Albert Einstein Healthcare Center/Marcus Jewish Genetic Disease Consortium	*Gene Screen* iPhone/iPad Application	12/10–12/12	31,900
Alfred P. Sloan Foundation	DNA Barcoding Experiments by New York City High School Students (*Urban Barcode Project*)	1/11–7/12	74,533
Howard Hughes Medical Institute	Pre-College Science Education Initiative: NYC Teacher Professional Development	9/07–8/12	145,319
Dana Foundation	*Harlem DNA Lab* Operating Support	3/09–2/12	27,700
William Townsend Porter Foundation	Scholarships for Minority and Underserved Students at *Harlem DNA Lab*	3/11–3/12	17,000
National Grid Foundation	Scholarships for Minority and Underserved Students in the Central Islip Union Free School District	1/12 –12/12	15,000
Spinal Muscular Atrophy Foundation	*Learn About SMA* Internet site	4/11–4/13	78,965

The following schools and school districts each contributed $1,000 or more for participation in the *Curriculum Study* Program:

Elwood Union Free School District	$ 1,500	Locust Valley Central School District	1,500
Fordham Preparatory School	3,000	Northport-East Northport Union Free School District	3,000
Great Neck Union Free School District	1,500	Oceanside Union Free School District	3,000
Half Hollow Schools Central School District	3,000	Port Washington Union Free School District	3,000
Herricks Union Free School District	1,500	Ramaz Upper School	1,500
Huntington Union Free School District	1,500	Sachem Central School District	3,000
Levittown Union Free School District	3,000	Yeshiva University High School for Girls	3,000

The following schools and school districts each contributed $1,000 or more for participation in the *Genetics as a Model for Whole Learning* Program:

Adelphi STEP	$ 1,050	Lynbrook Union Free School District	1,000
Bay Shore Union Free School District	2,800	Merrick Union Free School District	3,400
Bellmore Union Free School District	2,800	M.S. 447, Brooklyn	1,800
Bellmore–Merrick Union Free School District	10,000	North Bellmore Union Free School District	2,600
Cold Spring Harbor Central School District	14,400	North Shore Central School District	1,750
Commack Union Free School District	6,900	North Shore Hebrew Academy	1,050
East Meadow Union Free School District	3,730	Oceanside Union Free School District	1,625
East Williston Union Free School District	2,900	Oyster Bay–East Norwich Central School District	5,625
Elwood Union Free School District	3,425	Plainedge Union Free School District	2,100
Floral Park–Bellerose Union Free School District	7,800	Port Washington Union Free School District	9,600
Friends Academy	2,100	Rockville Centre Union Free School District	6,240
Garden City Union Free School District	4,000	Roslyn Union Free School District	4,200
Great Neck Union Free School District	6,000	Sachem Union Free School District	2,925
Half Hollow Hills Union Free School District	9,150	Saint Dominic Elementary School	4,550
Herricks Union Free School District	2,275	Scarsdale Union Free School District	12,600
Hofstra STEP	1,050	Smithtown Union Free School District	1,680
Holy Child Academy	3,100	Syosset Union Free School District	36,600
IHM Home School Program	1,050	Three Village Central School District	4,200
Huntington Union Free School District	4,600	Trinity Regional School	1,300
Jericho Union Free School District	8,250	Valley Stream 13 Union Free School District	1,050
Locust Valley Central School District	8,859	Yeshiva of Flatbush	2,000

[†]Includes direct and indirect costs.

BANBURY CENTER GRANTS

Grantor	*Program*	*Duration of Grant*	*2012 Funding*
FEDERAL SUPPORT			
Centers for Disease Control and Prevention	Decoding Clinical Trials to Improve Treatment of ME/CFS	2012	$18,440
NIH–National Institute of Mental Health	The Fourth Annual NIMH Brain Camp	2012	35,662
National Science Foundation through a grant to Stony Brook University	Grand Challenges in Organismal Biology	2012	9,045
NONFEDERAL SUPPORT			
Astellas-OSI Oncology	Cell Plasticity in Cancer Evolution	2012	49,602
Baxter Healthcare Corporation	Patenting Genes: New Developments, New Questions	2012	5,000
Boehringer Ingelheim Fonds	Communicating Science	2012	50,549
Certerra, Inc.	Systems Biology of Autism: From Basic Science to Therapeutic Strategies	2012	4,266
CFIDS Association of America	Decoding Clinical Trials to Improve Treatment of ME/CFS	2012	39,107
Cold Spring Harbor Laboratory Corporate Sponsor Program	Transcription and Cancer	2012	43,261
Cold Spring Harbor Laboratory Corporate Sponsor Program	Inflammation, Cancer, and Metabolism	2012	46,068
Cold Spring Harbor Laboratory Library and Archives	Envisioning the Future of Science Libraries at Academic Research Institutions	2012	4,512
Cold Spring Harbor Laboratory/DuPont Pioneer Joint Collaborative	Plant–Environment Interactions	2012	50,000
Discovery Network	Inspire2Live	2012	21,203
DRI Capital, Inc.	Patenting Genes: New Developments, New Questions	2012	7,500
GangaGen, Inc.	Phage and Phage-Based Therapies	2012	39,857
Genentech, Inc.	Patenting Genes: New Developments, New Questions	2012	10,000
Individual participants	The Fourth Annual NIMH Brain Camp	2012	8,070
Individual participants	Patenting Genes: New Developments, New Questions	2012	1,335
Individual participants	Emerging Approaches in Oncology: A Brainstorming Think Tank	2012	2,000
Individual participants	Grand Challenges in Organismal Biology	2012	1,005
Individual participants	Decoding Clinical Trials to Improve Treatment of ME/CFS	2012	2,982
Jones Day LLP	Patenting Genes: New Developments, New Questions	2012	2,000
Kaye Scholer LLP	Patenting Genes: New Developments, New Questions	2012	5,000
King & Spalding, LLP	Patenting Genes: New Developments, New Questions	2012	5,000
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Haig R. Nalbantian	Interdisciplinary Symposium on Literature, Memory, and Neuroscience	2012	1,000
Novartis Pharma AG	Patenting Genes: New Developments, New Questions	2012	5,000
Novo Nordisk Inc.	Patenting Genes: New Developments, New Questions	2012	5,000
Pfizer, Inc.	Systems Biology of Autism: From Basic Science to Therapeutic Strategies	2012	5,000
Mr. and Mrs. Howard Phipps, Jr.	Interdisciplinary Symposium on Literature, Memory, and Neuroscience	2012	10,000
Marie Robertson Research Fund	Systems Biology of Autism: From Basic Science to Therapeutic Strategies	2012	20,000
Robertson Fund	Systems Biology of Autism: From Basic Science to Therapeutic Strategies	2012	10,000
The Rockefeller University	Envisioning the Future of Science Libraries at Academic Research Institutions	2012	15,000

BANBURY CENTER GRANTS (*Continued*)

Grantor	*Program*	*Duration of Grant*	*2012 Funding*
Ropes & Gray	Patenting Genes: New Developments, New Questions	2012	5,000
The Daniel and Joanna S. Rose Fund, Inc.	Interdisciplinary Symposium on Literature, Memory, and Neuroscience	2012	10,000
The Satenik and Adom Ourian Educational Foundation	Interdisciplinary Symposium on Literature, Memory, and Neuroscience	2012	1,000
Simons Foundation	Systems Biology of Autism: From Basic Science to Therapeutic Strategies	2012	10,000
The Alfred P. Sloan Foundation	A History of the Human Genome Project	2012	50,000
The Alfred P. Sloan Foundation	Envisioning the Future of Science Libraries at Academic Research Institutions	2012	20,000
University of Louisville, Brown Cancer Center	Regulation of Metabolism in Cancer	2012	45,625
University of Southern California, NCI Physical Sciences in Oncology Center	Emerging Approaches in Oncology: A Brainstorming Think Tank	2012	32,663

CORPORATE SPONSOR PROGRAM FOR MEETINGS SUPPORT

The Corporate Sponsor Program continues to provide critical funding for the vigorous meetings program held at Cold Spring Harbor Laboratory, whether at Grace Auditorium on the main Laboratory campus or at the Banbury Center. Without the strong foundation provided by the Program, we could neither plan with confidence for the year's meetings nor introduce new and unusual topics.

We are especially grateful to the companies that joined us in 2012 as the economic difficulties have persisted. The year 2013 is going to be especially challenging as the number of companies shrinks through takeovers, and companies and foundations adopt austerity measures. The members of the Program receive special privileges in acknowledgment of their contributions. We waive all on-site fees for eight representatives of the Corporate Sponsors at our meetings. Three scientists from Sponsors may attend meetings at the Banbury Center, where attendance is otherwise only by invitation of the organizers. Member companies also receive gratis copies of Cold Spring Harbor Laboratory Press publications, including the journals *Genes & Development*, *Learning & Memory*, *CSH Protocols,* and *Genome Research*.

