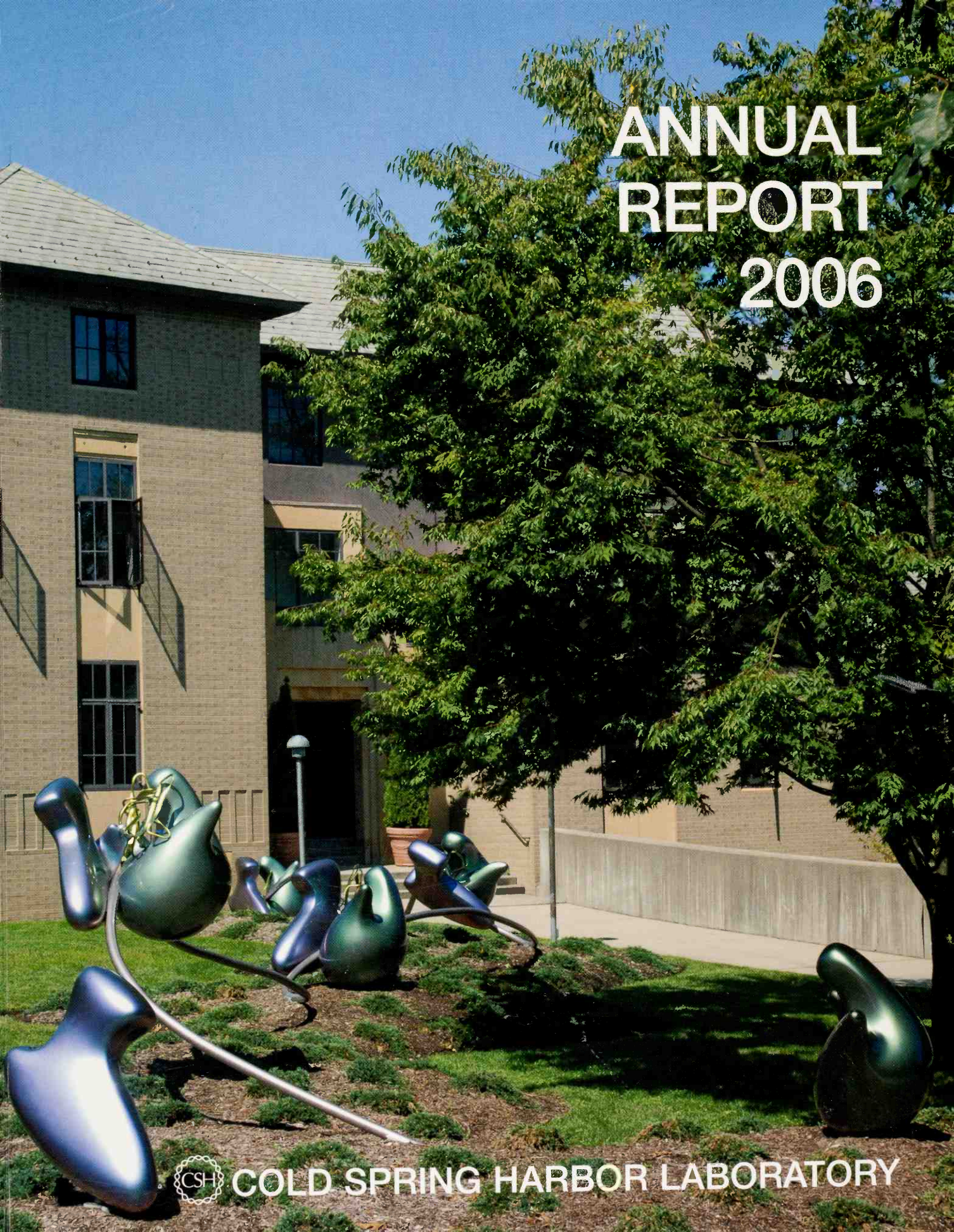
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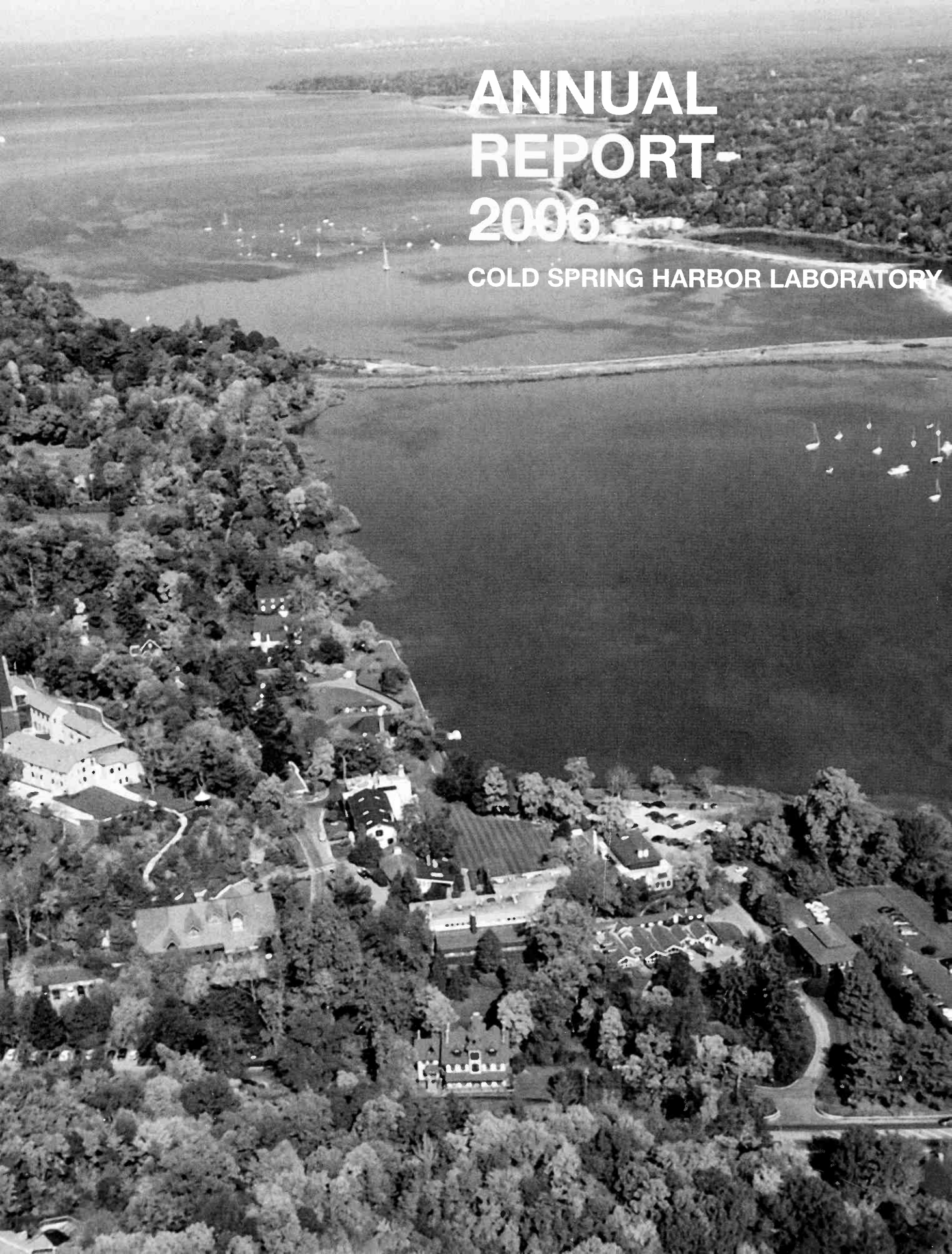
COLD SPRING HARBOR LABORATORY



ANNUAL REPORT- 2006

COLD SPRING HARBOR LABORATORY

ANNUAL REPORT 2006

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Front cover: "The Waltz of the Polypeptides," a sculpture by Mara G. Haseltine, was installed on the CSHL campus on the south side of Dolan Hall and dedicated on October 7, 2006. (Photo by Miriam Chua.)

Back cover: The cornerstone to commemorate the Hillside Campus of CSHL was dedicated on October 15, 2006. (Photo by Miriam Chua.)

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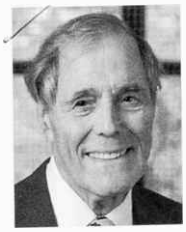

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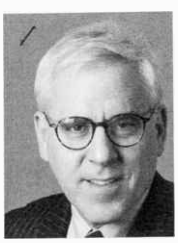

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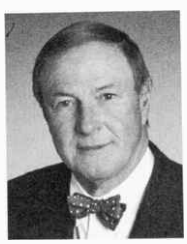

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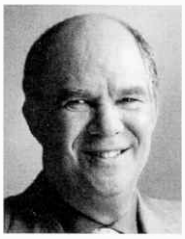

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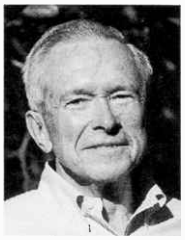

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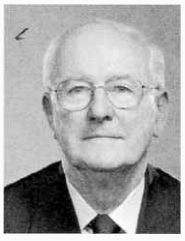

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The Laboratory is chartered as an educational and research institution by the Board of Regents of the Education Department of the State of New York. It is authorized to operate a graduate program under the name "Cold Spring Harbor Laboratory, Watson 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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Wendy Vander Poel Russell (1919–2006)

Wendy Vander Poel Russell, whose inherent cheerfulness, good taste, and devotion to the public good long graced the North Shore of Long Island, died at age 86 on March 9, 2006. As a participant in one of our early summer Nature Study courses, she waded in the waters, coming out of St. John's pond never having reason to think that she would later assume a major role in helping chart the Lab's development into a major worldwide center for biological research.

Born February 6, 1919, she was the daughter of Gretchen Billings and Halsted Vander Poel, a descendent of one of New York's original Dutch settlers. After spending her grammar school years at the Nightingale Bamford School in New York City, she prepared for her future as a thoughtful member of New York society at The Ethel Walker School in Simsbury, Connecticut. In 1940, just before the United States entered World War II, she married George Montagu Miller (Monty), and soon served as a Red Cross nurse's aide, first at Presbyterian Hospital and then at Halloran General Hospital, Staten Island.

From their marriage, two daughters—Wendell Miller Steavenson of London, England, and Gretchen Miller Elkus, of Ligonier, Pennsylvania, were born. Although her parents' main home was in New York City, it was her family's Long Island estate on Mill River Road and Route 25A and membership in the Piping Rock Club that provided Wendy her assemblage of lifelong friends, golf, and bridge partners who helped her pass through years of domestic life that she wished might have gone differently. Equally important and stabilizing to her long, productive life was her long role as President of Miller Richard, Inc. decorators. In 1977, after a divorce from her first husband, she married Sinclair Hatch, a partner in the New York law firm Milbank, Tweed, Hadley, and McCloy. Upon his death in 1989, she married William Francis Russell, who also predeceased her on April 9, 1996.

In New York City, her main philanthropy was directed toward the Manhattan Eye and Ear Hospital of which she was a vice president, serving for many years as President of the Eye Bank for Sight Restoration (a parent foundation for 95 eye banks). On Long Island, she was already long associated with Hutton House, the adult education outreach program of C.W. Post College, when she joined our Board of Trustees in November 1984. At that time, the Laboratory had just purchased land at Uplands Farm from the Nature Conservancy to create an agricultural research station for maize research. To raise funds for the station, Wendy organized a visit from executives of the Ira W. DeCamp Foundation, which resulted in a $100,000 grant for plant genetics research. Soon after, she greatly assisted Ed Pulling with his task to raise monies for the Lab's new Grace Auditorium.

During her three successive four-year terms on the Board, including two periods as secretary (1985–1987 and 1992–1997), she became a legendary fund-raiser for the Lab, describing herself as the best pickpocket

the Lab ever had. Wendy's dinner parties, which introduced potential donors to visiting and resident Cold Spring Harbor scientists, regularly attracted new Lab supporters. Already seasoned in the Lab's goals of converting basic research into medical breakthroughs, she was key to the success of the Second Century Campaign, serving as head of its "major gift" committee that raised from her vast circle of friends more than $2 million for the construction of the Neuroscience Center. Upon its dedication in 1991, Wendy's main focus turned the renovation of Cold Spring Harbor's 1926 brick school building into the highly functional DNA Learning Center.

Toward its objective, she brought into existence a Corporate Advisory Committee, whose members came from prominent Long Island industrial and commercial groups. By 1997, its ever-increasingly successful June golf tournament at the Piping Rock Club raised $98,000 for the DNA Learning Center programs. Equally important were her fund-raising efforts to bring into existence, in 1997, the Mary D. Lindsay Child Care Center, which includes a playground that bears Wendy's name.

Upon retiring from our Board of Trustees in 1997, Wendy was elected an Honorary Trustee, taking much pleasure in continuing to participate in virtually all of the Lab's public activities. The coming into existence of the Lab's graduate program much excited her, with her happily participating in its first commencement exercises that awarded an Honorary Degree to her close friend David Luke for his key role in bringing the Watson School into existence.

Her inherent upbeat tone remained visible even after she was stricken with ovarian cancer that all too soon was seen as incurable. As our final tribute to her, the lovely colonial-style superintendent's house on Uplands Farm was completely remodeled into a residence for young scientists—the Wendy Vander Poel House. At its dedication ceremony, on a warm September evening, Wendy was totally her long vibrant self, much enjoying her daughter's retelling of 80 great things about Mummy on her 80th birthday.

In Wendy's passing, we take comfort in how long she was on our side.

James D. Watson

Regina Clarkson Quick (1927–2006)

When Laboratory Trustee Tom Quick lovingly eulogized his mother at a memorial service in St. Patrick's Cathedral last April, he said, "Indeed, there was an elegant simplicity to her." How true. And how fortunate her Laurel Hollow neighbors and the Laboratory community were to have known her for 25 years. Jean was the rarest of people in that she seemed to embody all of the most admirable human qualities—kindness, humility, selflessness, sense of humor, and generosity of spirit.

In many respects, her life story reflected the American ideal of her generation. Jean's grandparents sailed, penniless, from Cork, Ireland, to Ellis Island in search of a better life. She grew up in Queens, New York, one of five children of Thomas and Mary Clarkson. Thomas supported the family by laboring as an iron worker, contributing to the construction of New York City landmarks such as the Singer Building and the Queensboro Bridge. Jean was an excellent student who, apparently, only strayed from the straight and narrow when presented with the opportunity to skip school and see the young Frank Sinatra perform at the Paramount Theatre. She went on to work as a secretary at a local tool and die company.

It was during her childhood in Queens that Jean first met her eventual husband of 51 years—the late Leslie C. Quick, Jr. The story goes that young Les, a paperboy for the Long Island Press, convinced a colleague to switch delivery routes with him so that he could pass the Clarkson home each day in hope of catching a glimpse of his childhood sweetheart. The rest is history. After wartime service in the U.S. Army Air Corps, Les attended the Pennsylvania Military College, from which he graduated in 1950. Ten days later, he and Jean were married.

Their life together was an adventure. They outgrew their first Long Island home in Levittown as the family eventually expanded to seven children. As Jean dedicated herself tirelessly to child-rearing and community activities, Les was pursuing his legendary business career culminating with the founding of the discount brokerage Quick & Reilly, the firm for which all four of the Quick sons eventually worked. The business grew and prospered over three decades and the Quick family along with it. Tom fondly remembers the days when, despite the financial good fortune that the business brought to the family, Jean continued to clip grocery store coupons out of the daily newspaper. "You can take the girl out of Queens, but you can't take Queens out of the girl," says Tom. Indeed, Jean's charm stemmed from the fact that she forever maintained her humility. There was no pretense as she always seemed comfortable with herself and with others, whether in Queens or Palm Beach.

Fortunately for us, in 1978, Jean and Les moved from Woodbury to a beautiful house in Laurel Hollow directly up the hill from the Laboratory campus. Equally fortuitous and totally by coincidence, my wife Sophie and I bought the house directly next door to the Quicks in 1985. Jean and Les welcomed and befriended us with an unexpected graciousness. In 1997, Laboratory Chairman David Luke and I approached Les about serving on the Board and that began a wonderful relationship between the Laboratory and the entire Quick

family. Les developed a love for the Laboratory and worked on its behalf with great energy and distinction until his untimely death from cancer in 2001. After his death, I asked Jean whether she thought any of the children would like to take Les' place on the board. Without hesitation, she replied "Les loved the Lab—it's what he would have wanted." She suggested son Tom who immediately agreed and has served on the board ever since. More recently, son Peter, a resident of Mill Neck, has joined the Lab's Capital Campaign Committee.

The death of Les was a devastating blow to Jean. She had become physically frail as a result of her long battle with progressive arthritis. Yet she remained tenaciously strong in mind and spirit, most likely due to her strong faith in God and her love of family. She never complained. In 2004, Jim Watson and I visited Jean and asked her to consider memorializing the Quick family legacy at Cold Spring Harbor Laboratory by naming one of the two new cancer buildings to be built as part of the main campus expansion. When Jean invited us back to the house to inform us of her decision to grant the request, I don't know who wore the broadest smile—Jim or Jean. This spring, it will be wonderful for all of us to see the Leslie and Jean Quick Building for Cancer Research begin to rise on the hillside overlooking the harbor that they both loved.

As Tom said at St. Patrick's, "she asked little, but gave so much." Certainly, in addition to Cold Spring Harbor Laboratory, Jean gave a great deal of herself and her resources to many groups and institutions including the Dana Farber Cancer Center, The Breast Cancer Research Foundation, The Arthritis Association, St. Bonaventure University, Fairfield University, and St. Francis Hospital. Most of all though, I believe Jean would have liked to be remembered for her love of family. She was unfailingly proud of her husband, her seven children, and her twenty-six grandchildren. Whenever any of them were in the room, her eyes twinkled and her laugh was ready.

Dill Ayres

PRESIDENT'S REPORT

For much of its 116-year history, and particularly during the last thirty-five years, Cold Spring Harbor Laboratory has provided an environment for science of the highest quality. As we stand on the verge of the largest single expansion in the Laboratory's history, it is worth reflecting on how this culture of achievement is fostered.

The value of science can be assessed subjectively by the benefits it brings to society, such as advances in human health. There are more objective measures, such as the rate at which a research group's publications are cited by other scientists. By this measure, the Laboratory stands at or near the top of an elite group of international institutes and universities. But important science can also be so visionary that it is overlooked or misunderstood. The pioneering genetics research done at Cold Spring Harbor in the 1940s and 1950s by Barbara McClintock was not highly cited because it was so forward-looking, and in fact, two decades had passed before the scientific community as a whole came to appreciate the work for which Barbara eventually won a Nobel Prize in 1983. Even today, our plant biologists are still discovering molecular intricacies of the phenomena Barbara described.

Outstanding science is produced when the most able investigators work in an intellectual culture of the right kind. A successful research scientist needs talent but must also have a passion for exploring by asking questions and doing experiments. And passion is truly required, for the life of a scientist is demanding on time, intellectual effort, and family life. Thus equipped, a successful scientist must work in an environment that promotes freedom of exploration, because it is the imagination of an individual that generates new ideas and testable hypotheses. The Laboratory's environment, aside from being one of the most scenically beautiful in science, contains many elements that promote excellence.

One is our very capable administration that nurtures science while minimizing obstacles. Another is our internationally renowned meetings and courses program and the Banbury Conference Center, which bring to our campus many of the world's leading investigators to share new data and technologies. Many of our staff members take full advantage of the opportunities to learn from our visitors, recruit new members of research teams, and initiate collaborations. As a new Postdoctoral Fellow in 1979, I benefited enormously from the Cold Spring Harbor meetings and gained knowledge that has helped my own research and my ability to oversee a broad research program.

Another contribution to the Laboratory's research success has been the balance between creating diversity in lines of pursued research and building a critical mass of investigators with shared intellectual and technical interests. In maintaining such balance, it is important that the appointment of new faculty complements the skills of existing faculty, adding strength and depth to the research program as a whole. The combination of faculty turnover and arrival of new staff keeps Cold Spring Harbor at the cutting edge of research.

The collaboration and interaction among our scientists is one of our strongest assets. We actively foster such interactions through formal and informal discussion groups, joint laboratory meetings, and "in-house" seminars that encourage the exchange of ideas. In addition, some of the best ideas emerge in informal locations, such as the cafeteria, coffee shop, or bar. Postdoctoral Fellows and students gain valuable practical help and advance their careers by getting to know our experienced and successful senior scientists.

But even an institution such as ours, with so many elements conducive to creative science, needs the crucial addition of sufficient financial resources to enable researchers to pursue their ideas without constant worry about future funding. Modern science is expensive, time-consuming, and labor-intensive. Our scientists need the money to take on bold challenges—the kind that get federal funding only after an initial breakthrough. For this reason, a large percentage of the highly innovative research at Cold Spring Harbor is now funded by philanthropy. Such funds are much appreciated, but unrestricted endowment would provide essential funds for research infrastructure, start-up costs for new investigators, and support for new projects.

Maintaining the financial resources necessary to sustain outstanding science is our greatest challenge. Because federal support for science has fallen dramatically and competition for grants has intensified, unrestricted funds are required that can be directed to areas of need. Being financially lean can force scientists to think seriously about the most important questions to be addressed, but one cannot be too lean for too long. To maintain Cold Spring Harbor Laboratory as a place of outstanding science, we must be able to recruit the very best young investigators and provide them with start-up funds, and we must be able to support our successful senior scientists when they need it.

Perpetuation of our culture also requires contributions from our senior staff, especially as I devote more time in the future to raising funds to support our science and education programs. Advice from a Board of Trustees subcommittee (Robert Lindsay, Eduardo Mestre, Titia de Lange, and Robert Tjian) has helped us to put in place a management structure that will ensure that our culture of excellence will continue. A key appointment has been the promotion of Dr. David Spector to Director for Research. David is an internationally respected leader in cell biology and continues to make notable contributions to the understanding of the structure of the mammalian nucleus and its influence on gene expression. He has a deep understanding of our culture and will be vital in maintaining it.

Challenging times are ahead for the sciences in the United States due to reductions in research federal funding. With constant pressure from a public that wants its major diseases cured, Congress should now act to reverse this trend. But at Cold Spring Harbor, we must find a path that ensures that our way of doing science can be preserved independently of national trends. As this Annual Report demonstrates, our science continues to produce surprising and unexpected insights into the fundamentals of cancer, neurobiology, and the ways in which genes work. Sustaining this productivity is our most important goal.

HIGHLIGHTS OF THE YEAR

Research

Cancer

Liver cancer is the fifth most frequent neoplasm worldwide. However, owing to the lack of effective treatment options, it is the third leading cause of cancer deaths. By generating tumors in laboratory mice that mimic human liver cancer and by comparing the DNA of mouse and human tumors, researchers at CSHL have identified two genes that are likely to have a role in this form of cancer. The study also establishes an efficient and adaptable method for exploring the biology of liver cancer, for validating potential therapeutic targets, and for testing new treatments.

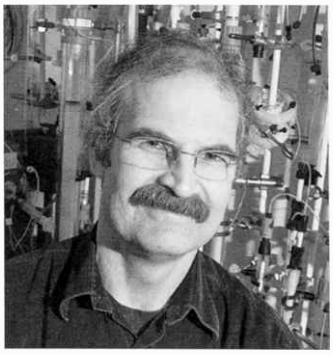
To gain a better understanding of the molecular causes of liver cancer, the researchers (see below) devised a strategy for genetically engineering liver stem cells, harvested from mouse embryos, and subsequently transplanting the cells into adult mice. Following transplantation, the cells can become part of the recipient mouse's liver.

The scientists engineered the liver stem cells to mimic the genetic lesions that are known to occur in human liver and other cancers (namely, deletion of the *p53* gene and activation of the *myc* gene). Transplanted cells lacking the *p53* gene and bearing an activated version of the *myc* gene rapidly gave rise to aggressive, invasive liver tumors. Scanning the DNA of these tumors revealed that a specific segment of mouse chromosome 9 was amplified—or present in excess copies—compared to the DNA of healthy mouse liver cells. Because this segment of mouse DNA carried several genes, the researchers turned to the human genome to help them narrow down which gene (or genes, as it turned out) was the culprit in liver cancer. In parallel with their analysis of the mouse liver tumors, they scanned the DNA of human liver and other tumors. Remarkably, they found that a region of human chromosome 11 that is evolutionarily related to the segment of mouse chromosome 9 was amplified in several of the human tumors.

Additional experiments revealed that two genes—*Yap* and *cIAP1*—were both consistently overexpressed in both mouse and human tumors. Thus, when produced at abnormally high levels, proteins encoded by the *Yap* and *cIAP1* genes contribute significantly to human liver and other cancers. Therefore, these proteins and others in the pathways they control are attractive candidates for the development of novel cancer therapies.

The study involved scientists from five institutions in Europe, Asia, Australia, and the United States and five research groups at CSHL led by Scott Lowe, Mike Wigler, Greg Hannon, Rob Lucito, and Scott Powers.

In some cases, the fusion of human cells is a normal process that leads, for instance, to the formation of muscle and bone. Seemingly innocuous infections by common viruses can also cause cells in our bodies to fuse. Such fused or "hybrid" cells are widely considered to be harmless because they are generally believed to die and be cleared from the body without consequences to our health. This view of cells fused by viruses as being harmless may need to be revised, and revised dramatically. According to a recent study led by Yuri Lazebnik and postdoctoral fellow Dominik Duelli, cell fusion triggered by common viral infections may be a significant factor in the development of human cancer.



Y. Lazebnik

The idea that aberrations in the number or structure of human chromosomes can spur tumor formation is more than a century old. Such aberra-

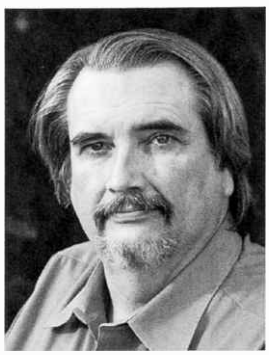
tions—known collectively as "aneuploidy"—arise in two principal ways: as a consequence of abnormal cell division or as a result of cell fusion. In both cases, aneuploid cells have an abnormal genetic makeup (e.g., too few or too many copies of a particular chromosome or chromosome segment) and they frequently die. But not always.

Researchers have long known that cancer cells—very much alive—are often aneuploid. Whether aneuploidy is a cause or a consequence of a cancerous state is still being debated. But in any case, given that cell fusion causes aneuploidy and that aneuploidy may cause cancer, it follows that cell fusion may cause cancer. This is where "innocuous" viral infections come in.

Dominik and Yuri first observed that cultured human cells are fused through the action of a particular virus (Mason-Pfizer monkey virus [MPMV], one among many "fusogenic" viruses). As expected, the resulting hybrid cells are aneuploid and fail to grow. However, the researchers next showed that if one of the cell fusion partners is engineered to carry a particular mutation in an oncogene or a tumor suppressor gene, then a significant number of the resulting hybrid cells grow land are thus potentially cancerous. Yuri's group is currently exploring whether such proliferating fused cells are produced by viral infections in animal models. If they are, then the work of sorting out which of the many known fusogenic viruses might contribute to human cancer will likely begin in many laboratories.

Genomics and Bioinformatics

Rice feeds more than half of the world's human population. Estimates indicate that the rice crop yields will need to be increased by about 30% over the next two decades to meet a projected increase in demand.



W.R. McCombie

W. Richard McCombie, his CSHL colleagues, and other members of the 10-nation International Rice Genome Sequencing Project have reported a highly accurate, "finished" DNA sequence of the entire rice genome. The complete rice genome sequence—which reveals some 38,000 genes on the 12 chromosomes of rice—provides the raw material for many studies aimed at improving the agricultural yield of the world's most important food source. Moreover, because the rice genome is closely related to that of other major cereal grasses (including corn, wheat, barley, rye, sorghum, and millet), the complete rice genome sequence is an extraordinarily useful resource for identifying genes of interest in a group of crop plants that collectively supply two thirds of humanity's food supply.

The study revealed thousands of genetic markers in the rice genome that are of immediate use to plant breeders and others working to improve rice agriculture. It also generated the first finished genome sequence of any crop plant, making rice a powerful model for how to use genome sequence information to improve many other aspects of agriculture. The finished rice genome sequence builds upon draft sequences previously published by the private companies Monsanto and Syngenta. As such, it is an excellent example of a successful public-private partnership that saved the public consortium both time and money.

By enabling scientists to identify genes that underlie agriculturally important traits, a draft of the rice genome sequence released by the public consortium in 2002 has already spurred both biotechnological and conventional plant-breeding approaches to increasing rice yields. The new, finished rice genome sequence has the potential to accelerate those efforts. The availability of the sequence should greatly speed the hunt for genes that increase yield, protect against disease and pests, and improve other traits of rice and several other cereal crops.

Plant Molecular Genetics

Although most people probably do not give too much thought to leaves, they are in fact crucial light-harvesting and gas-exchange organs, without which agriculture as we know it, not to mention life on Earth itself, would be very different.

To the naked eye, the top and bottom surfaces of leaves look rather similar. Closer inspection reveals that they are highly specialized regions that arise through a complex series of molecular events. Marja Timmermans has recently made a number of important discoveries concerning these events and how they instruct unspecialized stem cells to form the specialized top (light-harvesting) and bottom (gas-exchanging) surfaces of leaves.

M. Timmermans

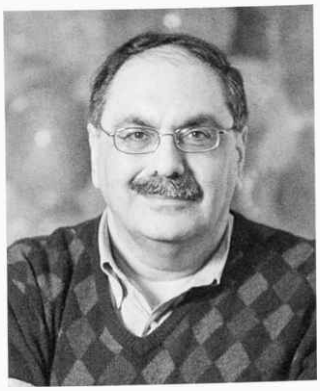
One of Marja's projects explores the role of a gene called *leafbladeless1* in plant development. Corn plants with a normal *leafbladeless1* gene develop broad, flat leaves with distinct top and bottom surfaces, as usual. In contrast, mutant plants lacking a functional *leafbladeless1* gene develop long, threadlike leaves that are "all bottom, no top." This means that the *leafbladeless1* gene is somehow required to specify the formation of top surfaces of leaves. To find out how, in parallel with other experiments (see below), Marja and her colleagues, including postdoctoral fellow Fabio Nogueira, isolated the *leafbladeless1* gene. They discovered that *leafbladeless1* is similar to another gene with a known role in generating biologically powerful snippets of RNA called "*trans*-acting small interfering RNAs" (ta-siRNAs). This is clue #1, i.e., *leafbladeless1* specifies "top" by promoting ta-siRNA formation. Clue #2 came from examining a different sort of small regulatory RNA called "microRNA166" (miR166). Marja's group had previously shown that in normal plants, miR166 is present in the cells that generate the bottom surfaces of leaves but is absent from adjacent cells that generate the top surfaces of leaves. In short, miR166 means "bottom."

If ta-siRNAs mean "top" and miR166 means "bottom," then what might ta-siRNAs and miR166 mean to each other? Does one control the other? Marja and her colleagues answered this question by determining whether the pattern of miR166 expression is altered in plants (*leafbladeless1* mutants) that lack ta-siRNAs. The result (clue #3 and a major discovery): In the absence of ta-siRNAs, miR166 is present both in its usual "bottom" cells *and* in the cells that normally generate the top surfaces of leaves. This is consistent with the idea that in normal plants, ta-siRNA activity blocks miR166 expression in the "top" cells. It also explains why the leaves of *leafbladeless1* mutants are "all bottom, no top." In such mutants, the "bottom promoting" activity of miR166 is abnormally present in the "top" cells and transforms the fate of those cells from top to bottom.

Through the work of other scientists, including some of CSHL's own (Greg Hannon, Leemor Joshua-Tor, Rob Martienssen), small RNAs akin to miR166 and ta-siRNAs have recently been shown to have important roles in the biology of many organisms, including humans. Therefore, the discovery by Marja and her colleagues that the opposing activity of two small RNAs can control major developmental events in plants establishes a paradigm that is likely to have broad implications for the biological and biomedical sciences.