We acknowledge our Sponsors in all relevant publications, including the books of abstracts given to each of the 7000 participants who come to the meetings each year. The names of the sponsoring companies are listed on the poster describing the meetings, and this is mailed to approximately 17,000 scientists throughout the world. In addition, the companies are listed on the Cold Spring Harbor Laboratory website on the Meetings Office and Banbury Center pages. Members in 2012 were the following:

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DEVELOPMENT

With the support of our many generous donors, Cold Spring Harbor Laboratory had one of the most successful years in our history. For the first time, more than $6 million was raised for unrestricted support. The 2012 Double Helix Medals dinner was also hugely successful as we honored Michael J. Fox, Art Levinson, and Mary Lindsay. In their unique ways, each of these individuals has made the world a better place, and we were very proud to recognize their achievements.

In July, John Cleary's family and friends gathered to celebrate the dedication of a laboratory in his memory. The *John P. Cleary Laboratory* is located in the DeMatteis building and is home to Dr. Michael Wigler's lab. John was involved with the Laboratory for many years as CSHL Association's President, Trustee, and then as an Honorary Trustee. His friendship to the Laboratory, the scientists, the employees, and all who walked on our grounds will never be forgotten.

With support from the Lustgarten Foundation, Cold Spring Harbor Laboratory welcomed Dr. Dave Tuveson, one of the world's most preeminent researchers and clinicians in the field of pancreatic cancer research. Dave was formerly at the Cancer Research UK Cambridge Research Institute, and he came to the Laboratory to direct our Cancer Therapeutics Initiative. This ambitious initiative will focus on moving advanced-stage drug candidates from the laboratory to the clinic. With more space needed to accommodate sophisticated technology and additional people, plans are set to expand our Woodbury facility. We will be breaking ground on the project in the second quarter of 2013.

All our successes during this past year could not have happened without the support of our donors and we are extremely thankful for all of their contributions.

Charles V. Prizzi, *Vice President for Development*

Cold Spring Harbor Laboratory is a nonprofit research and educational institution chartered by the State of New York. Less than half of the Laboratory's annual revenues are derived from federal grants and contracts, and thus, it relies heavily upon support from the private sector: foundations, corporations, and individuals. CSHL takes this occasion to sincerely thank each and every donor whose generous contributions have made possible the discoveries we report in this publication. There are a variety of ways to give to the Laboratory:

Capital and Endowment Campaign Support: Donations help to secure the financial stability of CSHL and provide resources to expand the facilities and staff.

Research Support: Donations in specific areas of research help to increase the speed at which genetic discoveries are translated into diagnostic tests and therapeutics.

Annual Fund: Donations provide funding for some of the most innovative young researchers in science today and constitute an important investment in groundbreaking research in cancer, neuroscience, plant biology, and bioinformatics.

Science Education: Donations support programs at the DNA Learning Center and the Watson School of Biological Sciences, where the next generation learns about genetics in an exciting and interactive environment.

Planned and Estate Gifts: Individuals who inform us of their intention to make a gift to CSHL from their estate are invited to become members of the Harbor Society. Estate gifts help to ensure that CSHL will continue to pursue its mission for many years to come.

For additional information, please contact Charlie Prizzi, Vice President for Development, Cold Spring Harbor Laboratory, One Bungtown Road, Cold Spring Harbor, New York 11724. Phone number: 516-367-6961. E-mail: prizzi@cshl.edu

President's Council

This year's topic—Ethical Dilemmas in Medicine—enabled us to shine light on CSHL's human genetics research, strides that we are making in cancer profiling, and the Laboratory's early role in the 20th-century eugenics movement. Three of our October talks were presented by key CSHL individuals: Dave Micklos, founder and head of CSHL DNA Learning Center; David Spector, CSHL Director of Research; and Lloyd Trotman, Assistant Professor of cancer research. Gholson Lyon, M.D., PhD., a new CSHL Assistant Professor of neuroscience, spoke at the Council reception in May hosted by Claudia and Gunnar Overstrom in their magnificent New York City apartment.

Dartmouth Professor H. Gilbert Welch, M.D., opened the October meeting with a captivating presentation on the downside of routine preclinical medical screening. Saturday talks featured a thoughtful lecture on informed consent of incapacitated human subjects and the ethics of gene patenting. In addition, an extraordinary keynote lecture by Andrew Solomon moved most of our guests to tears with his moving account of the love and special meaning that parents find in raising children who are different, the topic of his newest book *Far from the Tree: Parents, Children and the Search for Identity.*

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President's Council speaker, Andrew Solomon signing copies of his books for attendees

Freddie Staller, Gilbert Welch, and Erwin Staller

Cold Spring Harbor Laboratory Association

With 1200 members, the Cold Spring Harbor Laboratory Association (CSHLA) continues to increase community awareness of CSHL, and this year they helped raise more than $6 million in unrestricted support for the Laboratory. Events spearheaded by the Directors of the Association included Labapalooza, the 77th Symposium dinner parties, the Women's Partnership for Science luncheon, and a variety of receptions and dinners that introduced new friends to the exceptionally talented young scientists at CSHL. Members also had a significant role in the success of the Double Helix Medals Dinner in New York City and the 19th annual golf tournament at the Piping Rock Club. Special thanks go to Sandy Tytel, president, as she continues to enthusiastically lead the CSHLA directors to new levels of participation.

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The Corporate Advisory Board (CAB) is a vital liaison between Cold Spring Harbor Laboratory and the tri-state business community. Established more than 20 years ago, the CAB is the driving force behind the Laboratory's annual golf outing at Piping Rock Club; this outing raises critical unrestricted funding for research and educational programs.

Serving as CAB Chair for more than 10 years and Golf Chair for 6 years, Eddie Chernoff continues to provide leadership and support and we truly appreciate all of his efforts.

CAB Chairman Eddie Chernoff with his wife Donna and Drs. Mona and David Spector

CAB member Michael Aboff and his son Matthew present a check to Dr. Bruce Stillman for breast cancer research

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CSHL Trustee Leo Guthart (*left*) with Jim Simons

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The Don Monti Memorial Research Foundation provides critical support to CSHL's cancer research program. Featured are President Caroline Monti-Saladino and Chairmen Richard Monti (*left*) and Artie Saladino

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CSHL researchers Mikala Egeblad and Nick Tonks with Jason Gladowsky and Alison Gladowsky of the Joni Gladowsky Breast Cancer Foundation

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Dr. Robert and Marjorie Feeney
James R. Feramisco
Douglas and Christine Fox
Mr. and Mrs. Roger Fradin
E. Maxwell Geddes, Jr.
**Robert Joy Glaser, M.D.
Mr. and Mrs. William H. Grover
Frank and Lynn Gundersen
Rev. Linda Peyton Hancock
Gil and Sheila Henoch
Allan and Carole Herzog
Mr. and Mrs. Joseph Heyenoort–Cold Spring Motors
Lori and Chris Homer–A.L.L. Caring Foundation
Theodora W. Hooton
Mr. and Mrs. Robert E. Hughes
Joan M. Hutchins
Frederic B. Ingraham
Virginia P. Jacobsen
Stephen Juelsgaard
Dolores Fredrich and Jeffrey Keller
Edward M. Lamont
Mr. and Mrs. James Large
Claudia Lawrence
Mr. and Mrs. Anthony V. Leness
Brett MacInnes
Mr. and Mrs. Patrick H. Mackay
Mr. and Mrs. Robert G. MacLean
Kevin G. Mahony
Simone Mailman
Mr. and Mrs. John Maroney
Marsh USA, Inc.
Marshs
Victoria S. Meagher
Mercer Health & Benefits LLC
MetLife
Dr. Jeffrey H. Miller
Leo Model Foundation
Mrs. Keith M. Moffat
Kathleen Mooney
John Moore
Mr. and Mrs. Stephen J. Moore
Catherine Moraetis
Mr. and Mrs. Francis W. Murray III
Mr. and Mrs. Paul Napoli
Richard P. and Faye Nespola Charitable Foundation
New England Foundation
Mr. and Mrs. James H. Norris
Dr. Larry Norton
Mr. and Mrs. Michael S. O'Brien
Hugh & Patricia O'Kane Family Foundation
Mr. and Mrs. Richard S. Overton
Tracy Dellomo and Clifford Packingham
Anthony Panebianco
Rhoda Parmet
Mr. and Mrs. Nicholas B. Paumgarten
Dave and Terry Peikon
Cecile Pickart Student Travel Award
Louise S. Pickart
Mr. and Mrs. Robert A. Pilkington
Carol and George C. Pratt
Mark Ptashne and Lucy Gordon
Mr. and Mrs. Thomas L. Pulling
K.J. Quinn Charitable Foundation
Curtis S. Read
Russell S. Reynolds, Jr.
Mr. and Mrs. Arthur M. Rogers, Jr.
Susan & Elihu Rose Foundation, Inc.
Michele Rosenberg
Mr. and Mrs. J. Wright Rumbough, Jr.
Drs. Maria and Charles S. Ryan
Raju Sarwal, M.D.
Mr. and Mrs. Lee Schalop
Robin and Enrique Senior
Dr. Jack Skalicky
Mr. and Mrs. Trevor A. Smith
Drs. Mona and David Spector
Mr. and Mrs. James L. Spingarn
Dr. Wesley I. Sundquist
Judith and Irwin Tantleff
Mrs. David S. Taylor
Pamela M. Thye
Mr. and Mrs. Bart T. Tiernan
Lynn and Pat Tone
Robert H. Trust and Sarah Vermylen Trust
George Vande Woude
Helene P. Victor
Jackie and Warren Wasp
Mr. and Mrs. Ted Weiss
Mr. and Mrs. Karl G. Wellner
Neil Westreich
Gerald I. White
Wolfensohn Family Foundation

**Deceased

CORPORATE AND FOUNDATION MATCHING GIFT CONTRIBUTORS

American Express Charitable Fund
American Express PAC Match Program
Bank of America Matching Gifts
Bristol-Myers Squibb Foundation
Dow Jones & Company, Inc.
FBR Capital Markets Charitable Foundation
Honeywell Matching Gift Program
iStar Financial, Inc.
The J.P. Morgan Chase Foundation
McKinsey & Company, Inc.
David and Lucile Packard Foundation
Pfizer Foundation
RLI Charitable Fund
United Way of New York City
Wells Fargo Foundation

IN HONOR OF

Elinor Bettencourt
Jamie Nicholls and Fran Biondi
Bonnie and Steve Brenner
Eddie Chernoff
Rhonda Federman
Jessie Friedman's Bat Mitzvah
Dr. Candido Fuentes
Mary Goldberg
Mrs. Oliver R. Grace
Dr. Nancy Israeli
Suzanne Leeds
Mary D. Lindsay
Cathryn MacInnes
Justin Rosati
Scheman-Villani
Edith Seligson
Drs. Marilyn and James Simons
Page Underwood
Dr. James D. Watson
Dr. Michael Wigler
Dr. Jan Witkowski

IN MEMORY OF

Elaine Abrams
T.J. Arcati
C. A. Beach
Anthony Biondolillo
Richard Blackman
Patricia Boudreau
Ellen Brenner
Ahmad Bukhari
Carol A. Buonaiuto
Robert Cafferkey
Mark A. Calace
Patricia May Duke Card
Josephine and Ted Chodkowski
John P. Cleary
Tina Duque Corbett
Randie Switko Delmont
Dr. Milislav Demerec
George and Evelyn Diehl
James Eisenman
Daniel Fishman
Ardell Bierman Fox
Albert L. Freedman
Paul Garrett
Nancy Slaughter Gay
Joni Gladowsky
Dr. Christopher G. Goff
Teresa Haire
Skip Hargraves
Walter Hein
Dr. Andy Kaplan
Townsend Knight
Lydia A. Krawec
James Laas
Cathy Lipstein
Lisa MacInnes
Carol Marcincuk
Carissa Maringo
Elizabeth McFarland
Steven Michalik
Rose M. Mooney
Monica Mowery
Donald J. Parmet
Dr. Cecile Pickart
Dorothy Pirozzi
G. Jeffrey Poletti
Christina Renna
Elizabeth Roberts
Dolores Ann Roman
Dr. Susan Rose
Diane Edmin Sachs
Howard Roy Schwartz
Iris Schwartzman
Martha Seidel
Andrew Seligson
Arnold Stanley Solomon
Becky Stephens
Edward M. Thomas
Harold Umansky
Linda Vernam
Dr. Uta von Schwedler
Fay Wallace

FINANCE

FINANCIAL STATEMENTS

CONSOLIDATED BALANCE SHEET
December 31, 2012
(with comparative financial information as of December 31, 2011)

		2012	2011
Assets:			
Cash and cash equivalents	$	75,471,404	58,055,329
Grants receivable		10,012,401	5,733,104
Contributions receivable, net		145,289,626	163,144,645
Publications inventory		2,767,427	3,292,898
Investments		296,611,329	269,786,326
Restricted use assets		3,597,846	2,882,590
Other assets		15,891,942	15,027,787
Land, buildings, and equipment, net		240,625,332	241,828,796
Total assets	$	790,267,307	759,751,475
Liabilities and net assets:			
Liabilities:			
Accounts payable and accrued expenses	$	10,456,412	11,135,584
Deferred revenue		5,258,525	5,467,566
Interest rate swap		35,556,347	37,726,697
Bonds payable		97,200,000	97,200,000
Total liabilities		148,471,284	151,529,847
Net assets:			
Unrestricted		243,281,390	205,967,407
Temporarily restricted		293,464,158	298,351,339
Permanently restricted		105,050,475	103,902,882
Total net assets		641,796,023	608,221,628
Total liabilities and net assets	$	790,267,307	759,751,475

CONSOLIDATED STATEMENT OF ACTIVITIES
Year ended December 31, 2012
(with summarized financial information for the year ended December 31, 2011)

	Unrestricted	*Temporarily Restricted*	*Permanently Restricted*	*2012 Total*	*2011 Total*
Revenue and other support:					
Public support–contributions and nonfederal grant awards	$ 19,618,621	40,079,983	1,147,593	60,846,197	113,684,017
Federal grant awards	28,711,510	–	–	28,711,510	34,490,267
Indirect cost allowances	24,445,726	–	–	24,445,726	25,623,148
Investment return utilized	23,396,825	–	–	23,396,825	22,585,474
Program fees	8,049,856	–	–	8,049,856	6,963,110
Publications sales	10,957,863	–	–	10,957,863	9,848,446
Dining services	4,614,691	–	–	4,614,691	4,309,694
Rooms and apartments	3,770,473	–	–	3,770,473	3,345,273
Miscellaneous	2,527,870	–	–	2,527,870	3,745,958
Net assets released from restrictions	58,423,282	(58,423,282)	–	–	–
Total revenue and other support	184,516,717	(18,343,299)	1,147,593	167,321,011	224,595,387
Expenses:					
Research	88,821,283	–	–	88,821,283	91,848,784
Educational programs	17,815,388	–	–	17,815,388	16,117,941
Publications	10,092,372	–	–	10,092,372	9,323,927
Banbury Center conferences	1,389,048	–	–	1,389,048	1,409,384
DNA Learning Center programs	1,578,211	–	–	1,578,211	1,857,078
Watson School of Biological Sciences programs	3,356,033	–	–	3,356,033	3,584,716
General and administrative	16,860,891	–	–	16,860,891	15,596,163
Dining services	5,642,788	–	–	5,642,788	5,717,278
Total expenses	145,556,014	–	–	145,556,014	145,455,271
Excess (deficiency) of revenue and other support over (under) expenses	38,960,703	(18,343,299)	1,147,593	21,764,997	79,140,116
Other changes in net assets:					
Investment (loss) return excluding amount utilized	(3,817,070)	13,456,118	–	9,639,048	(22,610,255)
Change in fair value of interest rate swap	2,170,350	–	–	2,170,350	(21,287,538)
Increase (decrease) in net assets	37,313,983	(4,887,181)	1,147,593	33,574,395	35,242,323
Net assets at beginning of year	205,967,407	298,351,339	103,902,882	608,221,628	572,979,305
Net assets at end of year	$ 243,281,390	293,464,158	105,050,475	641,796,023	608,221,628

CONSOLIDATED STATEMENT OF CASH FLOWS
Year ended December 31, 2012
(with comparative financial information for the year ended December 31, 2011)

	2012	2011
Cash flows from operating activities:		
Increase in net assets	$ 33,574,395	35,242,324
Adjustments to reconcile increase in net assets to net cash provided by operating activities:		
Change in fair value of interest rate swap	(2,170,350)	21,287,538
Depreciation and amortization	15,422,891	14,697,263
Net (appreciation) depreciation in fair value of investments	(29,322,335)	4,173,189
Contributions restricted for long-term investment	(10,527,878)	(13,475,213)
Changes in assets and liabilities:		
Grants receivable	(4,279,297)	1,992,555
Contributions receivable, net of financing activities	25,176,987	(53,517,765)
Publications inventory	525,471	262,295
Other assets	(912,934)	(5,008,296)
Restricted use assets	(715,256)	(302,119)
Accounts payable and accrued expenses, net of financing activities	(139,990)	1,835,064
Deferred revenue	(209,041)	(2,743,102)
Net cash provided by operating activities	26,422,663	4,443,733
Cash flows from investing activities:		
Capital expenditures	(14,219,427)	(17,748,180)
Proceeds from sales and maturities of investments	73,005,504	33,325,960
Purchases of investments	(70,508,172)	(30,860,745)
Net change in investment in employee residences	48,779	(97,394)
Net cash used in investing activities	(11,673,316)	(15,380,359)
Cash flows from financing activities:		
Contributions restricted for long-term investment	1,147,593	1,600,563
Contributions restricted for investment in capital	9,380,285	11,874,650
Increase in contributions receivable	(7,321,968)	(5,354,289)
(Decrease) increase in accounts payable relating to capital expenditures	(539,182)	104,669
Net cash provided by financing activities	2,666,728	8,225,593
Net increase (decrease) in cash and cash equivalents	17,416,075	(2,711,033)
Cash and cash equivalents at beginning of year	58,055,329	60,766,362
Cash and cash equivalents at end of year	$ 75,471,404	58,055,329
Supplemental disclosure:		
Interest paid	$ 3,683,436	3,614,370
Noncash investing and financing activity:		
Contributed property	$ 50,000	1,095,000

FINANCIAL SUPPORT OF THE LABORATORY

Cold Spring Harbor Laboratory, Banbury Center, and the Dolan DNA Learning Center receive a substantial portion of their funding through grants from the federal government and through grants, capital gifts, and annual contributions from New York State, private foundations, corporations, and individuals. The following section summarizes funding that occurred during 2012.