Neuroscience

CSHL neuroscientist Grigori (Grisha) Enikolopov and his colleagues have identified which cell type among several different kinds of neural precursor cells in the brain is the sole target of the widely prescribed antidepressant Prozac. This discovery might enable a new generation



G. Enikolopov

of more specific treatments for depression, with fewer side effects, to be developed. It also lays the foundation for many studies of the factors that control how, when, and where new neurons are generated from stem cells in the brain. Such work could eventually lead to cell replacement therapies for neurodegenerative and other brain disorders including Alzheimer's and Parkinson's disease.

It has been known for some years that Prozac (fluoxetine) is likely to relieve the symptoms of depression by somehow causing more neurons to be present in a particular region of the brain (the "dentate gyrus"). But the origins of these neurons, and how Prozac promotes their existence, have been a mystery. Until now. By profiling the telltale marker proteins produced by different kinds of cells in the brains of adult mice, Grisha's group—spearheaded by postdoctoral fellow Juan Manuel Encinas—first defined discrete steps in the complex process, called neurogenesis, that converts unspecialized stem cells into mature, specialized neurons.

Next, knowing that Prozac treatment somehow increases the number of neurons in the brain, the researchers tested which step in the neurogenesis pathway might be stimulated by Prozac. They found that Prozac treatment specifically stimulates the generation of a kind of cells they dubbed "amplifying neural progenitors" or ANPs—the second step in the neurogenesis pathway from stem cells to mature neurons.

To address the controversy surrounding the use of Prozac in children and in pregnant women, Grisha's group is currently testing the effects of the drug on brain neurogenesis in juvenile and pregnant mice. The results of those experiments should provide valuable information for assessing the possible effects of Prozac and related drugs on fetal and adolescent brain development. The researchers are also using the tools they have developed to explore whether other treatments for depression, including other drugs and deep brain stimulation, act in the same way as Prozac or in different ways. In addition, they are screening for new drugs that stimulate ANP cells to multiply and thus expand the production of brain neurons for the treatment of neurodegenerative diseases.

Z. Mainen

It is a classic upper-middle-class dilemma: Should we buy a perfect second home in an area that takes hours to get to or should we settle for something closer but not as nice? In the rodent world, an equivalent decision-making situation might be, "Was the food I liked better down this alley or over there?"

By discovering that particular rat brain neurons combine or "integrate" dissimilar pieces of information (e.g., location vs. reward), Zach Mainen and his colleagues have begun to learn how the brain controls decision-making and goal-oriented behaviors. Examples of these include foraging and navigation in animals and in humans, whether to buy a particular second home or, in general, whether to favor a long-term benefit over immediate gratification.

Zach's recent study represents the first time that brain neurons have been shown to integrate spatial and reward information. Its results contrast with a previous "pure economic" view that neurons in the orbitofrontal cortex (OFC) are involved solely in assessing value. Moreover, the study has implications for understanding pathological states in humans that affect decision-making, motivation, and emotions such as addiction, depression, obsessive-compulsive disorder, autism, and other disorders of thought or mood.

The research was spearheaded by graduate student Claudia Feierstein, who recorded the activity of OFC neurons while rats performed an odor discrimination task that they had previously learned to accomplish. In the task, the animal receives a test odor ("A" or "B") by pok-

ing its nose into a centrally located odor port. Next, the animal chooses odor A or odor B as being the same as the test odor by poking its nose into a choice port located to its right (odor A) or left (odor B). If the animal chooses correctly, it receives a reward (a drop of water). As expected, many of the OFC neurons actively signaled "I'm getting a reward" when the animal moved right or left, i.e., toward odor A or odor B. Surprisingly, however, several of the neurons signaled "I'm getting the reward to my right," whereas several others signaled "I'm getting the reward to my left."

One of Zach's next steps will be to examine what happens in the brain while the animals are first learning to recognize new odors. Through this work, the researchers hope to gain a greater understanding of learning and memory as well as the neural basis of perception, motivation, decision-making, and other aspects of behavior.

Cold Spring Harbor Laboratory Board of Trustees

The Board of Trustees was pleased this year to welcome four new members: John C. Phelan, Managing Partner and cofounder of MSD Capital, L.P.; Jamie C. Nicholls, recently a General Partner and currently a Limited Partner at Forstman Little & Co.; Donald Everett Axinn, writer, respected investor and builder in the New York area, and committed public servant; and Landon Clay, Managing Member of East Hill Management Company.

Concluding their terms as Trustees this year were Arthur M. Spiro and Susan Lee Lindquist. Mr. Spiro was first elected to the Board in November 1999 and was then reelected to a second term in 2002. He was active on several committees, including Audit, Executive, and Woodbury Genome Research Center, and he served for 6 years as the Chairman of the Dolan DNA Learning Center Committee. Dr. Lindquist was elected to the Board in 2002 and brought her expertise to bear on the Tenure and Appointments Committee throughout her term.

We said a sad goodbye to Wendy Vander Poel Russell, Honorary Trustee, who passed away in March, 2006. Mrs. Russell was an active member of the Board of Trustees since 1984, serving as Secretary from 1985 to 1987 and from 1992 to 1997. A legendary fundraiser, her pet project at the Laboratory was the Dolan DNA Learning Center, and she was instrumental in the establishment of its Corporate Advisory Board.

The Cold Spring Harbor Laboratory Association (CSHLA) raised a total of $1,155,000 this year under the leadership of Association president Joe Donohue. We say thanks to Mr. Donahue who served his second term as president in 2006, doing double duty while also serving as a Trustee of the Board. New Directors in 2006 included Joe Amelia, Suzanne DiMaio, Nancy Edsparr, Larry Gellman, M.D., and Scott J. Ratner, M.D.

Hillside Campus Dedication

The Hillside Campus Cornerstone Dedication Ceremony on October 15 marked the transition from constructing the infrastructure for new facilities to construction of the facilities themselves. Much of the work done this year has been groundwork for the construction of six buildings dedicated to scientific disciplines that fulfill the core research mission:

- The David H. Koch Building
- The DeMatteis Family Building
- The William L. and Marjorie A. Matheson Building

- The Leslie and Jean Quick Building
- The Donald Everett Axinn Building
- The Wendt Family Building

The bright, crisp afternoon of the ceremony brought dozens of well-wishers, including CSHL faculty, staff, dignitaries, and most importantly the donors and their families whose names will grace these facilities. Once complete, the Laboratory's research space will increase by nearly 40%.

The festive day included a solemn note with fond memories of Jean Quick who passed away earlier this year after naming the Leslie and Jean Quick Building for Cancer Research after her late husband and CSHL Trustee Leslie C. Quick, Jr. Long-time residents of Laurel Hollow and neighbors of the Laboratory, their legacy at CSHL lives on through their gifts and the continued involvement of the family.

In planning for the Hillside Campus, CSHL has worked to be environmentally and aesthetically sensitive to the unique environment of Cold Spring Harbor. The new facilities have been designed to encourage efficiency and easy communication between buildings and scientists. Together, they will function as an academic village at the southern end of the campus, stylistically within the broader village of science that now exists.

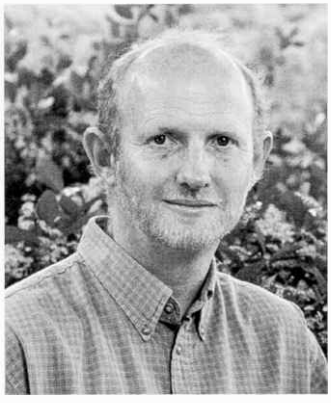
Much of the infrastructure work completed this year consisted of ensuring adequate storm water drainage for the previously wooded site. Rather than employ the conventional approach of installing an enormous quantity of dry wells to accommodate storm water, our civil engineers adopted an ingenious approach: They designed water quality rain gardens and bioretention ponds to collect and treat storm water runoff before it enters the harbor. This approach not only provides an environmentally sound means of treating storm water, but also creates additional naturalized water features on the campus, adding beauty and a wildlife habitat.

Hillside Campus Dedication Ceremony

Awards and Honors

Rob Martienssen was elected a Fellow of the Royal Society, distinguished for fundamental discoveries on the epigenetic mechanisms that regulate transposon silencing, gene control, and stem cell function in plants. He was also noted by the Royal Society as a major contributor to sequencing the genome of *Arabidopsis*, the first plant genome sequence completed. This is one of the highest honors that can be accorded a scientist, and CSHL now adds Martienssen to its list of previously elected fellows: Jim Watson, Nick Tonks, and myself.

The Leukemia & Lymphoma Society selected Bill Tansey as one of five researchers to receive its prestigious Stohllman Scholar Award, recognizing his outstanding contributions to the advancement of blood cancer research. The focus of his work is a protein, Myc, known to contribute to the growth of leukemia and lymphoma cancer cells.



R. Martienssen

Sandra J. Kuhlman and Eleonore Real each received the National Alliance for Research on Schizophrenia and Depression Young Investigator Award. Sandra is studying in an animal model the role GABAergic synapses in the prefrontal cortex have in memory impairment in people with schizophrenia. Eleonore is working on glutamate receptor trafficking and synaptic plasticity, since glutamate abnormalities have been implicated in psychotic disorders.

CSHL Fellow Ira Hall received a 2006 Burroughs Wellcome Fund Career Award in the Biomedical Sciences. This award provides early-career biomedical researchers with funding over a 5-year period to foster their development and help them make the critical transition to independent investigators. Ira is using DNA microarray technology to explore DNA copy-number fluctuations and epigenetic inheritance in the mouse, an important model system for many diseases including cancer.

Thomson-ISI added the CSHL Press journal *Genes & Development* to its "Top 10 Scientific Journals in All Areas" list for the decade 1995–2005. Edited by Terri Grodzicker, this journal presents research papers of broad general interest and biological signficance in molecular biology, molecular genetics, and related fields. Thomson-ISI provides a service that measures the impact of some 7 million papers published in 11,000+ journals in 22 major scientific fields.

Inside Cancer—a comprehensive, user-friendly Web guide to cancer biology created by the BioMedia Group of CSHL's Dolan DNA Learning Center—was selected as an official "Site of the Day" by Adobe Systems Incorporated, joining the ranks of other winners that included Nike, Cartier, and Bentley Motors.

A publication by Leemor Joshua-Tor and her colleagues Niraj Tolia, Fabiola Rivas, and Greg Hannon was selected as the "New Hot Paper" by Thomson Scientific's Essential Science Indicators. "Purified Argonaute2 and an siRNA form recombinant human RISC" won this distinction by virtue of it being cited more frequently than 99.9% of all other studies in numerous journals surveyed.

CSHL was selected to be part of a consortium that will benefit from a $100 million grant from the Starr Foundation. CSHL, The Broad Institute of MIT and Harvard, Memorial Sloan-Kettering Cancer Center, The Rockefeller University, and Weill Cornell Medical College will collaborate on research aimed at understanding cancer at its most fundamental levels and at developing new approaches to the prevention, diagnosis, and treatment of many forms of the disease.

Starr Cancer Consortium members (*left to right*) A.M. Grotto, Jr., E. Lander, M. Greenberg, H. Varmus, B. Stillman, F. Davis

D. Spector

David Spector will lead CSHL's involvement in the establishment of the Nanomedicine Center for Nucleoprotein Machines. The National Institutes of Health awarded a $10 million grant to CSHL, the California Institute of Technology, Emory University, Georgia Institute of Technology, The German Cancer Research Center, the Medical College of Georgia, Massachusetts Institute of Technology, and the New York University Medical Center. The focus of the center will be to understand the molecular machines that enable cells to detect and repair damaged DNA. David was also the first recipient of the Winship Herr Award for Excellence and Creativity in Teaching in the Watson School of Biological Sciences.

CSHL President Bruce Stillman was honored by the Society of Surgical Oncology, receiving the American Cancer Society Basic Science Award and Lecture.

Development

This year has proven to be another year of successful fund-raising at Cold Spring Harbor Laboratory. Our most generous donors have provided much needed support to both capital and research projects as well as our endowment.

Cold Spring Harbor Campaign

Capital

The following major donors have generously contributed new gifts and pledges of $100,000 or more to support the Laboratory's Hillside Campus expansion project: Mr. and Mrs. Donald Everett Axinn, David H. Koch Charitable Trust, Mary D. Lindsay, and The Perkin Fund.

Faculty Recruitment Support and Equipment

We gratefully acknowledge support of $100,000 or more from Mr. and Mrs. Landon T. Clay, Mr. and Mrs. Norris Darrell, The Shelby Cullom Davis Foundation, The Coleman Fung Foundation, Jeff Hawkins and Janet Strauss, Jamie Nicholls and Fran Biondi, The Robertson Foundation, Dr. and Mrs. James Stone, and The Roy J. Zuckerberg Family Foundation.

Watson School of Biological Sciences

Support of the Dean's Chair, fellowships, and lectureships enable the Watson School to continue to grow and influence the field of biological sciences. We appreciate new gifts and pledges of $100,000 or more this year by Mr. Michel David-Weill, Mr. Curt Engelhorn, and Mr. and Mrs. Robert D. Lindsay and Family.

Dolan DNA Learning Center

New gifts and pledges to support the Dolan DNA Learning Center endowment were gratefully received from The Lessing Family Foundation and the OSI Pharmaceuticals Foundation.

Carnegie Library

The Genentech Center for Molecular Biology and Biotechnology was established with a generous gift from Genentech. Other supporters of $100,000 or more include the New York State Office of Parks and Historic Preservation and Dr. Norton Zinder (see the Chancellor's Report).

Program Support

Private funding is essential to maintain the Laboratory's innovative research programs. We appreciate new gifts and pledges of $100,000 or more from the following donors: an anonymous donor, Mr. and Mrs. Donald Everett Axinn, Ms. Kathryn Wasserman Davis, The DeMatteis Family Foundation, Mr. and Mrs. Leo A. Guthart, The Thomas Hartman Foundation for Parkinson's Research, Jo-Ellen and Ira Hazan, The Lita Annenberg Hazen Foundation, Hope for Depression Foundation, The Keck Foundation, The Miracle Foundation, The Don Monti Memorial Research Foundation, and Pam and Pierre Omidyar.

Robertson Research Fund

The primary in-house support for our scientists for more than three decades, the Robertson Research Fund in 2006 supported research in the labs of Alexei Koulakov, Cordula Schulz, Leemor Joshua-Tor, Rui-Ming Xu, Wolfgang Lukowitz, Rob Martienssen, Marja Timmermans, and David Jackson. Start-up research support was also provided by the Fund to two new investigators: Glenn Turner and Hiroyasu Furukawa. In addition, the Robertson Research Fund continues to support the annual CSHL In-House Symposium and our programs for postdoctoral fellows, graduate students, the laboratory seminar program, and faculty recruitment.

Breast Cancer Research Support

The Laboratory greatly appreciates the many supporters of our breast cancer research program. This includes several local grassroots groups that provide not only much needed funds, but also public awareness and outreach. This year, we were fortunate to receive support from Breast Cancer Awareness Day in memory of Elizabeth McFarland, Breast Cancer HELP, Glen Cove Cares, The Breast Cancer Research Foundation, the Cold Spring Harbor Main Street Association, Find A Cure Today (F.A.C.T.), Mr. and Mrs. Richard Gordon, Long Island 2-Day Walk, Long Islanders Against Breast Cancer (L.I.A.B.C.), the Manhasset Women's Coalition Against Breast Cancer, the Pierre and Pam Omidyar Fund, the Judi Shesh Memorial Foundation, the Waldbaum Foundation, the West Islip Breast Cancer Coalition for Long Island, the Women's Insurance Network of Long Island, and the Clear Channel/WALK for Women Breast Cancer Fund.

Building Projects

The year 2006 was a busy one for the Facilities Department, with multiple simultaneous construction projects being undertaken in addition to the work on the Hillside Campus.

The James Laboratory renovation—a multiyear project in which nearly the entire building has been reconstructed to meet modern needs—continued from 2005, with only one laboratory and two offices remaining to be completed in 2007. The replacement of the Grace Auditorium bluestone patio, begun in the fall of the previous year, was completed in time for the meeting and course season, and the groundwater-cooled chiller plant that was to service the Grace and Harris Buildings was completed, meeting the increased cooling demand with greater efficiency. This also paved the way for the complete renovation of the Harris Building mechanical systems, which, when completed in 2007, will increase the building's capacity by more than 40%. Additionally, the Demerec Building chiller, running above its design capacity and beyond its useful life, was replaced with a new unit of increased capacity and far greater efficiency.

2006 also saw a continuation of the Laboratory's program to upgrade and improve its residential properties. The final Hooper apartment, the remaining two Firehouse apartments, and the Rose cottage were all renovated during this year. Additionally, the caretaker quarters of the Robertson House—previously composed of two cramped rooms—were expanded and renovated into a comfortable apartment for the live-in caretaker.

Other small projects include those in support of meetings, courses, and special events. Restrooms in Grace were enlarged to accommodate the increased size of meetings. Power and lighting were improved in the Bush Auditorium, and offices were constructed in Blackford Hall to accommodate the increased size of the events planning staff.

Less visible, but equally as important, several key infrastructure projects were completed as well. Several sections of the Laboratory's underground high-voltage power mains were replaced. Underground fiber optic network cables were extended to areas not previously serviced. And the Laboratory's water main was extended both to accommodate future expansion into the Upper Campus and to connect to residential properties at the north end of the campus, which were previously fed by well water. Two highly visible infrastructure projects are the curbing and stabilizing of the Davenport lawn parking lot and a major drainage project intended to divert the stream running through the campus around the foundations of the Demerec Laboratory during 100-year floods, two of which occurred within a single month the previous year.

Special Events

Symposium

The 71st Symposium—"Regulatory RNAs"—once again included the annual Dorcas Cummings Lecture. Ron Pasterk's outstanding lecture on "The Emerging World of Small RNAs" was presented to a mixed audience of scientists and lay friends and neighbors of the Laboratory. Following the lecture, more than 20 of our neighbors graciously opened their homes and hosted dinner parties for Symposium participants and Laboratory friends alike.

Gavin Borden Visiting Fellows

The 12th Annual Gavin Borden Visiting Fellow Lecture—in memory of the publisher of *Molecular Biology of the Cell*—was held on Tuesday, May 9. Dr. Michael Levine, Professor of Molecular and Cell Biology at the University of California, Berkeley, presented the lecture entitled "Gene Networks for Fly Gastrulation and Heart Formation in Sea Squirts."

Delbrück 100th Birthday Celebration

On August 26 and 27, the Laboratory commemorated the centennial of the birth of Max Delbrück (September 4, 1906). Delbrück, who frequently visited during the 1940s through the 1960s, was a scientific leader who conducted breakthrough research and began Cold Spring Harbor Laboratory's Phage Course. With Salvador Luria and Alfred Hershey, he founded the "Phage Group" to research bacteriophage (viruses that attack bacteria) in order to understand the nature of the gene, work for which the three shared the Nobel Prize in 1969.

The meeting brought together several of Delbrück's colleagues, students, friends, and family members to share stories and discuss his contributions to 20th century science. The 2-day conference included talks by Ernst Peter Fischer, Delbrück's former student and biographer; Matt Meselson, who conducted experiments that helped show how DNA replicates; and Delbrück's longtime collaborator, Gunther Stent; among others. The meeting continued with a symposium on current research related to topics that had interested Delbrück, concluding with a review of the burgeoning area of Systems Biology by Lee Hood, a longtime colleague of Delbrück's.

The First Double Helix Medal

The Laboratory held its inaugural Double Helix Medal Dinner on November 9 at the Mandarin Oriental, New York. Medals were presented to Muhammad Ali for his public campaign against Parkinson's disease; to Suzanne and Bob Wright (NBC Universal) for their work in bringing attention to autism through Autism Speaks; and to Nobel laureate and former CSHL faculty member Phillip Sharp for his lifelong contributions to biomedical research leading to a deeper understanding of cancer and other genetic diseases. This special event, which raised nearly $2.5 million for the Laboratory, was cochaired by CSHL Trustees Tom Quick, David Rubenstein, and Roy Zuckerberg and NBC Universal President, Jeff Zucker.

Double Helix Medal

Public Lectures

The CSHL Cultural Series is a tradition in which an eclectic mix of artists, writers, and scientists present lectures, concerts, and exhibits that provide compelling glimpses of how we experience, discover, live in, and make sense of our world. Open to the public, the aim of the Cultural Series is to stimulate, inspire, and entertain.

S. Lowe

March 14
Simon Baron-Cohen, Professor of Developmental Psychopathology at University of Cambridge and Director of the Austism Research Centre in Cambridge: *Seeking the Cause of Autism*.

April 25
Scott Lowe, HHMI Investigator/Professor, Cold Spring Harbor Laboratory: *Recent Progress in Cancer Research*.

May 16
Irene Pepperberg, Adjunct Associate Professor, Brandeis University; Research Associate, Harvard University; Leader, The Alex Foundation: *In Search of King Solomon's Ring: Cognitive and Communicative Abilities of Grey Parrots*.

M. Ridley

June 13
Matt Ridley, F.R.S., Visiting Professor, Watson School of Biological Sciences: *Francis Crick: Discoverer of the Genetic Code*.

September 12
Jeffrey Friedman, HHMI Investigator/Professor, The Rockefeller University: *Leptin and the Biological Basis of Obesity*.

September 26
Paul Liam Harrison, artist-in-residence, Human Genome Organization: *Pertaining to Origins: Organization of Form and Function*.

J. Friedman

October 24
Tim Tully, St. Giles Professor of Neuroscience, Cold Spring Harbor Laboratory: *Recent Progress in Neuroscience Research*.

Public Concerts

March 18
Alexandre Pirojenko, piano

March 25
Rui Shi and Chris Gaudi, piano and oboe

April 29
Martin Kasik, piano

May 6
Asmira Woodward-Page, violin

May 20
Gleb Ivanov, piano

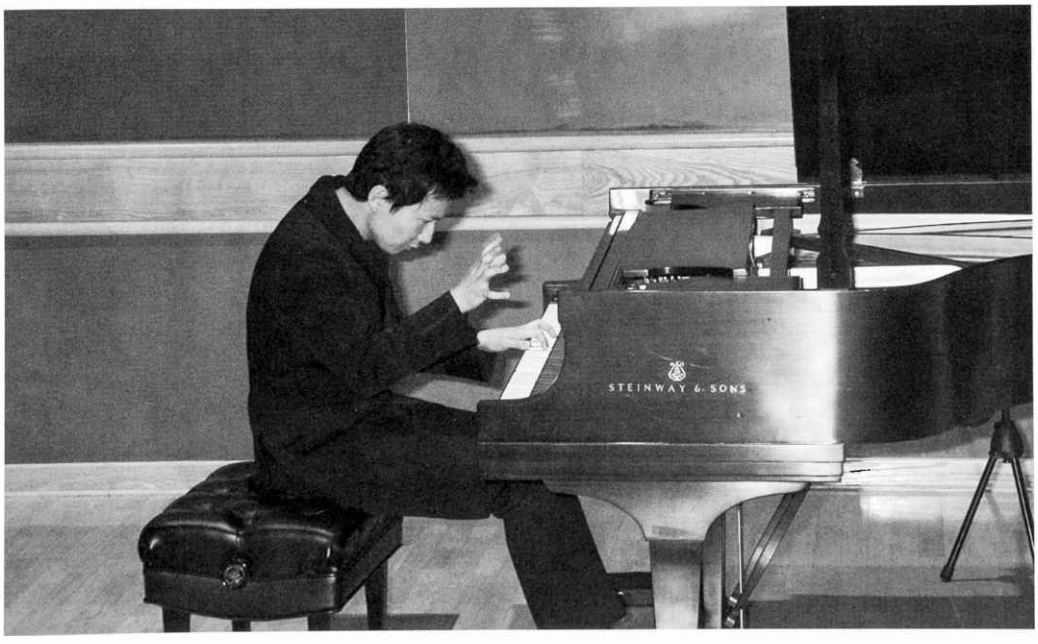
August 26
Julie Albers, cello

September 2
Orion Weiss, piano

September 16
Efe Baltacigil, cello

September 30
Wonny Song, piano

O. Weiss



W. Song

Jupiter String Quartet

October 14
Thomas Meglioranza, baritone

November 18
Daniel Phillips, violin

December 2
Jupiter String Quartet

Exhibits

The 2005 Photographer-in-Residence Ryan Brenizer exhibited his works in Bush Auditorium throughout the month of July. The photographs of many CSHL researchers were captured during his residency the previous summer.

Paul Liam Harrison, Artist and Printmaker, exhibited his work in a show entitled "Pertaining to Origins," held in the Racker Room of Blackford Hall from September 26 through October 1.

Laboratory Employees

New Staff

Glenn Turner joined the faculty in the winter of 2006 to study the association between smell and taste; for example, how the brain encodes the association between spoiled milk and the memory of that awful taste. He is using the fruit fly's association between a given odor and a bitter taste along with the electrophysiology techniques that he developed at the California Institute of Technology (CalTech) and the powerful ability to manipulate genes in the fruit fly using molecular genetics. Turner did postdoctoral research in Dr. Gilles Laurent's laboratory at CalTech, where he collaborated on a method to measure electrical activity and hence the activity of neurons in the fruit fly brain. Using this technology, he found that olfactory information is represented in an area of the fruit fly brain called the mushroom body that is essential for learning and memory.

Hiro Furukawa, who joined the faculty of CSHL in December 2006, is interested in the connection between the NMDA receptors and proteins involved in Alzheimer's disease. His research both as a graduate student in Dr. Tatsuya Haga's laboratory at The University of Tokyo and as a postdoctoral fellow in Dr. Eric Gouaux's laboratory at the Vollum Institute at Columbia University focused on understanding how these neurotransmitter receptors work. He is studying γ-secretase, which cuts amyloid precursor protein into a plaque-forming γ-amyloid fragment found in Alzheimer's patients and may affect neuronal activity by associating with NMDA receptors. Understanding these interactions may aide in defining targets for developing new drugs to treat Alzheimer's disease.

A new faculty member as of September, Raffaella Sordella is interested in exploring the molecular mechanism of why particular mutations in the oncogene called epidermal growth factor receptor (EGF-R) result in cancer cell addiction, and why other EGF-R mutations are resistant to the drug *Iressa*. Continuing the work that she did at Massachusetts General Hospital Cancer Center as a postdoctoral fellow, she hopes to identify other cellular components to which cancer cells become addicted that potentially can be targeted by cancer therapies. Sordella received her Ph.D. from Turin University, Italy, where she worked with Dr. Paolo Comoglio studying the role of growth factor receptors in cancer development.

Dr. Walter Goldschmidts joined the laboratory as the Executive Director of Sponsored Programs, bringing extensive federal and private sector management and research funding experience to our campus. In this newly created role, his Department facilitates new and continuing sponsored research initiatives to promote the research, education, and scholarly mission of the Laboratory and its investigators.

Promotions

Congratulations to David Jackson and Linda Van Aelst who were both promoted to Professor.

Departures

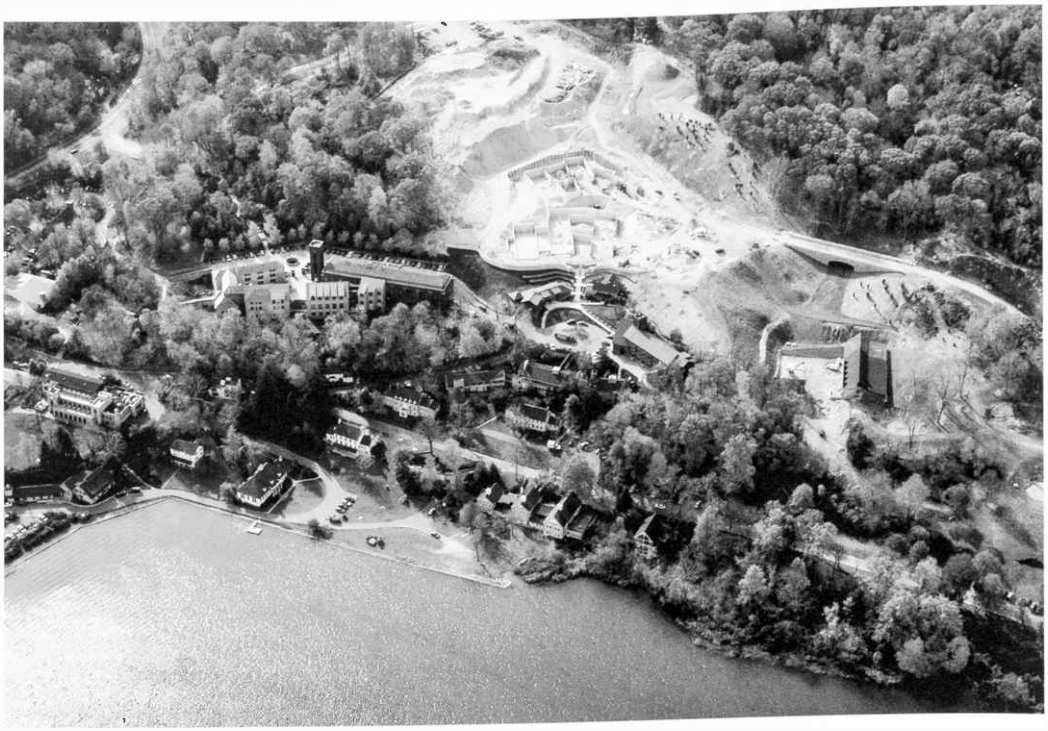
During the course of the year, several faculty took on new challenges at other institutions. Associate Professor Carlos Brody moved to Princeton University, but we are pleased to have him continue at CSHL as an Adjust Professor. Eli Hatchwell moved further east on Long Island to Stony Brook University, and Andrew Neuwald began a faculty appointment at The Instititute for Genomic Research (TIGR).

Jeff Picarello, the Director of Public Affairs for Cold Spring Harbor Laboratory and former managing editor of the Annual Report, took a position with Edelman Public Relations.

Peter Sherwood, who was the Director of Research Communications and Editor-in-Chief of the Harbor Transcript, moved on to Hofstra University.

We are saddened by the passing of Teresa Haire, a talented graduate student in Senthil Muthuswamy's laboratory. Several events were held to celebrate Teresa's life, and a fund has been established in her honor to support graduate student education at the Watson School of Biological Sciences.

The CSHL community also mourned the death of Enrique (Henry) Cepero, a postdoctoral fellow in Scott Lowe's laboratory who had battled cancer for three years. He is survived by his wife Jennifer and two newborn daughters, for whom college funds were established by friends here.



Hillside Campus expansion

Community Outreach

Cold Spring Harbor Laboratory participated in a number of community outreach events, including the sixth annual Pancreatic Cancer Walk at Old Westbury Gardens; the Long Island 2-Day Walk to Fight Breast Cancer; the Long Island Prom Boutique; the Long Island Cares Food Drive; and numerous activities to support the Ronald McDonald House at Schneider Children's Hospital in New Hyde Park.

Looking Forward

The year 2006 was one that propelled Cold Spring Harbor Laboratory forward. The foundations for the future were firmly set this year. Structurally, we moved mountains to set the cornerstone for the Hillside Campus expansion whose buildings will proudly bear the names of some of our most generous supporters. We celebrated the history and legacy of our Long Island campus with yet another successful symposium and numerous other concerts and lectures. The Double Helix Medal begins a new legacy that received national recognition for CSHL's dedication to raising awareness about the importance of genetics research for improving the health of people everywhere. The year 2006 should inspire us all to continue to move forward and realize the full potential of this institution. Thanks to our Trustees, our faculty and staff, and to our supporters for making this possible.

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

CHANCELLOR'S REPORT

Several years ago, I began donating to the Cold Spring Harbor Laboratory Archives an extensive collection of letters, manuscripts, and books related to my scientific career, including academic and literary efforts from the 1950s to the present. Vital to our Archives receiving and digitizing many of these records was a much-needed series of gifts totaling $400,000 from Lewis Lehrman, who has already done so much to preserve key documents of our nation's Civil War. In addition, instrumental in digitizing many photographs and other historical unpublished materials from mine and other unique scientific collections was a $500,000 grant from the Josiah Macy, Jr. Foundation.