GRANTS January 1–December 31, 2012

COLD SPRING HARBOR LABORATORY

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2012 Funding*[1]	
FEDERAL GRANTS					
NATIONAL INSTITUTES OF HEALTH					
Program Project and Center Support	Drs. Hannon/Krainer/Spector/Stillman	05/25/12	12/31/16	$ 4,486,042	*
	Dr. Stillman, Cancer Center Core	08/17/11	07/31/16	4,346,720	
Cooperative Research Agreement Support[2]	Dr. Gingeras	09/21/12	07/31/16	1,975,096	*
	Drs. Lowe/Hannon/Hicks/Powers	09/01/09	08/31/14	564,230	
	Drs. Powers/Hannon/Krasnitz/Sordella	05/01/12	04/30/17	1,013,392	*
Equipment Support	Dr. Hannon	04/01/12	03/31/13	519,814	*
Research Support	Dr. Churchland	09/01/10	08/31/13	247,403	
	Dr. Dubnau	09/15/09	08/31/14	466,125	
	Drs. Enikolopov/Koulakov	09/15/11	08/31/16	411,276	
	Dr. Furukawa	03/01/10	02/28/15	457,875	
	Dr. Hannon	09/01/09	08/31/13	378,401	
	Dr. Huang	07/01/11	03/31/16	560,565	
	Drs. Kepecs/Huang	07/01/11	05/31/16	411,250	
	Drs. Koulakov/Enikolopov	07/15/10	02/28/14	356,621	
	Dr. Krainer	04/01/12	03/31/17	728,529	*
	Dr. Krainer	09/01/12	08/31/14	235,416	*
	Dr. Li	07/01/10	03/31/15	560,038	
	Dr. Martienssen	09/15/11	08/31/15	344,865	
	Dr. Martienssen	01/20/12	11/30/15	737,743	*
	Dr. McCombie	07/23/10	02/28/15	1,069,587	
	Dr. Mills	12/26/07	11/30/12	37,605	
	Dr. Mitra	09/30/09	03/31/14	975,957	
	Dr. Mitra	08/01/10	05/31/13	406,574	
	Dr. Muthuswamy	02/01/09	12/31/13	405,930	
	Dr. Osten	04/01/12	03/31/17	485,438	*
	Dr. D. Spector	04/01/11	03/31/15	740,360	
	Dr. Stenlund	12/01/06	06/30/13	470,000	
	Dr. Stillman	06/01/12	05/31/16	696,400	*
	Dr. Tonks	07/01/10	04/30/15	761,643	
	Dr. Tonks	01/11/10	12/31/13	434,648	
	Dr. Trotman	04/01/10	01/31/14	301,376	
	Dr. Turner	07/15/10	06/30/15	454,960	

[1]Includes direct and indirect costs

[2]Cooperative research agreement funding amounts include only CSHL portion of award

*New or competing renewal grants awarded in 2012

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2012 Funding*[1]	
	Dr. Van Aelst	07/01/08	03/31/14	$ 417,658	
	Dr. Van Aelst	01/01/09	12/31/13	376,384	
	Dr. Zador	09/18/08	07/31/13	354,339	
	Dr. Zador	09/27/10	05/31/15	464,475	
	Dr. Zador	09/01/12	08/31/17	400,209	*
	Dr. Zhong	06/01/10	05/31/13	368,154	
Research Subcontracts					
NIH/Allen Institute for Brain Science Consortium Agreement	Dr. Mitra	09/15/09	08/31/13	186,076	
NIH/Cornell University Consortium Agreement	Dr. Mitra	04/03/08	01/31/13	21,091	
NIH/Georgia Institute of Technology Consortium Agreement	Dr. D. Spector	09/30/06	07/31/15	290,000	
NIH/The Johns Hopkins University Consortium Agreement	Dr. Schatz	09/21/11	08/31/14	161,013	
NIH/The Scripps Research Institute Consortium Agreement	Dr. Osten	09/30/12	08/31/13	75,600	*
NIH/University of California - San Francisco Consortium Agreement	Dr. Egeblad	09/01/09	08/31/14	174,905	
NIH/University of Southern California Consortium Agreement	Dr. Hannon	07/01/10	06/30/14	117,450	
NIH/University of Texas Consortium Agreement	Dr. Krainer	08/01/10	03/31/13	44,732	
NIH/University of Texas Consortium Agreement	Dr. Furukawa	09/30/11	05/31/15	50,514	
Fellowship Support	Dr. Ipsaro	09/01/11	08/31/13	52,190	
	Dr. Jansen	01/01/11	07/31/14	47,756	
	J. Tucciarone	09/16/12	09/15/15	32,582	*
Institutional Training Program Support	Dr. Joshua-Tor, Watson School of Biological Sciences	07/01/12	06/30/17	267,927	*
	Dr. Mills	09/01/11	08/31/16	181,564	
Course Support	Advanced Immunocytochemistry: In Situ Hybridization and Live Cell Imaging	09/01/10	08/31/15	100,323	
	Advanced Sequencing Technologies and Applications	04/10/12	03/31/15	52,885	*
	Cell and Developmental Biology of *Xenopus*	04/01/09	03/31/14	63,345	
	Cellular Biology of Addiction	03/01/11	02/29/16	80,694	
	Computational and Comparative Genomics	08/01/08	07/31/13	51,792	
	Drosophila Neurobiology: Genes, Circuits, and Behavior	07/15/12	06/30/17	35,000	*
	Eukaryotic Gene Expression	04/01/12	03/31/17	114,306	*
	Mouse Development, Stem Cells, and Cancer	04/01/12	03/31/17	110,288	*
	Programming for Biology	09/01/09	08/31/14	63,654	
	Protein Purification and Characterization	04/01/12	03/31/17	95,019	*
	Proteomics	08/01/12	04/30/17	105,068	*
	X-Ray Methods in Structural Biology	09/01/12	08/31/17	81,540	*
	Yeast Genetics and Genomics	09/18/10	06/30/13	53,462	

[1]Includes direct and indirect costs

*New or competing renewal grants awarded in 2012

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2012 Funding*[1]	
Meeting Support	Automated Imaging and High-Throughput Phenotyping	04/01/12	03/31/13	$ 5,000	*
	Axon Guidance, Synapse Formation, and Regeneration	07/01/12	06/30/13	10,000	*
	Bacteria, Archaea, and Phages	05/15/12	04/30/13	6,000	*
	The Biology of Genomes	04/01/08	03/31/13	39,339	
	Evolution of *Caenorhabditis* and Other Nematodes	04/01/12	03/31/13	15,000	*
	Gene Expression and Signaling in the Immune System	04/01/08	03/31/13	10,000	
	Germ Cells	07/01/08	06/30/13	6,000	
	Mechanisms and Models of Cancer	08/01/08	07/31/13	8,500	
	Molecular Chaperones and Stress Responses	05/01/10	04/30/13	20,602	
	Molecular Genetics of Aging	03/01/08	02/28/13	31,600	
	Neurodegenerative Diseases: Biology and Therapeutics	09/26/12	09/25/13	23,000	*
	Neuronal Circuits	03/01/12	02/28/13	15,000	*
	Nuclear Receptors and Disease	09/21/12	08/31/13	20,025	*
	Pharmacogenomics and Personalized Therapy	09/30/10	08/31/15	10,000	
	PTEN Pathways and Targets	09/30/11	08/31/12	3,000	
	Retroviruses	03/15/12	02/28/17	36,645	*
	Systems Biology: Global Regulation of Gene Expression	03/01/10	02/28/15	5,000	
	Vertebrate Organogenesis	04/01/11	03/31/15	6,000	
NATIONAL SCIENCE FOUNDATION					
Multiple Project Award Support	Dr. Jackson	10/01/10	09/30/15	1,115,265	
	Drs. Lippman/Schatz	11/01/12	10/31/15	854,233	*
	Dr. Ware	06/01/12	05/31/17	1,762,326	*
Research Support	Dr. Churchland	08/01/11	07/31/14	43,156	
	Dr. Jackson	09/01/10	08/31/13	150,000	
	Dr. Timmermans	08/15/10	07/31/13	150,000	
	Drs. Timmermans/Hammell	05/01/12	04/30/16	181,550	*
Research Subcontracts					
NSF/Cornell University Consortium Agreement	Dr. Timmermans	09/01/08	08/31/12	16,000	
NSF/New York University Consortium Agreement	Drs. McCombie/Martienssen	08/01/10	07/31/14	407,632	
NSF/North Carolina State University Consortium Agreement	Dr. Martienssen	03/01/11	02/29/16	510,779	
NSF/University of Arizona Consortium Agreement	Dr. Ware	08/15/10	07/31/14	583,136	
NSF/University of Arizona Consortium Agreement	Drs. Ware/Micklos/Schatz/Stein	02/01/12	01/31/13	1,863,578	*
NSF/University of California - Berkeley Consortium Agreement	Dr. Jackson	08/01/06	07/31/12	269,815	
NSF/University of Maryland Consortium Agreement	Dr. Schatz	02/01/12	03/31/13	60,000	*