Now magnifying my collection's importance is the recent decision of Sydney Brenner to donate to our Archives virtually all the documentation from his eminent scientific career of the past 50 years. Toward this end, our ever-resourceful Director of Library and Archives, Mila Pollock, has already made several trips to his East Anglian home in Ely, England to facilitate transfer of these records cataloging his career at Cambridge. There for more than 20 years, his and Francis Crick's desks faced each other in the same offices—first at the Cavendish Laboratory and then at the Laboratory of Molecular Biology. Using funds from the sale of duplicate documents from my life, many of Sydney's key letters and manuscripts have already been digitized.

Sydney's and my archives together comprise what we anticipate to be the beginning of a much more extensive future collection of important letters and lab notebooks documenting key advances in molecular biology and biotechnology that followed the discovery of the double helix. To properly house this collection, we have prepared architectural plans for a two-storied addition to our 1904 library building that was constructed to serve as the first laboratory for the Carnegie Institute of Washington's Cold Spring Harbor Station for Experimental Evolution. Long the center of genetic experiments, it became our library following the 1953 occupancy of Demerec Laboratory.

The need to expand our library was already obvious in 1994 when I ceased directing the science here at the Laboratory to assume the new position of President. However, almost a decade passed before a gift of $1 million from Waclaw Szybalski, who was on our staff in the 1950s, allowed us to begin planning this annex. The subsequent architectural plans generated by Bill Grover of Centerbrook, Essex, Connecticut, successfully preserved an early-20th-century atmosphere—just what we wanted. With essentially all of the required building and environmental permits now in place, we intend to start construction during late summer 2007, with occupancy expected in the spring of 2009.

We initially thought that some $3.5 million would cover the cost of our addition, but all too soon we realized that we must renovate and restore the original 1904 building. Now we know that approximately $6 million is needed to complete the project. To help raise this money, I traveled to San Francisco last March to the pioneering biotech firm Genentech and returned with much more assistance than I had requested. Genentech President Art Levinson authorized a $2.5 million grant to create the *Genentech Center for the History of Molecular Biology and Biotechnology*. Later, we also received a much-welcomed $287,500 contribution from New York State's Historic Preservation Office to help preserve the long-admired ambience of our first year-round building for science. To help the Center acquire unique historical material, an Archives Advisory Committee has been formed. This Committee, comprised of leading scientists, including three Nobel laureates as well as prominent historians and archivists, is working to collect and preserve materials on specific research topics and make them available online. Last year, with endowed support from

Philip Goelet and Sydney Brenner, the Sydney Brenner Scholar Research Program was begun, for which the first annual scholarship was awarded to Robert Olby to assist in his research on a biography of Francis Crick.

I have also been preoccupied with my current desire to make our Laboratory a major player in teaching and research on schizophrenia and other major mental health diseases. Greatly aided by Sydney Gary at the Banbury Center, during the summer of 2006, as in 2005, we used monies provided by my Oliver R. Grace Professorship to support 2-week-long summer workshops on schizophrenia, with an aim to entice more young neuroscientists to focus their research on this devastating human disease. Key to my belief that now is the time for us to commit our resources to research on mental-health disorders are the new advances in genome technology that offer the prospect of revealing the complete genetic underpinnings of mental disease. Fortunately, we were able to induce David Porteous of Edinburgh University to head this year's teaching staff, bringing with him the latest facts from his lab's study of balanced chromosomal exchanges that pinpointed several critical genes whose malfunctioning leads to psychiatric disorders. Grace funds also allowed us to initiate a new course on social cognition taught by David Skuse of the Institute of Neurology at University College London and by Ralph Adolphs from the California Institute of Technology. Failure to have suitable social interaction leads to much human misery, and considerable evidence demonstrates that misappropriate gene functioning is once again involved.

Rendering of the expanded Carnegie Building, which will house the existing Library, Archives, and the new Genentech Center.

My goal is for Cold Spring Harbor Laboratory to be as effective in identifying genes behind behavioral disorders as it has been in discovering key genes whose misfunctioning leads to cancer. A most-welcomed late-2005 grant of $1 million from the Forrest and Frances Lattner Foundation allowed Laboratory scientist Jonathan Sebat to move beyond finding copy-number variations involved in autism to also search for such genetic changes behind schizophrenia. Soon afterward, a noteworthy December 2005 visit to the Laboratory by Ted and Vada Stanley inspired their donation of $5 million. This has allowed us to expand such efforts to include bipolar disease and unipolar depression. Critical to the successful deployment of our latest DNA genome technologies is to obtain DNA samples from properly documented psychiatric patients. Toward this end, we are collaborating with Anil Malhotra of Hillside Hospital here on Long Island, Francis McMahon of the National Institute of Mental Health, James Potash of Johns Hopkins Hospital, and David Porteous of Edinburgh University.

Also at the center of our immediate efforts are the new high-throughput low-cost DNA sequencing technologies. The cost of resequencing a human genome has already fallen to $1 million, with the sum during the next several years likely to fall by an additional factor of 5. When the dollars needed to resequence a human genome become no more than the cost of a Chevrolet (by 2010?), the long-argued controversies regarding the roles of nature versus nurture in human lives will no longer be settled by rhetorical might. Then, happily, facts—not prejudices—will prevail.

I much anticipate the genomic zing at which we will move through the immediate future.

James D. Watson
Chancellor

CHIEF OPERATING OFFICER'S REPORT

A literal and figurative "changing of the landscape" began at the Laboratory in 2006. Phase I of the Hillside Campus expansion project was completed, laying the infrastructure and preparing the way for the construction of six research buildings totaling 100,000 square feet of new laboratory and common space. The buildings will provide much needed room for our programs in bioinformatics, cancer diagnosis and therapy, genomics, and neuroscience. A total of 180,000 cubic yards of soil were removed and sold for reuse, and new gas, water, and electrical systems were installed. Construction sites are not pretty and we are sensitive to the fact that the pristine vistas of our neighbors across the harbor have been affected by this work. Consequently, 6 acres of cleared area have already been restored and 300 trees have been planted to begin the reforestation process. As the buildings go up in the coming months, we will continue to work diligently to restore the site. We are proud of the fact that the Laboratory has been recognized for its environmentally friendly engineering of the project. An innovative system for managing storm water runoff was engineered by creating a series of attractive planted retention ponds in which storm water is organically purified before dissipating into the surrounding soil. The high-capacity "chiller building," containing the equipment needed to cool the water supply to all of the new laboratories, has the look of an authentic antique barn. As has been our practice over time, every effort is being made to preserve the character and natural beauty of our surroundings.

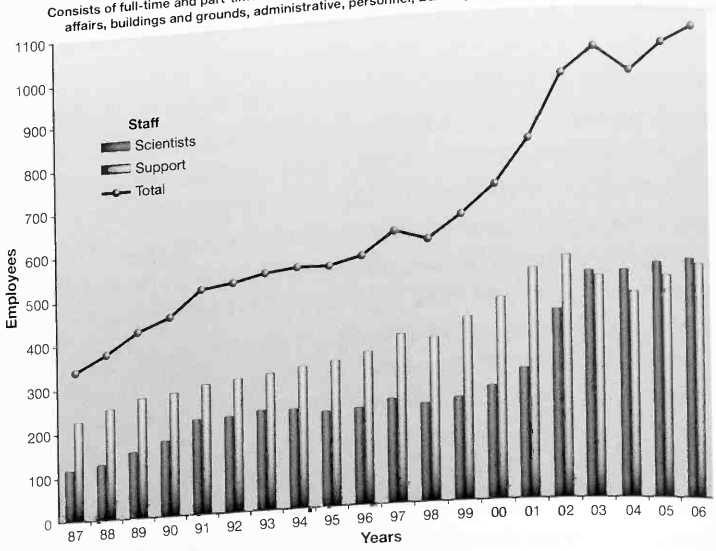
Fund-raising for the project has been a rigorous and rewarding process. Although the cost of infrastructure and buildings is approximately $100 million, we must allocate substantial funds to start-up expenses, recruitment of new investigators, and endowment. There is still much hard work to be done, but we are pleased to be well past the halfway point toward our $200 million goal. We are grateful for the generous support of our friends, donors, and Trustees who have made this possible, as well as for the dedication and effort of Charlie Prizzi and the Development team.

In addition to fund-raising, the Laboratory reaffirmed its A+ credit rating and publicly issued $55 million in tax-exempt bonds to support the construction. The offering, underwritten by J.P. Morgan, was well-received by investors and fully subscribed. The Investment Committee of the Board of Trustees favored mitigation of the interest rate risk on the floating rate obligations, and so a "swap contract" was executed to effectively fix the interest rate on all of the Laboratory's debt at 3.8% for the 35-year life of the bonds.

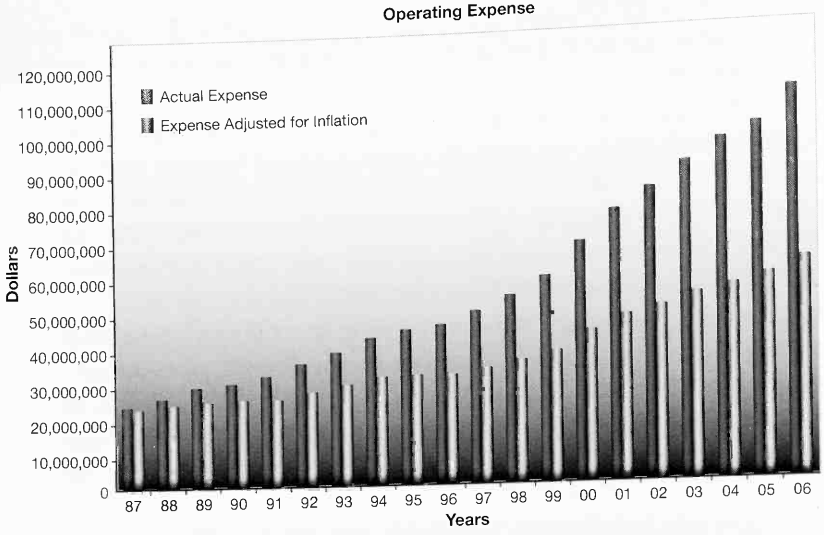
During the course of the year, we also saw the research "landscape" change in some very positive and not so positive respects. The programs in bioinformatics, genomics, cancer, and neuroscience have never been stronger, marked by important discoveries, distinguished publications, and awards to our faculty. There is great excitement over the fact that much of the work here is evolving from basic to more translational science, meaning that the results are contributing to advances in diagnostics and therapeutics in addition to the understanding of the underlying mechanisms and causes of problems like cancer. The challenging aspect of the current research environment is that the federal government, through the National Institutes of Health, has cut back substantially on funding for biomedical research. Federal grants are fewer, smaller, and harder to come by. This causes very real budgetary problems for institutions like ours, both by hindering the progress of innovative programs and by restricting the amount of funds available to cover the indirect cost or administrative and overhead expenses. The frustration level is high for dedicated professionals in a field where so much is suddenly possible, due to available data, knowledge, and technology, yet resource-limited.

The Laboratory's educational programs continue their vibrancy and excellence. Attendance at Cold Spring Harbor Meetings and Courses increased again this year, and the visiting scientists and scholars clearly contribute to the intellectual dynamics of the campus. The same can be said for the

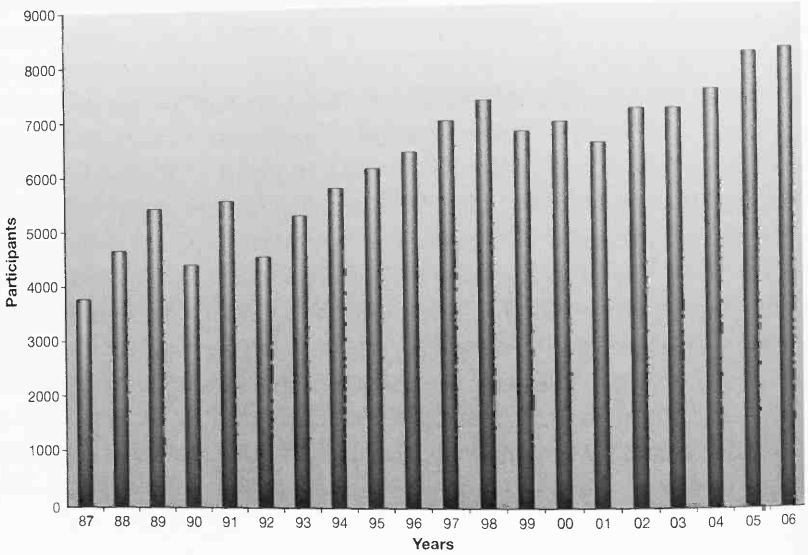
Consists of full-time and part-time technical support, core services, publications, meetings, library, public affairs, buildings and grounds, administrative, personnel, Banbury Center, and DNA Learning Center



Operating Expense



Meetings and Courses Participants



ongoing success of the Dolan DNA Learning Center, the Banbury Center, and the Watson School of Biological Sciences. The Cold Spring Harbor Laboratory Press has become a substantial enterprise that is adapting creatively to the digital publishing environment by developing new distribution strategies for its intellectual property.

For the fiscal year ended 12/31/06, the Laboratory budget reached $112 million—an 8% increase over the prior year. We had anticipated and forecasted a difficult year on the bottom line as we began to feel the effects of the federal funding reductions and increases in energy and insurance costs. As is often the case, funding from grants and from endowment support were not going to be sufficient to cover the direct cost of the research in 2006 and the institutional overhead expense. Despite rigorous and effective efforts to contain administrative expenses, it was necessary to access our reserve of biotech stocks in an effort to balance the budget after depreciation and interest expense. These measures, combined with the unrestricted proceeds from the wonderfully successful "Double Helix Award" gala in New York City, allowed us to avoid what might have been a substantial operating cash deficit for the year.

Fortunately, we saw another positive year in the financial markets and our endowment fund performed accordingly, returning 13.6%. Since the initial endowment gift by the Robertson family in 1973, the total fund has grown in value from $8 million to $289 million at December 31, 2006. This healthy appreciation is attributable to successful fund-raising, proactive commercialization of intellectual property, above average investment performance, and prudent spending policy. Investment strategy is determined by the Investment Committee of the Board of Trustees with the assistance of a consultant. The strategy has remained consistent, with the objective of achieving capital appreciation within acceptable risk parameters. The above average returns, over the years, are the result of good manager selection and diversity, as well as disciplined balancing of the portfolio. Historically, the portfolio has been allocated primarily to equities and fixed-income investments in a mix ranging from 70%/30% to 60%/40%. In 2006, two new international equity managers and one domestic value manager were added, all of whom achieved positive results. Also at year end, the Board and the Investment Committee determined that it would be in the Laboratory's interest to increase its allocation to "alternative" asset investments in order to further diversify the portfolio, enhance returns, and limit volatility. Execution of this strategy will be a priority in the coming year.

Looking ahead, the next several years are likely to be the most challenging in the modern era of the Laboratory. The cost of doing cutting-edge research is escalating at a time when the pool of federal funds for research is shrinking. Operating costs that are difficult to control, such as utilities, insurance, and healthcare, are increasing at double-digit rates. The expansion of our facilities is essential and necessary in order for the Laboratory to continue as a worldwide leader in research and education. However, the new buildings will add substantially to overhead expense and the recruitment of new investigators is expensive. It is imperative that we operate as efficiently as possible and that we redouble our efforts to attract research support from private foundations and individuals.

All of this requires the dedication of many. Our Board of Trustees, consisting of prominent scientists and business/community leaders, provides leadership, oversight, and invaluable advice to the senior management of the institution. This year, we thank two retiring trustees—Dr. Susan Lindquist and Arthur Spiro—for their years of service. We also recognize Joe Donohue for his years of extraordinary service in the dual role of Trustee and President of the Cold Spring Harbor Laboratory Association. Welcome are four new trustees elected in 2006—John Phelan, Landon Clay, Jamie Nicholls, and Don Axinn.

Finally, we recognize an extraordinary staff—nearly 1000 strong—whose great effort and dedication cannot possibly be adequately rewarded in a nonprofit environment.

Dill Ayres
Chief Operating Officer

LONG-TERM SERVICE

(*Standing*) Bruce Stillman, Michael Regulski, Pat Wendel, John Maroney, Martha Daddario, Delia King, Rodney Chisum, Adrian Krainer, Peter Stahl, Bill Keen, Tim Mulligan, Phil Renna, Peter Schwind, Chris Hubert, Frank Russo. (*Seated*) Susan Lauter, Mary Cozza, Margaret Wallace, Patricia McAdams, Mary Ann Miceli, Ingrid Benirschke-Perkins.

The following employees celebrated milestone anniversaries in 2006:

35 years	Bill Keen, John Maroney
30 years	Joseph Ellis, Peter Stahl, Margaret Wallace, Pat Wendel
25 years	Rodney Chisum, Philip Renna
20 years	Mary Cozza, Chris Hubert, Adrian Krainer, Susan Lauter, Vincent Meschan, Tim Mulligan, Jacek Skowronski
15 years	Ingrid Benirschke-Perkins, Kathy Cirone, Martha Daddario, Delia King, Patricia McAdams, Mary Ann Miceli, Chris Oravitz, Michael Regulski, Frank Russo, Claudia Schmid, Peter Schwind, Tim Tully, Linda Van Aelst, Michael Zhang


RESEARCH

See previous page for photos of the following scientific staff:

Row 1: R. Slotkin (Martienssen Lab); H. Oviedo (Zador Lab); M. Galli (Lukowitz Lab); I. Hall (CSHL Fellow)

Row 2: L. Manche (Krainer Lab); J. Brennecke (Hannon Lab); K. McJunkin (WSBS Student); J. Zuber (Lowe Lab)

Row 3: J. Hicks (Wigler Lab); S. Hearn (Spector Lab), Y. Benitez Alfonso (Jackson Lab); D.C. Gao (Mittal Lab); I. Bureau (Svoboda Lab)

Row 4: P. Kumar (Joshua-Tor Lab); C. Kopec (WSBS Student); V. Aranda Calleja (Muthuswamy Lab); J. Boehm (Malinow Lab)

Row 5: A. Mazurek (Stillman Lab); R. Thakkar (Spector Lab); F. Rivas (Hannon Lab); J. Li (Cline Lab)

CANCER: GENE EXPRESSION

Certain types of cancer and other diseases have been linked to defects in a cellular editing process called premessenger RNA splicing. Adrian Krainer's lab continues to study the process of pre-mRNA splicing in human cells, to understand its basic mechanism, regulation, and relevance to human genetic disorders and cancer. Krainer and his colleagues are studying key RNA-binding proteins that are part of the cellular RNA splicing machinery. They found that the gene coding for one of those splicing factors is an oncogene. Thus, elevated expression of this gene in a variety of tumors can cause the cancer in the first place and is necessary for continued tumor growth. The Krainer lab also studies spinal muscular atrophy (SMA), a pediatric neurodegenerative disease that is the leading genetic cause of infant mortality. Krainer and colleagues have identified the genetic defect that causes this disorder and are developing a mouse model to test whether they can restore correct gene expression.

When DNA makes a copy of itself, two complementary DNA strands are separated and each becomes a template for the construction of a new complementary strand. Strand separation is mediated by a helicase enzyme, a molecular machine that uses the energy derived from a process called ATP hydrolysis to separate DNA strands while moving along the DNA. Leemor Joshua-Tor determined a crystal structure of a viral replicative helicase bound to single-stranded DNA and nucleotide molecules at the ATP-binding sites. This structure demonstrates that a single strand of DNA passes through a molecular structure called the hexamer channel and that the DNA-binding hairpins of each subunit collectively form a spiral staircase that sequentially tracks the DNA backbone. It also demonstrates a correlation between the height of each DNA-binding hairpin in the staircase and the ATP-binding configuration, suggesting a straightforward mechanism for DNA translocation. This discovery has been hailed as a dramatic insight into the mechanistic details of how a protein works.

The focus of research in William Tansey's lab is a protein called Myc that is known to contribute to the growth of leukemia and lymphoma cancer cells. Tansey and his team have established that normal cells control the levels of Myc by destroying it soon after synthesis, but that this process is abnormal in lymphomas, leading to elevated levels of Myc in the tumor cells. The Tansey lab continues to study the mechanisms that cells use to control Myc destruction and how this process goes awry in cancer. In 2006, Professor Tansey was honored by the Leukemia & Lymphoma Society with its prestigious Stohlman Scholar Award in recognition of his outstanding contributions to the advancement of blood cancer research.

Michael Myers is using the accumulated knowledge of how proteins and protein complexes regulate cell physiology to create global network maps of all of the interacting proteins in a cell. These maps display how protein-protein interactions contribute to normal cell physiology, and how the interactions are disrupted in cancer. His global protein network map is beginning to reveal why cells can tolerate certain kinds of errors, whereas other defects lead to cancer. Myers aims to understand how such networks generate robustness (error tolerance) and adaptability in cells.

Jacek Skowronski studies how the AIDS virus exerts control over protein sorting and signaling machineries in human cells. He has recently identified several human proteins that associate with the AIDS viral proteins Nef and Vpr/Vpx. Skowronski's group is now working to verify these interactions and to determine their relevance to previously known roles of the Nef and Vpr/Vpx accessory factors in AIDS pathogenesis.

STRUCTURAL BIOLOGY OF NUCLEIC ACID REGULATORY PROCESSES

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

We study the molecular basis of cell regulatory processes by using the tools of structural biology and biochemistry to examine proteins and protein complexes associated with these processes. X-ray crystallography enables us to obtain the three-dimensional structures of individual proteins and their interactions with other molecules. Biochemistry and molecular biology allow us to study properties that can be correlated with protein structure and function. Our efforts largely center on protein machines involved in nucleic acid regulatory processes.

DNA Translocation in a Replicative Hexameric Helicase

E.J. Enemark

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

Papillomaviruses are tumor viruses that cause benign and cancerous lesions in their host. Replication of papillomavirus DNA within a host cell requires the viral E1 protein, a multifunctional protein. E1 initially participates in recognizing a specific replication origin DNA sequence as a dimer with E2, another viral protein. Subsequently, further E1 molecules are assembled at the replication origin until two hexamers are established. These hexamers are the active helicases that operate bidirectionally in the replication of the viral DNA. To unwind DNA, helicases must separate the two strands while moving along, or translocating, on the DNA. On the basis of structures of the DNA-binding domain of E1 bound to DNA that we determined a few years ago in collaboration with Arne Stenlund's lab here at CSHL, we suggested a mechanism for DNA strand separation. However, the mechanism that couples the ATP cycle to DNA translocation has been unclear. The E1 hexameric helicase adopts a ring shape with a prominent central channel that has been presumed to encircle substrate DNA during the unwinding process, but the atomic details of this binding have been uncertain, including whether the ring encircles one or both strands of DNA during unwinding.

Our crystal structure of the E1 hexameric helicase bound to single-stranded DNA (Fig. 1) demonstrates that only one strand of DNA passes through the central channel and reveals the details of DNA binding (Fig. 2). The β hairpins (DNA-binding hairpins) of each subunit sequentially track the sugar-phosphate backbone of the DNA in a one nucleotide per subunit increment. This configuration resembles a spiral staircase (Fig. 2).

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

A straightforward "coordinated escort" DNA-translocation mechanism is inferred from the staircased DNA binding and its correlation with the configuration at the ATP-binding sites. Each DNA-binding hairpin maintains continuous contact with one unique nucleotide of single-stranded DNA and migrates downward via ATP hydrolysis and subse-

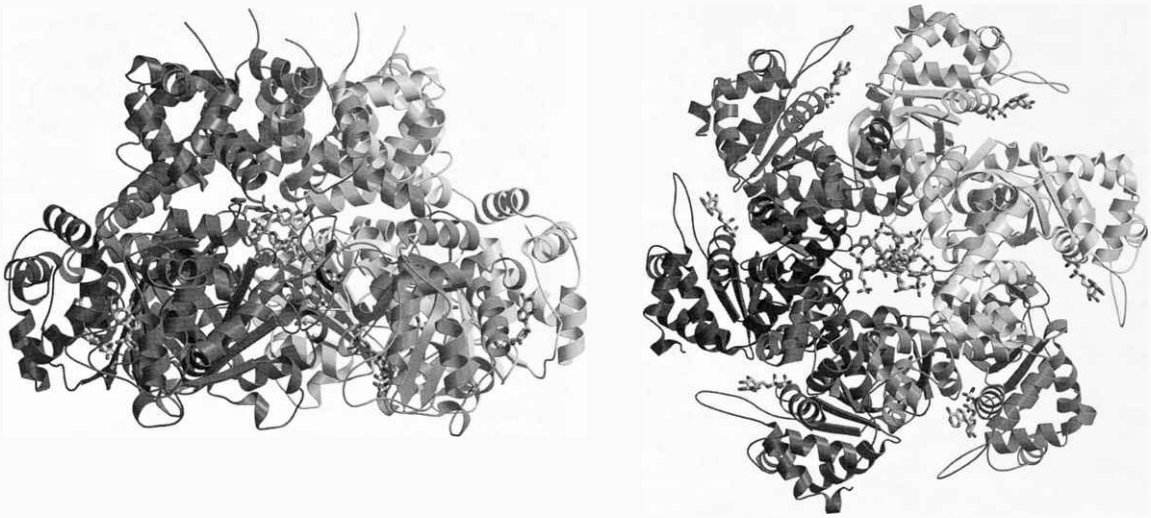


FIGURE 1 Views of the E1 hexamer parallel and perpendicular to the central channel with individual subunits. Single-stranded DNA is bound discretely within the channel, and nucleotides are present at the subunit interfaces.

quent ADP release at the subunit interfaces. ATP hydrolysis occurs between subunits located toward the top of the staircase, whereas ADP release occurs between subunits located toward the bottom of the staircase. The hairpin at the bottom of the staircase releases its associated single-stranded DNA phosphate to conclude its voyage through the hexameric channel. Upon binding a new ATP molecule, this subunit moves to the top of the staircase to pick up the next available single-stranded DNA phosphate, initiating its escorted journey through the channel and repeating the process. For one full cycle of the hexamer, each subunit hydrolyzes one ATP molecule, releases one ADP molecule, and translocates one nucleotide of DNA through the interior channel. A full cycle therefore translocates six nucleotides with associated hydrolysis of six ATPs and release of six ADPs.

Mechanisms of RNAi

N. Tolia, T. Schalch, C. Faehnle, C. Aksoy, D. Chitwood,
S. Smith [in collaboration with G.J. Hannon and
R.A. Martienssen, Cold Spring Harbor Laboratory;
C. Mello, University of Massachusetts]

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

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

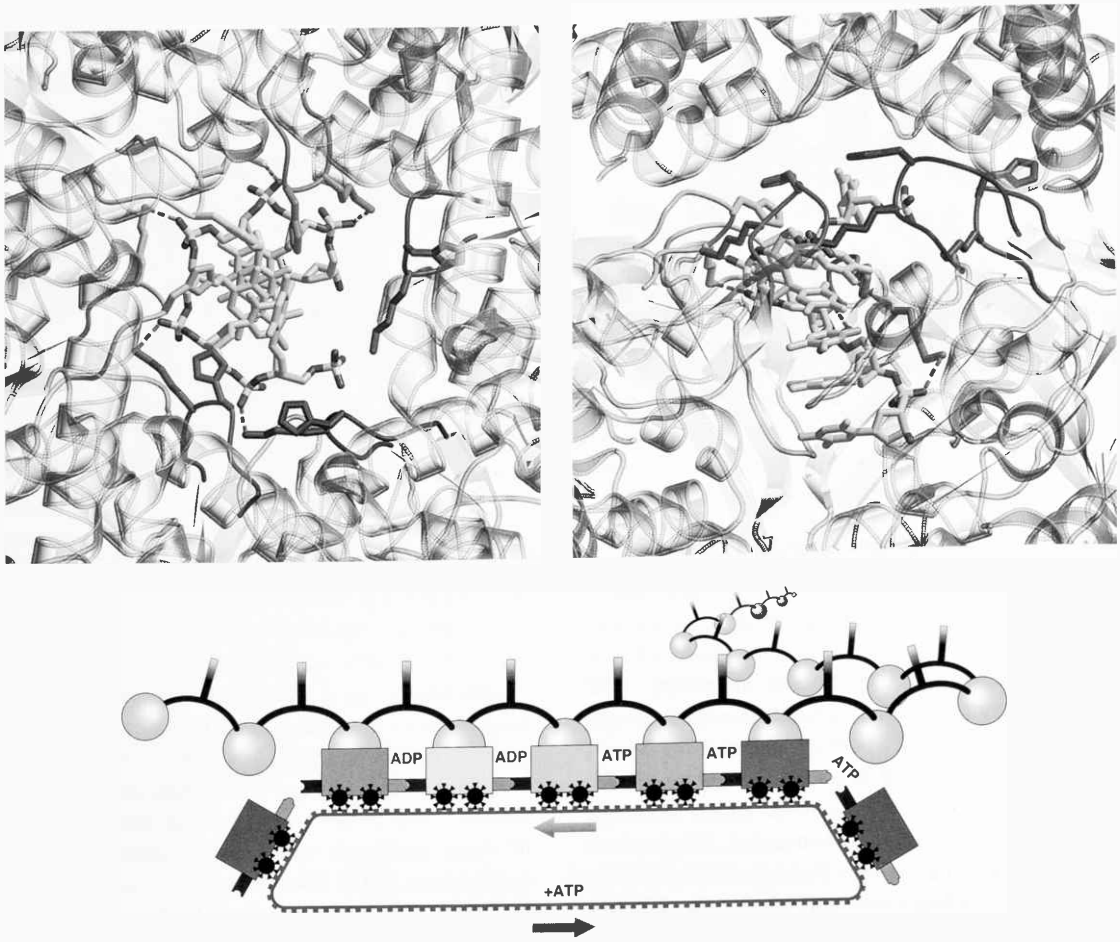


FIGURE 2 Details of DNA coordination viewed parallel (*top left*) and perpendicular (*top right*) to the hexamer channel and a cartoon (*bottom*) depicting the "coordinated escort" mechanism for DNA translocation.

Argonaute protein, features that serve to define the RISC complex.

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

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

We have been examining several Argonaute family members and found that the sole Argonaute protein in *Schizosaccaromyces pombe* is an active slicer. Importantly, together with Rob Martienssen's group,

we showed that slicing is required for TGS in *S. pombe*.

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

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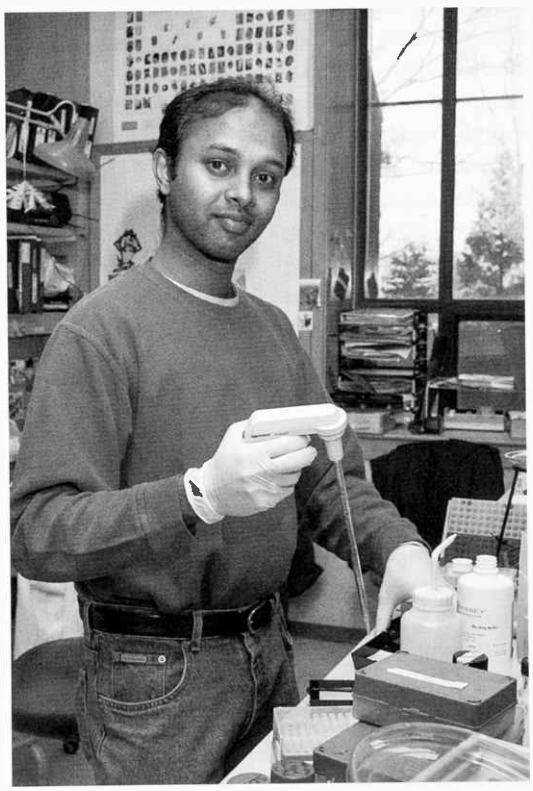

Prakash Kumar

RNA SPLICING

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MECHANISMS OF CONSTITUTIVE AND ALTERNATIVE PRE-mRNA SPLICING

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

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

MECHANISMS OF EXON RECOGNITION IN THE SPINAL MUSCULAR ATROPHY GENES *SMN1* AND *SMN2*

Spinal muscular atrophy (SMA) is a neurodegenerative genetic disorder caused by the deletion or mutation of the survival-of-motor-neuron gene, *SMN1*. An *SMN1* paralog, *SMN2*, differs by a C to T transition in exon 7 that causes substantial skipping of this exon, such that *SMN2* expresses only low levels of functional protein. A better understanding of *SMN* splicing mechanisms should facilitate the development of drugs that increase SMN protein levels by improving *SMN2* exon 7 inclusion. In addition, exonic mutations that cause defective splicing give rise to many genetic diseases, and the *SMN1/2* system is a useful paradigm for understanding exon identity determinants and alternative splicing mechanisms. Skipping of *SMN2* exon 7 was previously attributed either to the loss of an SF2/ASF-dependent exonic splicing enhancer (ESE) or to the creation of an hnRNP A/B-dependent exonic splicing silencer (ESS), as a result of the C to T transition. We extensively tested the enhancer-loss and silencer-gain models by mutagenesis, RNA interference, overexpression, RNA splicing, and RNA-protein interaction experiments. Our results supported the enhancer-loss model but also demonstrate that hnRNP A/B proteins antagonize SF2/ASF-dependent ESE activity and promote exon 7 skipping by a mechanism that is independent of the C to T transition and is therefore common to both *SMN1* and *SMN2*. These findings explain the basis of defective *SMN2* splicing, illustrate the fine balance between positive and negative determinants of exon identity and alternative splicing, and underscore the importance of antagonistic splicing factors and exonic elements in a disease context.