[1]Includes direct and indirect costs

*New or competing renewal grants awarded in 2012

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2012 Funding*[1]	
NSF/Washington University Consortium Agreement	Dr. Ware	03/01/10	02/28/13	$ 92,150	
Fellowship Support	Dr. Liberatore	06/01/10	05/31/13	43,500	
	Dr. Carlston	06/01/10	05/31/13	42,000	*
Undergraduate Training Program Support	Dr. Lippman	04/01/12	03/31/15	115,000	*
Course Support	Advanced Bacterial Genetics	07/01/09	06/30/14	95,634	
	Computational Cell Biology	08/01/12	07/31/15	70,000	*
	Frontiers and Techniques in Plant Science	05/15/12	04/30/15	97,675	*
	Neurobiology of *Drosophila*	08/01/12	07/31/14	20,000	*
UNITED STATES DEPARTMENT OF AGRICULTURE					
Research Support	Dr. Jackson	01/15/11	01/14/15	120,963	
	Dr. McCombie	09/11/12	09/10/13	224,106	*
UNITED STATES DEPARTMENT OF THE ARMY					
Research Support	Dr. Hannon	09/15/12	09/14/17	1,069,481	*
	Dr. Hannon	09/01/08	08/31/13	709,165	
	Drs. Hicks/Trotman	09/30/12	09/29/14	276,672	*
	Dr. Zhong	06/15/10	06/14/13	302,572	
Research Subcontracts					
US Army/University of Pittsburgh Consortium Agreement	Dr. Hicks	09/01/12	08/31/13	152,921	*
Fellowship Support	E. Nakasone	01/01/11	03/13/12	10,800	
	Dr. Park	04/01/12	03/31/15	187,500	*
	Dr. Sheppard	09/01/12	08/31/15	38,927	*
UNITED STATES DEPARTMENT OF ENERGY					
Research Subcontracts					
DOE/Brookhaven National Laboratory Consortium Agreement	Drs. Ware/Schatz	11/29/11	09/30/16	1,076,238	
MISCELLANEOUS SOURCES OF FUNDING					
Equipment Support					
Academy for Medical Development and Collaboration (AMDeC)	Dr. Stillman	01/01/12	12/31/12	60,000	*
Edward P. Evans Foundation	Dr. Vakoc	08/01/12	07/31/13	120,000	*
Memorial Sloan-Kettering Cancer Center/Helena Rubinstein Foundation	Dr. Wigler	03/30/12	03/29/13	300,000	*
New York State Urban Development Corporation	Dr. Stillman	05/01/12	04/30/13	2,000,000	*
Edith and Alan Seligson	Dr. Wigler	06/01/11	05/31/14	250,000	

[1]Includes direct and indirect costs

*New or competing renewal grants awarded in 2012

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2012 Funding*[1]
Program Project Support				
Pfizer Inc.	Dr. Stillman	01/01/12	12/31/13	$ 2,050,000 *
Pioneer Hi-Bred International, Inc.	Drs. Jackson/Lippman/Martienssen/ Timmermans/Ware	07/01/12	06/30/17	1,600,000 *
The Simons Foundation/Autism	Dr. Wigler	01/01/12	12/31/15	6,557,422 *
The Simons Foundation/Center for Quantitative Biology	Dr. Wigler	09/01/08	08/31/15	1,500,000
Theodore R. and Vada S. Stanley	Drs. Watson/McCombie	06/01/11	05/31/14	5,000,000
Research Support				
Aboff's Inc.	Dr. Tonks	12/19/12	12/18/13	10,318 *
Paul G. Allen Family Foundation	Dr. Zador	10/01/10	09/30/13	575,200
Rita Allen Foundation	Dr. Hammell	09/01/12	08/31/17	110,000 *
American Association for Cancer Research	Dr. Egeblad	07/01/12	06/30/14	100,000 *
American Cancer Society	Dr. Wigler	01/01/12	08/31/12	10,000 *
	Dr. Wigler	01/01/11	08/31/13	47,000
American Legion Auxiliary	Dr. Egeblad	11/30/12	11/29/13	250 *
Anonymous	Drs. Mitra/Huang	02/01/12	01/31/14	300,000 *
Banfi Vineyards	Dr. Martienssen	01/01/12	12/31/12	5,679 *
The Breast Cancer Research Foundation	Drs. Wigler/Hicks	10/01/12	09/30/13	240,000 *
Burroughs Wellcome Fund	Dr. Vakoc	09/01/11	08/31/16	151,250
Caring for Carcinoid Foundation	Dr. Tuveson	07/01/12	06/30/14	150,000 *
The H.A. and Mary K. Chapman Foundation	Dr. Churchland	12/27/12	12/26/13	170,000 *
The Mary K. Chapman Foundation	Dr. Osten	12/31/11	12/30/13	150,000
Coferon Inc.	Dr. Vakoc	09/17/12	09/16/13	115,316 *
Dart Neuroscience LLC	Dr. Dubnau	12/01/12	11/30/13	250,000 *
Dysautonomia Foundation Inc.	Dr. Krainer	07/01/12	06/30/14	90,000 *
Lee MacCormick Edwards, Ph.D.	Dr. Stillman	12/01/12	11/30/13	5,000 *
The Ellison Medical Foundation	Dr. Enikolopov	09/09/09	09/08/13	252,000
Entertainment Industry Foundation	Dr. Hannon	11/01/12	10/31/13	100,000 *
Edward P. Evans Foundation	Dr. Vakoc	11/01/12	10/31/13	500,000 *
Charitable Lead Annuity Trust under the Will of Louis Feil	Drs. Albeanu/Kepecs/Li/Osten	12/15/12	12/14/13	1,000,000 *
Find a Cure Today Long Island Foundation	Dr. Egeblad	03/01/12	02/28/13	5,000 *
The Joni Gladowsky Breast Cancer Foundation	Dr. Tonks	05/01/12	04/30/13	55,000 *
Glen Cove C.A.R.E.S., Inc.	Dr. Egeblad	02/01/12	01/31/13	10,000 *
The Irving A. Hansen Memorial Foundation	Dr. Tonks	07/01/12	06/30/13	25,000 *
Jo-Ellen and Ira Hazan	Drs. Osten/Tonks/Krainer	12/01/12	11/30/13	200,000 *
Hearts for Cancer	Dr. Egeblad	04/01/12	03/31/13	28,456 *
The Hope Foundation	Dr. Egeblad	07/01/12	06/30/14	125,000 *
Howard Hughes Medical Institute–Gordon and Betty Moore Foundation	Dr. Martienssen	12/01/11	11/30/16	333,333
Isis Pharmaceuticals, Inc.	Dr. Krainer	11/01/11	10/26/13	150,000
Japan Science and Technology Agency	Dr. Taniguchi	03/01/12	07/31/12	54,799 *
Sidney Kimmel Foundation for Cancer Research	Dr. Zheng	07/01/11	06/30/13	100,000

[1]Includes direct and indirect costs

*New or competing renewal grants awarded in 2012

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2012 Funding*[1]	
F.M. Kirby Foundation, Inc.	Dr. Vakoc	12/15/12	12/14/13	$ 150,000	*
The Susan G. Komen Breast Cancer Foundation, Inc.	Dr. Egeblad	04/13/10	04/12/13	150,000	
Mara and Thomas Lehrman Charitable Fund	Drs. Hammell/Schatz	03/01/12	02/28/13	65,000	*
The Lehrman Institute	Dr. Hammell	03/01/12	02/28/13	200,000	*
Long Island Cruzin' For a Cure Inc.	Drs. Hicks/Trotman	08/01/12	07/31/13	20,000	*
The Long Island 2-Day Walk to Fight Breast Cancer	Dr. Egeblad	08/15/12	08/14/13	33,000	*
The Lustgarten Foundation	Dr. Hannon	01/01/10	12/31/12	835,531	
	Dr. Tuveson	06/15/12	06/14/17	50,000	*
	Drs. Tuveson/Pappin	09/01/12	08/30/17	1,000,000	*
The Manhasset Women's Coalition Against Breast Cancer, Inc.	Dr. Egeblad	01/01/11	12/31/12	50,744	
Carol Marcincuk Fund	Dr. Tonks	01/01/12	12/31/12	6,800	*
In Honor of Carissa Maringo	Dr. Egeblad	10/01/12	09/30/13	5,310	*
The G. Harold and Leila Y. Mathers Charitable Foundation	Dr. Mitra	09/01/10	08/31/13	203,920	
Breast Cancer Awareness Day in Memory of Elizabeth McFarland	Dr. Wigler	01/01/12	12/31/13	64,805	*
The McKnight Endowment Fund for Neuroscience	Dr. Churchland	07/01/12	06/30/15	75,000	*
The Melanoma Research Alliance	Drs. Hannon/Hammell/Vakoc	08/01/11	07/31/14	200,000	
The John Merck Fund	Dr. Churchland	06/01/11	05/31/15	75,000	
	Dr. Kepecs	06/01/10	05/31/14	75,000	
The Don Monti Memorial Research Foundation	Dr. Stillman	03/01/12	02/28/13	400,000	*
Louis Morin Charitable Trust	Dr. Stillman	12/01/12	11/30/13	75,000	*
National Alliance for Research on Schizophrenia and Depression (NARSAD)	Dr. Li	07/15/11	07/14/13	30,000	
Pancreatic Cancer UK	Dr. Tuveson	10/23/12	10/22/15	65,000	*
Panera Bread/Doherty Breads, LLC	Dr. Tonks	12/15/12	12/14/13	8,466	*
The Pew Charitable Trusts	Dr. Albeanu	08/01/12	07/31/17	60,000	*
	Dr. Hannon	01/01/11	12/31/15	349,452	
Pioneer Hi-Bred International, Inc.	Dr. Ware	03/15/12	06/30/15	65,385	*
The Hazen Polsky Foundation	Dr. Vakoc	01/01/12	12/31/14	50,000	*
The Prostate Cancer Foundation	Dr. Vakoc	01/20/12	01/19/14	150,000	*
Christina Renna Foundation Inc.	Dr. Van Aelst	05/01/12	04/30/13	20,300	*
The Research Foundation for the State University of New York	Dr. Furukawa	10/10/12	03/31/14	25,000	*
	Dr. Jackson	10/10/12	03/31/14	22,500	*
Marie Robertson Memorial Fund	CSHL Neuroscience Program Support	01/01/12	12/31/12	137,500	*
Damon Runyon Cancer Research Foundation	Dr. Sordella	01/01/10	12/31/12	150,000	
Diane Emdin Sachs Memorial Fund	Dr. Sordella	09/01/12	08/31/13	8,461	*
Eleanor Schwartz Charitable Foundation	Dr. Churchland	07/15/11	07/14/14	200,000	
The Seraph Foundation	Dr. Vakoc	12/21/12	12/20/13	125,000	*
Ms. Constance Silveri	Dr. Tonks	12/27/12	12/26/13	15,000	*
The Simons Foundation	Dr. Mills	07/01/10	06/30/13	350,000	
	Drs. Mitra/Huang	12/01/11	11/30/14	300,000	