We also collaborated with Dr. Brage Andresen (Aarhus University Hospital) to help characterize defective splicing due to a "missense" mutation in exon 5 of the *MCAD* gene, leading to medium-chain acyl-CoA dehydrogenase deficiency. The relevant region of this exon is remarkably similar to the above-mentioned region of *SMN2* exon 7. The *MCAD* missense mutation likewise inactivates a presumptive SF2/ASF-dependent ESE, resulting in exon skipping. However, there is also a juxtaposed ESS in exon 5 that is inactivated by a synonymous A to C single-nucleotide polymorphism (SNP). The C SNP

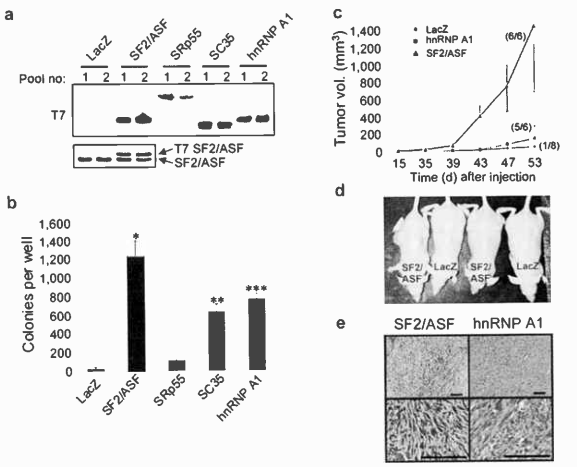

FIGURE 1 SF2/ASF transforms immortal cells and is tumorigenic in nude mice. (*a*) Total proteins from duplicate pools of NIH-3T3 cells stably transduced with retroviruses expressing the T7-tagged splicing factors SF2/ASF, SRp55, SC35, or hnRNP A1, or LacZ as a control, were analyzed by western blotting with anti-T7. The first four samples were also analyzed with anti-SF2/ASF (below) to compare the expression of endogenous and transduced SF2/ASF. (*b*) Quantification of soft-agar colony formation by the stably transduced cell lines. The mean ± S.D. for each pair of pooled lines is shown. *P* values in pairwise comparisons to the LacZ control: $*P = 4 \times 10^{-8}$; $**P = 10^{-7}$; $***P = 7 \times 10^{-8}$. (c) Tumor growth curve in mice injected with 2×10^6 cells from the indicated NIH-3T3 pooled lines. The number of tumors formed per number of injections is shown in parentheses. SRp55 and SC35 cell lines (six each) did not form tumors during the same time course (data not shown). Error bars: S.D. (*d*) Representative mice injected with LacZ-expressing contol cells or with SF2/ASF-overexpressing cells. (*e*) Light micrographs of formalin-fixed, paraffin-embedded tissue sections from tumors derived from NIH-3T3 cells overexpressing SF2/ASF or hnRNP A1, stained with hematoxylin and eosin. Bars, 100 μm.

has no effect on splicing by itself, but because of the antagonism between the ESE and the ESS, it suppresses the splicing defect due to the ESE-inactivating mutation. This study demonstrated that SNPs can have strong context-dependent effects on posttranscriptional gene expression, and thus the effects of clinically relevant mutations that potentially affect splicing should be evaluated in the context of the relevant haplotype.

ANTISENSE-CORRECTION OF DISEASE-ASSOCIATED SPLICING DEFECTS

We are exploring strategies to increase the extent of exon 7 inclusion during splicing of *SMN2* transcripts for eventual therapeutic use in SMA, a genetic neuromuscular disease. Antisense oligonucleotides (ASOs) that target an exon or its flanking splice sites typically promote exon skipping. However, when we systematically tested a large number of ASOs with a 2′-*O*-methoxyethyl ribose (MOE) backbone that hybridize to different positions of *SMN2* exon 7, we identified several that promote greater exon inclusion, others that promote exon skipping, and still others that were neutral. This approach provides positional information about presumptive exonic elements or secondary structures with positive or negative effects on exon inclusion. The ASOs are effective not only in cell-free splicing assays, but also when transfected into cultured cells, where they affect splicing of endogenous SMN transcripts. The ASOs that promote exon 7 inclusion increase full-length SMN protein levels, demonstrating that they do not interfere with mRNA export or translation, despite hybridizing to an exon. Some of the ASOs we identified are sufficiently active for us to proceed now with experiments in available SMA mouse models. This work was done in collaboration with Isis Pharmaceuticals.

We also pursued antisense modulation of splicing in the context of apolipoprotein B (APOB) expression. The *APOB* gene comprises 29 constitutively spliced exons. APOB protein, which is an integral part of various lipoprotein particles, exists as two natural isoforms: the full-length APOB100, secreted by the liver, and the carboxy-terminally truncated APOB48, secreted by the intestine. APOB48 is generated by cytidine-to-uridine mRNA editing, which introduces a stop codon in exon 26. Down-regulation of APOB100 is a potential therapy to lower circulating low-density lipoprotein (LDL) and cholesterol levels, whereas down-regulation of APOB48 gives unwanted side effects. We investigated the ability of 2′-*O*-methylribose ASOs to induce the skipping of exon 27 in endogenous APOB mRNA in HepG2 cells. These ASOs were complementary to the 5′ and 3′ splice sites of exon 27, the branchpoint sequence (BPS) of intron 26, or several predicted splicing enhancers within exon 27. ASOs targeting either the 5′ or 3′ splice site, in combination with the BPS, were the most effective. The mRNA skipping exon 27 was translated into a truncated isoform, dubbed APOB87SKIP27. The induction of APOB87SKIP27 expression in vivo is expected to reduce LDL and cholesterol levels, by analogy to patients with hypobetalipoproteinemia. As intestinal APOB mRNA editing and APOB48 expression rely on sequences within exon 26, exon 27 skipping should not affect APOB48 expression, in contrast to other methods of down-regulating APOB100 expression, which also down-regulate APOB48.

EXONIC AND INTRONIC SPLICING SIGNALS

Numerous disease-associated point mutations exert their effects by disrupting the activity of ESEs. We previously derived position weight matrices to predict putative ESEs specific for four human SR proteins. The score matrices are part of ESEfinder, an online resource to identify ESEs in query sequences. In collaboration with Michael Zhang here at CSHL, we carried out a refined functional SELEX screen for motifs that can act as ESEs in response to the human SR protein SF2/ASF. We derived an increased specificity score matrix that incorporates information from both of our SF2/ASF-specific matrices and that accurately predicts the exon-skipping phenotypes of deleterious point mutations.

We also collaborated with Ravi Sachidanandam here at CSHL to collect and classify more than 500,000 pairs of splice sites from five different species. We used these large genomic data sets to examine the phylogenetic conservation of U2-dependent and U12-dependent splice sites and associated sequence features and to draw mechanistic and evolutionary inferences. The data sets and analysis tools are also very useful for examining dependencies between individual positions of the splice sites and the frequencies of occurrence of actual wild-type or mutant splice sites in the genome, both of which help us to study the mechanisms and fidelity of splice site selection.

These ESE and splice site informatics tools have been very valuable for our collaborative studies with clinical geneticists. For example, we collaborated with Katharina Wimmer (Universitat Wien) to help analyze 36 *NF1* splicing mutations from neurofibromatosis type 1 patients. One goal of this study was to derive predictive rules for the precise outcome of mutations that disrupt 5′ splice sites. In another collaboration with Dr. Brage Andresen (Aarhus University Hospital) involving a 5′ splice site mutation in the *SBCAD* gene resulting in short/branched-chain acyl-CoA dehydrogenase deficiency, we addressed why a change from A to G at position +3 of a 5′ splice site can lead to aberrant splicing, even though both nucleotides match the degenerate 5′ splice site consensus.

A SPLICING-FACTOR ONCOGENE

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

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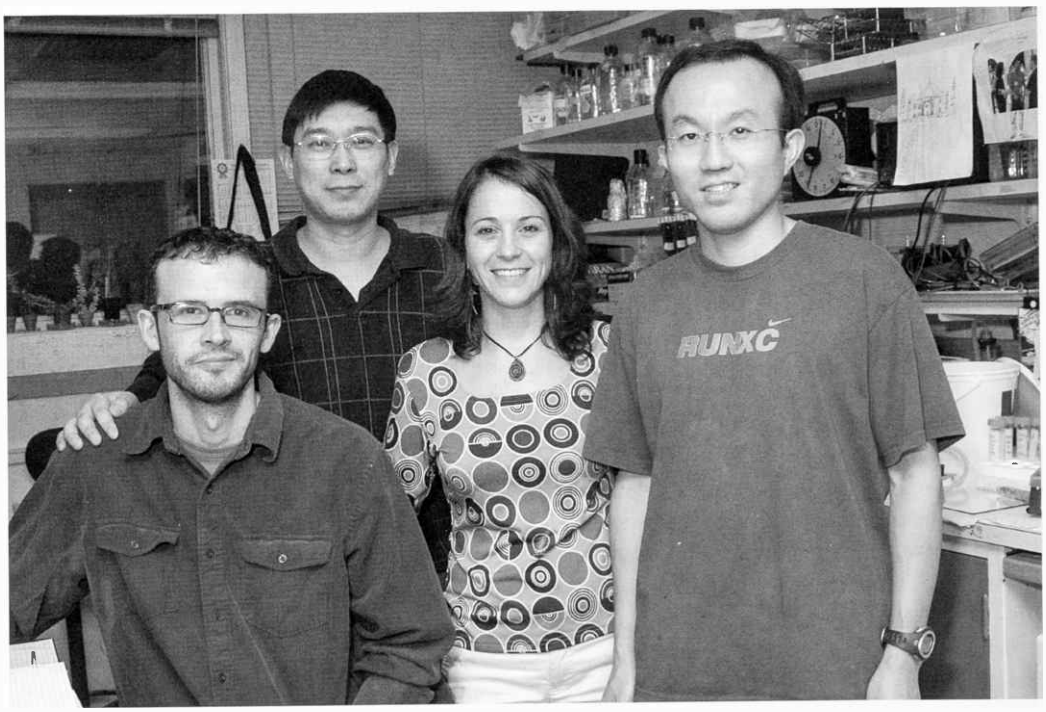

Francesc Roca, Yimin Hua, Lisa Manche, and Zuo Zhang

PROTEOMICS

M. Myers R. Bish L.L. Schmidt
O. Fregoso C. van der Meijden
D. Perkowski

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

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

Optimization of Protein Identification

D. Perkowski, L.L. Schmidt

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

As part of the optimization, we have found that nanoscale chromatography gives the best sensitivity. Using conventional reversed-phase chromatography media (packed beds of functionalized beads), we have been fabricating our own nanoscale (75-μm internal diameter) columns that operate at flow rates of 200–500 nl/min. We have switched over from large-bead media (>3 μm) to 1.8-μm media. The 1.8-μm media significantly increases the resolution of the chromatography and results in an approximately twofold increase in the number of proteins identified per run. A further increase in the number of identified proteins can be obtained by performing two rounds of chromatography. Traditionally, a prefractionation is performed using strong cation exchange (SCX) chromatography, which is then coupled to a reversed-phase separation; this multidimensional approach is frequently referred to as multidimensional protein identification technology (MudPIT). Although extremely powerful, this approach has several drawbacks for proteomic samples including the degradation of the reversed-phase separation by the salts required for the SCX chromatography and the trypsinization of the sample tends to produce peptides with identical behavior on SCX columns. Therefore, we have explored the coupling of two rounds of reversed-phase chromatography. Importantly, we have found that altering the pH of the separation dramatically alters the behavior of peptides on reversed-phase columns. This allows us to couple a reversed-phase column developed at pH 10 with a reversed-phase column developed at pH 2.5. Interestingly, extremely acidic peptides, such as phosphorylated peptides, are enriched in the early fractions of the high-pH separation, allowing for a simple, robust enrichment of phosphorylated peptides.

"Small World" Proteomics

R. Bish, C. van der Meijden [in collaboration with R. Sachidanandam, Cold Spring Harbor Laboratory]

We have modeled the data from several high-throughput protein interaction screens as a network. We have chosen to model these data as a network because it is one of the only ways to make sense of these large and complicated data sets. In this model, each protein is treated as a node and the interactions are treated as links between the nodes. In this way, the yeast protein network ends up looking very similar to the network of computers that make up the World Wide Web or the network of human social interactions that make this a "small world." On the basis of the network properties of the yeast protein network, we have been able to classify the yeast network as a scale-free network, in

which only a fraction of the proteins are responsible for making the lion's share of the connections. One prediction from the network model is that these highly connected proteins are essential for yeast viability. In fact, we find that this is the case, as essential proteins are highly enriched in the pool of highly connected proteins. Importantly, not all highly connected genes are essential, and we are focusing on trying to understand the differences between these proteins and those that are essential.

The overall goal of this study is to understand how these protein networks provide adaptability to genetic alterations, such as loss of a node (gene) that occurs during tumorigenesis. The large number of essential genes, 15–20% depending on the organism, suggests that the network is very intolerant of gene loss. Interestingly, we have found that the yeast nonessential and essential genes differ in their chromosomal distributions, indicative of a selective pressure excluding essential genes from some areas and enriching them in others. In contrast to normal proteome networks, the cancer network is expected to be robust to gene loss, largely because genomic instability is one of the hallmarks of human cancer.

We were hoping that our analysis of the yeast proteome network would reveal properties of mammalian networks and perhaps even how these networks become reprogrammed during tumorigenesis. One of the lessons from our work on yeast was that even this relatively small-scale network (6000 genes) was incredibly complex, suggesting that global approaches would result in few inroads. Therefore, we have focused on studying the DNA-damage response, partially due to our finding on the distribution of essential genes in yeast and partially because most tumors are initially sensitive to therapies based on DNA-damaging agents.

DNA damage elicits a signaling cascade that results in cell cycle arrest and recruitment of the DNA repair machinery to sites of DNA damage. Importantly, polyubiquitin chains have an important role in this recruitment. In fact, *BRCA1*, a gene identified from hereditary forms of breast cancer, is a critical factor for DNA repair and it functions as an ubiquitin ligase. Interestingly, *BRCA1* elaborates a specific form of polyubiquitin, K6-linked chains. However, the substrates and effectors of this chain are unclear, and we have attempted to identify potential *BRCA1* effectors by identifying proteins that are associated with K6-linked polyubiquitin chains. This resulted in the identification of a WHIP (Werner's helicase interacting protein). Importantly, WHIP binds to polyubiquitin via a previously uncharacterized polyubiquitin-binding domain. The yeast homolog of WHIP, MGS1, has previously been implicated in DNA repair, and we have found WHIP to be in a larger network of DNA-repair proteins, including Werner's helicase, ERCC1, ERCC4, and a human RuvB homolog. In addition, we also find WHIP to be associated with proteins involved in the unfolded protein response. These findings suggest that WHIP may function in both DNA-damage repair and an ubiquitin-dependent chaperone. Interestingly, RAD23 also has a dual role in DNA-damage repair and as an ubiquitin chaperone.