[1]Includes direct and indirect costs

*New or competing renewal grants awarded in 2012

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2012 Funding*[1]	
	Dr. Osten	10/01/11	09/30/13	$ 175,000	
	Dr. Osten	07/01/12	06/30/13	60,000	*
Mary F. Smith Family Foundation	Dr. Tonks	12/01/12	11/30/13	5,000	*
Smithtown Steakhouse LLC D/B/A Insignia Prime Steak: Sushi	Dr. Sordella	01/01/12	12/31/13	75,000	*
Starr Cancer Consortium	Dr. Hannon	08/01/11	07/31/13	216,000	
	Dr. Hannon	08/01/11	07/31/13	270,000	
	Drs. Hicks/Wigler	08/01/11	07/31/13	320,760	
	Dr. Sordella	08/01/11	07/31/13	216,000	
	Drs. Sordella/Pappin	08/01/11	07/31/13	221,988	
	Dr. Vakoc	08/01/11	07/31/13	270,000	
Swim Across America	Dr. Sordella	12/15/11	12/14/14	75,000	
U.S.–Israel Binational Agricultural Research and Development Fund	Dr. Krainer	10/01/10	09/30/14	10,000	
	Dr. Lippman	06/01/10	05/31/13	42,000	
	Dr. Zador	10/01/10	09/30/14	5,750	
University Health Network	Dr. Muthuswamy	01/01/11	12/31/12	28,339	
University of Pennsylvania	Dr. Krainer	08/22/12	08/31/14	150,000	*
The V Foundation	Dr. Vakoc	11/01/12	10/31/14	100,000	*
	Dr. Zheng	11/01/11	10/31/13	100,000	
Whitehall Foundation, Inc.	Dr. Albeanu	01/01/12	12/31/14	75,000	*
	Dr. Shea	09/01/11	08/31/15	75,000	
West Islip Breast Cancer Coalition for Long Island Inc.	Dr. Tonks	11/01/12	10/31/13	10,000	*
Elisabeth R. Woods Foundation Inc.	Dr. Sordella	08/16/12	08/15/13	27,000	*
Yale University/Gilead Sciences Inc.	Dr. Sordella	07/16/12	07/15/14	410,058	*
The Bradley Zankel Foundation, Inc.	Dr. Zheng	02/22/12	02/21/13	15,000	*
Fellowship Support					
American Cancer Society	Dr. Feigin	01/01/11	12/31/12	52,000	
	Dr. Preall	10/01/11	09/30/14	50,000	
Cashin Family Fund	Watson School of Biological Sciences	09/01/10	08/31/12	80,000	
CSHL Association Fellowship	Dr. Tuveson	01/01/12	12/31/12	280,000	*
Genentech Foundation	Watson School of Biological Sciences	10/01/12	09/30/15	58,751	*
German Academic Exchange Service	Dr. Bergmann	09/01/11	08/31/13	38,000	
Lola A. Goldring	Dr. Stillman	09/01/12	08/31/13	80,000	*
Hope Funds for Cancer Research	Dr. Knott	07/01/12	06/30/15	45,500	*
Human Frontier Science Program	Dr. Benkovics	05/01/12	04/30/13	39,400	*
Annette Kade Charitable Trust	Watson School of Biological Sciences	09/01/12	08/31/13	25,000	*
Karp Foundation Inc.	Dr. Penzo	08/01/12	07/31/13	50,000	*
Estate of Gale Kavaliuskas	Watson School of Biological Sciences	09/01/12	08/31/13	293,849	*
The Esther A and Joseph Klingenstein Fund Inc.	Dr. Shea	07/01/10	06/30/13	50,000	
Life Sciences Research Foundation	Dr. MacAlister	08/01/11	07/31/14	56,000	
Muscular Dystrophy Association	Dr. Sahashi	02/01/12	01/31/15	60,000	*
National Alliance for Research on Schizophrenia and Depression (NARSAD)	Dr. Mirrione	07/15/11	07/14/13	30,000	
National Agriculture and Food Research Organization (Japan)	Dr. Hiraga	09/17/12	09/16/13	14,642	*
Mr. and Mrs. John C. Phelan	Watson School of Biological Sciences	09/01/10	08/31/15	100,000	
Damon Runyon Cancer Research Foundation	Dr. Sabin	01/01/12	12/31/14	52,000	*

[1]Includes direct and indirect costs

*New or competing renewal grants awarded in 2012

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2012 Funding*[1]	
Sass Foundation for Medical Research Inc.	Dr. Vakoc	11/01/10	10/31/12	$ 8,752	
Lauri Strauss Leukemia Foundation	Dr. Kawaoka	09/01/12	08/31/13	10,000	*
The Swartz Foundation	Dr. Zador	01/01/12	12/31/12	75,000	*
	Drs. Koulakov/Wei	01/01/12	12/31/12	55,000	*
	Drs. Kepecs/Hangya	01/01/12	12/31/12	55,000	*
	Dr. Zador	01/01/12	12/31/12	15,000	*
The Swedish Society of Medicine	Dr. Ohlund	10/08/12	12/31/12	8,300	*
Training Support					
Clare College	Undergraduate Research Program	06/01/12	05/31/13	5,645	*
The Lita Annenberg Hazen Foundation	Undergraduate Research Program	05/01/08	04/30/18	10,000	
Howard Hughes Medical Institute	Undergraduate Research Program	06/01/12	05/31/13	8,250	*
William Townsend Porter Foundation	Undergraduate Research Program	04/01/12	03/31/13	12,000	*
Steamboat Foundation	Undergraduate Research Program	05/01/12	04/30/13	12,000	*
Trinity College	Undergraduate Research Program	06/01/12	05/31/13	3,970	*
Course Support					
Alzheimer's Drug Discovery Foundation	Workshop on Cognitive Aging	11/01/11	10/31/12	2,500	
American Brain Tumor Association	Brain Tumors	02/01/12	01/31/13	45,000	*
American Express Foundation	Leadership in Bioscience	11/01/11	10/31/14	65,000	
American Federation for Aging Research	Workshop on Cognitive Aging	01/01/12	12/31/12	2,500	*
Carl Zeiss Microscopy, LLC	Imaging Structure and Function in the Nervous System	06/15/12	06/14/13	5,000	*
The Ellison Medical Foundation	Workshop on Cognitive Aging	05/01/12	04/30/13	15,000	*
Howard Hughes Medical Institute	Course Support	01/01/07	12/31/12	600,000	
Nancy Lurie Marks Family Foundation	Workshop on Biology of Social Cognition	07/01/12	06/30/16	25,000	*
Roche Diagnostics Corporation	Advanced Sequencing Technologies and Applications	02/01/12	01/31/13	30,000	*
Society for Neuroscience/International Brain Research Organization	Summer Neuroscience Course	07/01/12	06/30/13	17,210	*
Meeting Support					
Abbott Laboratories	Gene Expression and Signaling in the Immune System	04/01/12	03/31/13	3,000	*
Amgen, USA	Molecular Pathways in Organ Development and Disease	09/01/11	08/31/12	5,000	
	Gene Expression and Signaling in the Immune System	04/01/12	03/31/13	5,000	*
Art Guild, Inc.	Corporate Exhibitors	03/01/12	02/28/13	5,000	*
Clontech Laboratories, Inc.	Single-Cell Analysis	11/01/12	10/31/13	5,000	*
The Company of Biologists LTD	Molecular Pathways in Organ Development and Disease	09/01/11	08/31/12	7,898	
Complete Genomics, Inc.	Corporate Exhibitors	03/01/12	02/28/13	14,000	*
Constellation Pharmaceuticals	Gene Expression and Signaling in the Immune System	04/01/12	03/31/13	2,000	*
Enzo Life Sciences	Molecular Chaperones and Stress Responses	04/01/12	03/31/13	2,500	*
Exiqon, Inc.	Corporate Exhibitors	03/01/12	02/28/13	500	*
Forest Research Institute Inc.	Blood Brain Barrier	12/01/12	11/30/13	25,000	*

[1]Includes direct and indirect costs

*New or competing renewal grants awarded in 2012

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2012 Funding*[1]	
The Gatsby Charitable Foundation	77th CSHL Symposium: The Biology of Plants	02/01/12	01/31/13	$ 3,150	*
Gilead Sciences Inc.	Retroviruses	05/01/12	04/30/13	12,500	*
Illumina, Inc.	Corporate Exhibitors	03/01/12	02/28/13	19,000	*
Life Technologies Corporation	Personal Genomes and Medical Genomics	12/15/12	12/14/13	10,000	*
March of Dimes Foundation	Molecular Pathways in Organ Development and Disease	04/01/12	09/30/12	5,000	*
MedImmune, LLC	Molecular Genetics of Bacteria and Phage	11/07/11	11/06/12	5,000	
Merck Sharp and Dohme Corporation	Molecular Pathways in Organ Development and Disease	03/01/12	02/28/13	15,000	*
Molecular Devices Corporation	Corporate Exhibitors	03/01/12	02/28/13	5,000	*
Gordon and Betty Moore Foundation	77th CSHL Symposium: The Biology of Plants	02/01/12	01/31/13	10,000	*
Nature America Inc.	Molecular Genetics of Bacteria and Phage	11/07/11	11/06/12	750	
Novartis Pharmaceuticals Corp.	PTEN Pathways and Targets	11/18/11	03/16/13	25,000	
Christine O'Neil	Corporate Exhibitors	03/01/12	02/28/13	6,000	*
Opgen, Inc.	Corporate Exhibitors	03/01/12	02/28/13	7,000	*
Phytotechnology Laboratories	Corporate Exhibitors	03/01/12	02/28/13	500	*
Promega Corporation	Molecular Genetics of Bacteria and Phage	11/07/11	11/06/12	750	
Martin Reese	Corporate Exhibitors	03/01/12	02/28/13	4,000	*
Rutgers, the State University of New Jersey	Protein Data Bank 40th Anniversary	12/01/10	11/30/12	2,300	
Society for Developmental Biology	Molecular Pathways in Organ Development and Disease	09/01/11	08/31/12	4,000	
Lemna Tec	Corporate Exhibitors	03/01/12	02/28/13	2,500	*
Library Support					
The Ellen Brenner Memorial Fund		12/15/12	12/14/13	2,000	*
The New York State Education Department		07/01/10	06/30/12	3,497	
The New York State Education Department		07/01/11	06/30/12	16,584	
Wellcome Trust		08/01/11	07/31/13	302,364	
Library Meeting Support					
Alfred P. Sloan Foundation		04/01/12	05/01/12	20,000	*
The Rockefeller University		04/01/12	03/31/13	15,000	*

[1]Includes direct and indirect costs

*New or competing renewal grants awarded in 2012

DNA LEARNING CENTER GRANTS

Grantor	*Program*	*Duration of Grant*	*2012 Funding*[†]
FEDERAL GRANTS			
National Science Foundation, University of Arizona	Educational Outreach for *iPlant Collaborative*: A Cyberinfrastructure for Plant Sciences	2/08–1/13	860,267
National Science Foundation, North Carolina State University	GEPR: Epigenome Dynamics during DNA Replication	3/11–2/13	73,108
National Science Foundation	Advanced Technology Education (ATE) Program: *Genomic Approaches in BioSciences*	4/11–3/14	157,894
NONFEDERAL GRANTS			
Victor Centers for Prevention of Jewish Genetic Diseases, Albert Einstein Healthcare Center/Marcus Jewish Genetic Disease Consortium	*Gene Screen* iPhone/iPad Application	12/10–12/12	31,900
Alfred P. Sloan Foundation	DNA Barcoding Experiments by New York City High School Students (*Urban Barcode Project*)	1/11–7/12	74,533
Howard Hughes Medical Institute	Pre-College Science Education Initiative: NYC Teacher Professional Development	9/07–8/12	145,319
Dana Foundation	*Harlem DNA Lab* Operating Support	3/09–2/12	27,700
William Townsend Porter Foundation	Scholarships for Minority and Underserved Students at *Harlem DNA Lab*	3/11–3/12	17,000
National Grid Foundation	Scholarships for Minority and Underserved Students in the Central Islip Union Free School District	1/12 –12/12	15,000
Spinal Muscular Atrophy Foundation	*Learn About SMA* Internet site	4/11–4/13	78,965

The following schools and school districts each contributed $1,000 or more for participation in the *Curriculum Study* Program:

Elwood Union Free School District	$ 1,500	Locust Valley Central School District	1,500
Fordham Preparatory School	3,000	Northport-East Northport Union Free School District	3,000
Great Neck Union Free School District	1,500	Oceanside Union Free School District	3,000
Half Hollow Schools Central School District	3,000	Port Washington Union Free School District	3,000
Herricks Union Free School District	1,500	Ramaz Upper School	1,500
Huntington Union Free School District	1,500	Sachem Central School District	3,000
Levittown Union Free School District	3,000	Yeshiva University High School for Girls	3,000

The following schools and school districts each contributed $1,000 or more for participation in the *Genetics as a Model for Whole Learning* Program:

Adelphi STEP	$ 1,050	Lynbrook Union Free School District	1,000
Bay Shore Union Free School District	2,800	Merrick Union Free School District	3,400
Bellmore Union Free School District	2,800	M.S. 447, Brooklyn	1,800
Bellmore–Merrick Union Free School District	10,000	North Bellmore Union Free School District	2,600
Cold Spring Harbor Central School District	14,400	North Shore Central School District	1,750
Commack Union Free School District	6,900	North Shore Hebrew Academy	1,050
East Meadow Union Free School District	3,730	Oceanside Union Free School District	1,625
East Williston Union Free School District	2,900	Oyster Bay–East Norwich Central School District	5,625
Elwood Union Free School District	3,425	Plainedge Union Free School District	2,100
Floral Park–Bellerose Union Free School District	7,800	Port Washington Union Free School District	9,600
Friends Academy	2,100	Rockville Centre Union Free School District	6,240
Garden City Union Free School District	4,000	Roslyn Union Free School District	4,200
Great Neck Union Free School District	6,000	Sachem Union Free School District	2,925
Half Hollow Hills Union Free School District	9,150	Saint Dominic Elementary School	4,550
Herricks Union Free School District	2,275	Scarsdale Union Free School District	12,600
Hofstra STEP	1,050	Smithtown Union Free School District	1,680
Holy Child Academy	3,100	Syosset Union Free School District	36,600
IHM Home School Program	1,050	Three Village Central School District	4,200
Huntington Union Free School District	4,600	Trinity Regional School	1,300
Jericho Union Free School District	8,250	Valley Stream 13 Union Free School District	1,050
Locust Valley Central School District	8,859	Yeshiva of Flatbush	2,000

[†]Includes direct and indirect costs.

BANBURY CENTER GRANTS

Grantor	*Program*	*Duration of Grant*	*2012 Funding*
FEDERAL SUPPORT			
Centers for Disease Control and Prevention	Decoding Clinical Trials to Improve Treatment of ME/CFS	2012	$18,440
NIH–National Institute of Mental Health	The Fourth Annual NIMH Brain Camp	2012	35,662
National Science Foundation through a grant to Stony Brook University	Grand Challenges in Organismal Biology	2012	9,045
NONFEDERAL SUPPORT			
Astellas-OSI Oncology	Cell Plasticity in Cancer Evolution	2012	49,602
Baxter Healthcare Corporation	Patenting Genes: New Developments, New Questions	2012	5,000
Boehringer Ingelheim Fonds	Communicating Science	2012	50,549
Certerra, Inc.	Systems Biology of Autism: From Basic Science to Therapeutic Strategies	2012	4,266
CFIDS Association of America	Decoding Clinical Trials to Improve Treatment of ME/CFS	2012	39,107
Cold Spring Harbor Laboratory Corporate Sponsor Program	Transcription and Cancer	2012	43,261
Cold Spring Harbor Laboratory Corporate Sponsor Program	Inflammation, Cancer, and Metabolism	2012	46,068
Cold Spring Harbor Laboratory Library and Archives	Envisioning the Future of Science Libraries at Academic Research Institutions	2012	4,512
Cold Spring Harbor Laboratory/DuPont Pioneer Joint Collaborative	Plant–Environment Interactions	2012	50,000
Discovery Network	Inspire2Live	2012	21,203
DRI Capital, Inc.	Patenting Genes: New Developments, New Questions	2012	7,500
GangaGen, Inc.	Phage and Phage-Based Therapies	2012	39,857
Genentech, Inc.	Patenting Genes: New Developments, New Questions	2012	10,000
Individual participants	The Fourth Annual NIMH Brain Camp	2012	8,070
Individual participants	Patenting Genes: New Developments, New Questions	2012	1,335
Individual participants	Emerging Approaches in Oncology: A Brainstorming Think Tank	2012	2,000
Individual participants	Grand Challenges in Organismal Biology	2012	1,005
Individual participants	Decoding Clinical Trials to Improve Treatment of ME/CFS	2012	2,982
Jones Day LLP	Patenting Genes: New Developments, New Questions	2012	2,000
Kaye Scholer LLP	Patenting Genes: New Developments, New Questions	2012	5,000
King & Spalding, LLP	Patenting Genes: New Developments, New Questions	2012	5,000
Eli Lilly & Company	Patenting Genes: New Developments, New Questions	2012	5,000
Haig R. Nalbantian	Interdisciplinary Symposium on Literature, Memory, and Neuroscience	2012	1,000
Novartis Pharma AG	Patenting Genes: New Developments, New Questions	2012	5,000
Novo Nordisk Inc.	Patenting Genes: New Developments, New Questions	2012	5,000
Pfizer, Inc.	Systems Biology of Autism: From Basic Science to Therapeutic Strategies	2012	5,000
Mr. and Mrs. Howard Phipps, Jr.	Interdisciplinary Symposium on Literature, Memory, and Neuroscience	2012	10,000
Marie Robertson Research Fund	Systems Biology of Autism: From Basic Science to Therapeutic Strategies	2012	20,000
Robertson Fund	Systems Biology of Autism: From Basic Science to Therapeutic Strategies	2012	10,000
The Rockefeller University	Envisioning the Future of Science Libraries at Academic Research Institutions	2012	15,000

BANBURY CENTER GRANTS (*Continued*)

Grantor	*Program*	*Duration of Grant*	*2012 Funding*
Ropes & Gray	Patenting Genes: New Developments, New Questions	2012	5,000
The Daniel and Joanna S. Rose Fund, Inc.	Interdisciplinary Symposium on Literature, Memory, and Neuroscience	2012	10,000
The Satenik and Adom Ourian Educational Foundation	Interdisciplinary Symposium on Literature, Memory, and Neuroscience	2012	1,000
Simons Foundation	Systems Biology of Autism: From Basic Science to Therapeutic Strategies	2012	10,000
The Alfred P. Sloan Foundation	A History of the Human Genome Project	2012	50,000
The Alfred P. Sloan Foundation	Envisioning the Future of Science Libraries at Academic Research Institutions	2012	20,000
University of Louisville, Brown Cancer Center	Regulation of Metabolism in Cancer	2012	45,625
University of Southern California, NCI Physical Sciences in Oncology Center	Emerging Approaches in Oncology: A Brainstorming Think Tank	2012	32,663

CORPORATE SPONSOR PROGRAM FOR MEETINGS SUPPORT

The Corporate Sponsor Program continues to provide critical funding for the vigorous meetings program held at Cold Spring Harbor Laboratory, whether at Grace Auditorium on the main Laboratory campus or at the Banbury Center. Without the strong foundation provided by the Program, we could neither plan with confidence for the year's meetings nor introduce new and unusual topics.

We are especially grateful to the companies that joined us in 2012 as the economic difficulties have persisted. The year 2013 is going to be especially challenging as the number of companies shrinks through takeovers, and companies and foundations adopt austerity measures. The members of the Program receive special privileges in acknowledgment of their contributions. We waive all on-site fees for eight representatives of the Corporate Sponsors at our meetings. Three scientists from Sponsors may attend meetings at the Banbury Center, where attendance is otherwise only by invitation of the organizers. Member companies also receive gratis copies of Cold Spring Harbor Laboratory Press publications, including the journals *Genes & Development*, *Learning & Memory*, *CSH Protocols,* and *Genome Research*.

We acknowledge our Sponsors in all relevant publications, including the books of abstracts given to each of the 7000 participants who come to the meetings each year. The names of the sponsoring companies are listed on the poster describing the meetings, and this is mailed to approximately 17,000 scientists throughout the world. In addition, the companies are listed on the Cold Spring Harbor Laboratory website on the Meetings Office and Banbury Center pages. Members in 2012 were the following:

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DEVELOPMENT

With the support of our many generous donors, Cold Spring Harbor Laboratory had one of the most successful years in our history. For the first time, more than $6 million was raised for unrestricted support. The 2012 Double Helix Medals dinner was also hugely successful as we honored Michael J. Fox, Art Levinson, and Mary Lindsay. In their unique ways, each of these individuals has made the world a better place, and we were very proud to recognize their achievements.

In July, John Cleary's family and friends gathered to celebrate the dedication of a laboratory in his memory. The *John P. Cleary Laboratory* is located in the DeMatteis building and is home to Dr. Michael Wigler's lab. John was involved with the Laboratory for many years as CSHL Association's President, Trustee, and then as an Honorary Trustee. His friendship to the Laboratory, the scientists, the employees, and all who walked on our grounds will never be forgotten.

With support from the Lustgarten Foundation, Cold Spring Harbor Laboratory welcomed Dr. Dave Tuveson, one of the world's most preeminent researchers and clinicians in the field of pancreatic cancer research. Dave was formerly at the Cancer Research UK Cambridge Research Institute, and he came to the Laboratory to direct our Cancer Therapeutics Initiative. This ambitious initiative will focus on moving advanced-stage drug candidates from the laboratory to the clinic. With more space needed to accommodate sophisticated technology and additional people, plans are set to expand our Woodbury facility. We will be breaking ground on the project in the second quarter of 2013.

All our successes during this past year could not have happened without the support of our donors and we are extremely thankful for all of their contributions.

Charles V. Prizzi, *Vice President for Development*

Cold Spring Harbor Laboratory is a nonprofit research and educational institution chartered by the State of New York. Less than half of the Laboratory's annual revenues are derived from federal grants and contracts, and thus, it relies heavily upon support from the private sector: foundations, corporations, and individuals. CSHL takes this occasion to sincerely thank each and every donor whose generous contributions have made possible the discoveries we report in this publication. There are a variety of ways to give to the Laboratory:

Capital and Endowment Campaign Support: Donations help to secure the financial stability of CSHL and provide resources to expand the facilities and staff.

Research Support: Donations in specific areas of research help to increase the speed at which genetic discoveries are translated into diagnostic tests and therapeutics.

Annual Fund: Donations provide funding for some of the most innovative young researchers in science today and constitute an important investment in groundbreaking research in cancer, neuroscience, plant biology, and bioinformatics.

Science Education: Donations support programs at the DNA Learning Center and the Watson School of Biological Sciences, where the next generation learns about genetics in an exciting and interactive environment.

Planned and Estate Gifts: Individuals who inform us of their intention to make a gift to CSHL from their estate are invited to become members of the Harbor Society. Estate gifts help to ensure that CSHL will continue to pursue its mission for many years to come.

For additional information, please contact Charlie Prizzi, Vice President for Development, Cold Spring Harbor Laboratory, One Bungtown Road, Cold Spring Harbor, New York 11724. Phone number: 516-367-6961. E-mail: prizzi@cshl.edu

President's Council

This year's topic—Ethical Dilemmas in Medicine—enabled us to shine light on CSHL's human genetics research, strides that we are making in cancer profiling, and the Laboratory's early role in the 20th-century eugenics movement. Three of our October talks were presented by key CSHL individuals: Dave Micklos, founder and head of CSHL DNA Learning Center; David Spector, CSHL Director of Research; and Lloyd Trotman, Assistant Professor of cancer research. Gholson Lyon, M.D., PhD., a new CSHL Assistant Professor of neuroscience, spoke at the Council reception in May hosted by Claudia and Gunnar Overstrom in their magnificent New York City apartment.

Dartmouth Professor H. Gilbert Welch, M.D., opened the October meeting with a captivating presentation on the downside of routine preclinical medical screening. Saturday talks featured a thoughtful lecture on informed consent of incapacitated human subjects and the ethics of gene patenting. In addition, an extraordinary keynote lecture by Andrew Solomon moved most of our guests to tears with his moving account of the love and special meaning that parents find in raising children who are different, the topic of his newest book *Far from the Tree: Parents, Children and the Search for Identity.*

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President's Council speaker, Andrew Solomon signing copies of his books for attendees

Freddie Staller, Gilbert Welch, and Erwin Staller

Cold Spring Harbor Laboratory Association

With 1200 members, the Cold Spring Harbor Laboratory Association (CSHLA) continues to increase community awareness of CSHL, and this year they helped raise more than $6 million in unrestricted support for the Laboratory. Events spearheaded by the Directors of the Association included Labapalooza, the 77th Symposium dinner parties, the Women's Partnership for Science luncheon, and a variety of receptions and dinners that introduced new friends to the exceptionally talented young scientists at CSHL. Members also had a significant role in the success of the Double Helix Medals Dinner in New York City and the 19th annual golf tournament at the Piping Rock Club. Special thanks go to Sandy Tytel, president, as she continues to enthusiastically lead the CSHLA directors to new levels of participation.

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The Corporate Advisory Board (CAB) is a vital liaison between Cold Spring Harbor Laboratory and the tri-state business community. Established more than 20 years ago, the CAB is the driving force behind the Laboratory's annual golf outing at Piping Rock Club; this outing raises critical unrestricted funding for research and educational programs.

Serving as CAB Chair for more than 10 years and Golf Chair for 6 years, Eddie Chernoff continues to provide leadership and support and we truly appreciate all of his efforts.

CAB Chairman Eddie Chernoff with his wife Donna and Drs. Mona and David Spector

CAB member Michael Aboff and his son Matthew present a check to Dr. Bruce Stillman for breast cancer research

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