Small world protein networks are typically built by mapping protein::protein interactions using either the yeast two-hybrid system or by immunoprecipitation coupled to mass spectrometry (IP-MS). This has proven to be very powerful in determining protein networks in yeast, but it has not been truly transferred to mammalian systems. Typically, mammalian systems require the overexpression of an epitope-tagged target (bait) protein. The overexpression can be a source of artifacts. Using adeno-associated virus (AAV), we have been developing a strategy to epitope-tag proteins in the genome. AAV directs the site-specific integration of the epitope tag into the genome and requires about 1 kb of homologous sequence. In addition, we have developed a novel selectable marker that couples the marker to the translation of the targeted protein, which results in at least a sixfold enrichment of targeted alleles over transcription-coupled (IRES)-based strategies.

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CELL SIGNALING IN HIV PATHOGENESIS

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Our interest lies in understanding the molecular mechanisms that underlie the pathogenesis of AIDS and, in particular, understanding the functional consequences of interactions between viral proteins and the regulatory machineries in the infected cells. The main focus of our research is to understand the functions of accessory proteins Nef and Vpr of human and simian immunodeficiency viruses (HIV and SIV). These proteins are important determinants of virulence. We directed a major effort toward the identification of mechanisms and downstream effectors that mediate the effects of Nef and Vpr in the infected cells, and our experiments have been concentrated in two main areas. First, we have focused heavily on the isolation and identification of cellular proteins that mediate the effects of Nef on signal transduction and endocytic machineries. This has led to the purification and microsequencing of several cellular factors that associate with Nef in T lymphocytes. Importantly, we found that Nef targets a critical molecular switch that regulates Rac GTPases downstream from chemokine- and antigen-initiated signaling pathways. This interaction enables Nef to influence multiple aspects of T-cell function and thus provides an important mechanism by which Nef impacts pathogenesis by primate lentiviruses. During the last year, we continued our studies of Nef interaction with various elements of the Rac-signaling pathways. We also continued to study novel interactions of Nef with other host-cell proteins identified by mass spectroscopic analyses and to evaluate their potential roles as downstream effectors of Nef. Second, we continued experiments aimed at purifying and identifying downstream effectors of lentiviral Vpr/Vpx accessory proteins. These experiments have led to the identification of several novel cellular proteins that tightly associate with Vpr in monocytes. Among them are E3 ubiquitin ligase complexes that regulate cell cycle progression and the repair of damaged DNA. The observation that Vpr tightly associates with E3 ubiquitin ligase components is interesting because HIV replication has been known to be restricted by protein ubiquitination. Current studies aim to verify these novel interactions and to address their involvement in the lentiviral life cycle and relevance to Vpr function. Here, we describe in more detail our studies of lentival Vpr.

PURIFICATION AND IDENTIFICATION BY MASS SPECTROSCOPY OF NOVEL EFFECTORS OF LENTIVIRAL VPR PROTEINS

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

To facilitate purification of the associated proteins, HIV-1 and SIVmac Vpr proteins were tagged at their amino termini with FLAG and hemagglutinin (HA) epitopes in tandem and expressed stably in U937 monocytes by retroviral transduction, or transiently in human embryonic kidney 293 (HEK 293) cells by calcium phosphate coprecipitation. Next, Vpr and their associated proteins were purified from detergent extracts by sequential immunoprecipitations with anti-HA and then anti-FLAG epitope antibodies, followed

each time by elution with the respective peptide epitope. The immunoprecipitates were analyzed by multidimensional protein identification technology (MudPIT), which is a combination of chromatographic and mass spectrometric procedures that allows unbiased and sensitive identification of proteins in complex mixtures. The MudPIT experiments were carried out in collaboration with Drs. Michael Washburn and Laurence Florens of Stowers Institute for Medical Research (Kansas City, Missouri). This led to the identification of several common polypeptides associated with Vpr proteins.

HIV-1 AND SIV Vpr PROTEINS BIND A COMMON SET OF POLYPEPTIDES

Significantly, three relatively abundant polypeptides, DDB1, DDA1, and VprBP, that were specifically associated with both HIV-1 and SIVmac Vpr proteins in both U937 and HEK 293T cells, and absent in purifications from negative control cells, were thus identified. Besides these proteins, several additional polypeptides, including those involved in chromatin remodeling, such as histone acetyltransferases, in transport across the nuclear membrane, such as karyopherins, or in repair of damaged DNA, were found associated preferentially and abundantly with HIV-1 Vpr, but only in HEK 293T cells. Some of such polypeptides, for example, adenine nucleotide translocator and karyopherins, have been implicated as important for Vpr function by previous studies. Others, such as histone acetyltransferases, are potentially relevant to known transcriptional effects of HIV-1 Vpr. Of particular interest was that among all of the polypeptides we identified, only DDB1, DDA1, and VprBP were found in complexes with Vpr proteins from both HIV-1 and SIVmac, which are two distantly related primate lentiviruses. Based on normalized spectral counts in HEK 293T cells, DDB1, DDA1, and VprBP were recovered from the affinity purifications at relative abundances similar to the epitope-tagged Vpr levels, suggesting the formation of a stochiometric quaternary complex. The conservation of these interactions suggested that they are important mediators of a common Vpr function. Therefore, we focused our studies on these proteins.

Vpr TARGETS A NOVEL Cul4 E3 UBIQUITIN LIGASE-LINKED COMPLEX

The finding that Vpr binds DDB1, VprBP, and DDA1 linked Vpr to Cullin-4 RING E3 (Cul4 E3) ubiquitin ligase complexes, as DDB1 is an obligatory subunit of Cul4 E3 ligases. These enzymes regulate DNA repair and replication through ubiquitination of key substrates in these processes. VprBP, a known HIV-1 Vpr-binding protein, and DDA1 have been found recently to bind DDB; however, their normal functions remain unknown. Therefore, to gain further insights into Vpr interactions with these polypeptides and their relation to Cul4 E3 ligases, we initiated a biochemical characterization of Vpr association with VprBP, DDB1, and DDA1. The observations that VprBP and DDA1, as well as DDB1, all copurified with Vpr and that VprBP and DDA1 can bind DDB1 suggested that they form a ternary complex, which is then targeted by Vpr. To address this possibility, protein complexes were immunopurified from HEK 293T cells expressing individual FLAG epitope-tagged VprBP, DDA1, DDB1, or HIV-1 Vpr. Immunoblot analysis confirmed that each protein was capable of associating with each other. Furthermore, analysis of the protein complexes purified via their VprBP or HIV-1 Vpr subunits by sedimentation in glycerol density gradients revealed that DDB1, DDA1, and VprBP form a ternary complex, which Vpr then binds.

The notion that Vpr proteins target a Cul4-based E3 ubiquitin ligase is interesting since previous evidence indicated that replication of primate lentiviruses is restricted to some extent by protein ubiquitination. Current studies address how the interaction between Vpr and Cul4 E3 ligase modulates the HIV life cycle and the consequences of this interaction on the infected cell.

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TRANSCRIPTIONAL CONTROL AND THE UBIQUITIN-PROTEASOME SYSTEM

W.P. Tansey A. Chakraborty, G. Collins, A. Daulny, F. Geng, D. Khalil, J. Kurland, A. Leung, A. Lowe, S. Salghetti, D. Simpson, L. Wang

The maintenance of normal cellular homeostasis requires that cells synthesize proteins when they are needed and destroy them when their function is no longer appropriate. These two "bookends" that define the life of any protein are, respectively, transcriptional regulation and ubiquitin (Ub)-mediated proteolysis. Despite the apparent dissimilarity of these processes, a growing body of evidence suggests that components of the Ub-proteasome system are intimately involved in regulating gene activity. Work in our laboratory takes advantage of the unexpected connection between the transcription and Ub-proteasome systems to achieve two objectives: To gain new insight into the actions of the oncoprotein transcription factor Myc and to probe the depth with which the factors normally associated with protein destruction are involved in regulating gene activity.

REGULATION OF MYC BY UBIQUITIN-MEDIATED PROTEOLYSIS

The c-*myc* oncogene encodes a basic helix-loop-helix leucine-zipper transcription factor that can act as both a transcriptional activator and a repressor. First identified as a cellular counterpart of the transforming gene from the avian myelocytomatosis virus MC29, Myc has been shown to have an extensive role in cancer: It can collaborate with other oncogenes to induce cellular transformation, and aberrant Myc expression is observed in many human malignancies, including adenocarcinoma, colon, breast, and lung cancer, as well as a variety of leukemias and lymphomas. Indeed, it has been estimated that one third of all human cancers display deregulated Myc activity.

The majority of evidence indicates that Myc exerts is biological effects by controlling transcription. Activation of Myc target genes depends on the association of Myc with its partner protein, Max, and is mediated via the actions of an amino-terminal transcriptional activation domain (TAD) that interacts with components of the transcriptional machinery. A large number (~1700) of Myc target genes have been identified, which include an impressive assortment of cell cycle regulators, metabolic enzymes, and components of the translational machinery. The ability of Myc to regulate so many genes important for cell proliferation and growth undoubtedly underlies its ability to promote ectopic S-phase entry, apoptosis, and cellular transformation.

Because of its potent growth-promoting properties, cells have evolved a number of mechanisms to limit Myc accumulation and activity. One of the most prominent of these mechanisms is Ub-mediated proteolysis, which typically destroys Myc within minutes of its synthesis. The rapid and controlled destruction of Myc keeps Myc levels low and has a major role in restraining Myc's function, a notion supported by the fact that tumor-derived mutations within Myc block its rapid destruction and are aggressively oncogenic. Our lab is interested in the mechanisms and consequences of Myc destruction by the Ub-proteasome system. We have defined the elements in Myc that control its stability, identified molecules that regulate Myc destruction, and investigated the consequences of aberrant Myc destruction to cellular growth control.

These studies have revealed that Myc destruction is important to the normal control of Myc function and, in fact, is intimately connected to Myc's activity as a transcriptional regulator: Not only is the transcriptional activation domain of Myc the primary element that signals Myc ubiquitylation, but a Ub-ligase that signals Myc destruction (SCF^{Skp2}) is also a potent stimulator of Myc's transcriptional activity. The intimate relationship between Myc destruction and function is typical of many transcriptional regulators (see below) and provides us with a unique opportunity to learn more about how Myc protein functions. Our thesis is that if activity and destruction are linked, then

we should be able to use one of these activities to learn about the other. This point is particularly relevant to Myc, which is a challenging protein to study in terms of transcriptional regulation (Myc typically activates or represses genes only a fewfold), but is robustly destroyed by the Ub-proteasome system in most settings.

Our current research on Myc is focused on exploiting the connection between activity and turnover to learn more about how Myc regulates gene expression programs relevant to oncogenic transformation. Much of our work is focused on the highly conserved elements within Myc known as "Myc boxes" (Mb). The first two Myc boxes—MbI and MbII—are within the transcriptional activation domain of Myc and have important roles in regulating Myc stability: MbI is the hot spot for tumor-derived mutations that stabilize Myc, whereas MbII is a major site of interaction of Myc with SCF^{Skp2}. MbI has also received considerable attention because other groups have found that it is the site of interaction of Myc with the SCF^{Fbw7} Ub-ligase. The ubiquitylation of Myc by SCF^{Fbw7}, in particular, appears to be a highly regulated process that is controlled by growth-factor-dependent phosphorylation within MbI. Specifically, phosphorylation at serine 62 (S62), which is signaled via the Ras/Raf/ERK pathway, protects Myc from proteolysis, but at the same time is required for phosphorylation of Myc at threonine 58 (T58) by GSK3β. Once T58 is phosphorylated, dephosphorylation at S62 occurs, creating a phosphodegron that is recognized by SCF^{Fbw7}. Thus, interdependent phosphorylation events at T58 and S62 are proposed to function, via SCF^{Fbw7}, as a "rheostat" that controls the metabolic stability of the Myc protein.

Despite the intensive work on Myc regulation by SCF^{Fbw7}, we have failed to find any evidence that this Ub-ligase complex is responsible for the turnover of the majority of Myc protein. Interestingly, the regulation of MbI by these phosphorylation events raises the distinct possibility that destruction of Myc by the SCF^{Fbw7} ligase may be under cell cycle control. If this were the case, then we might expect that subtle SCF^{Fbw7}-dependent changes in Myc turnover that occur during the cell cycle may not be obvious from analysis of asynchronous cultures. For this reason, we sought to develop a protocol that would allow us to take a comprehensive look at the influence of the cell cycle on Myc synthesis, location, and stability.

The recent development of laser-scanning cytometry (LSC) allows such cell cycle analyses to be performed in unperturbed, asynchronous, culture systems. The LSC, which is a technical merger between fluorescence microscopy and flow cytometry, allows for whole-cell quantification of fluorophores targeted to DNA, RNA, or protein. In this way, levels (and localization) of a particular RNA or protein can be measured in individual cells and expressed relative to the particular cell cycle stage, as determined by simultaneous analysis of DNA content. By compiling data from thousands of cells in this way, highly quantitative cell cycle analysis can be performed without any disruption to normal cellular physiology.

We have used LSC, together with protein synthesis inhibition and small interfering RNA (siRNA)-mediated gene knockdown of Fbw7, to ask whether Myc synthesis, localization, and stability are influenced by the cell cycle and to examine the contribution of SCF^{Fbw7} to Myc levels and stability. This analysis has found that Myc is predominantly nuclear at all phases of the cell cycle and that steady-state levels of Myc transcripts and protein are not appreciably different at different stages of the cell cycle. Importantly, we have also found that unlike for another SCF^{Fbw7} target, cyclin E, Fbw7 is not required to regulate Myc levels at any particular cell cycle stage (Fig. 1). These data support the notion that Myc synthesis and stability are constitutively regulated and reveal that SCF^{Fbw7} is not responsible for targeting a significant pool of the Myc protein for Ub-mediated destruction. We are currently investigating whether mechanisms are in place to actively protect Myc from Fbw7-dependent destruction or whether there is a smaller subpopulation of Myc (e.g., in a discrete subcellular compartment) that is subject to Fbw7-dependent turnover.

In addition to MbI, we are also interested in events that are occurring at the highly conserved MbIII region of Myc. We have recently found that MbIII is important for Myc function and destruction. MbIII was originally identified about 20 years ago but has not been studied extensively, leaving a considerable gap in our knowledge about Myc function. We have found that MbIII is important for Myc's ability to drive cellular transformation both in vitro and in vivo and that it has a crucial role in transcriptional repression by the Myc protein. Our current efforts on MbIII are centered on understanding how this element functions in transcriptional repression, identifying the cellular proteins that bind to this element to mediate its activity, and learning whether tumor-derived mutations within this element alter Myc stability and activity.

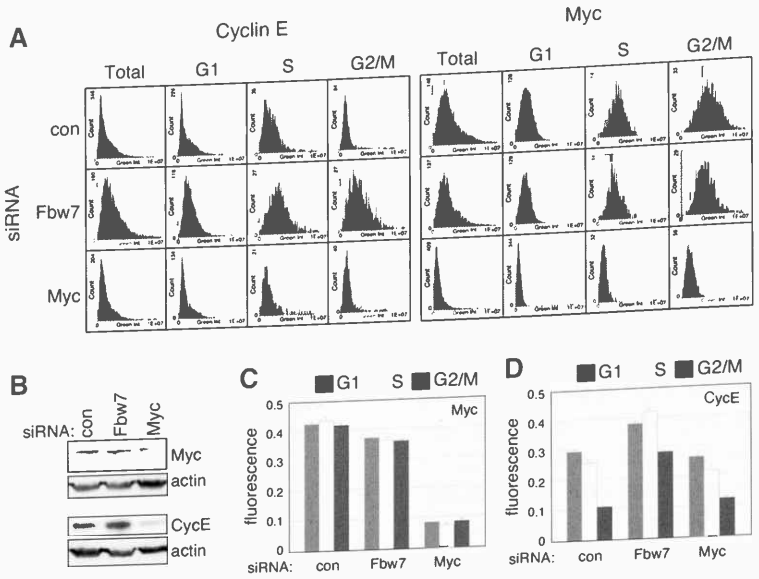

FIGURE 1 Differential regulation of Myc and cyclin E by Fbw7 during the cell cycle. (*A*) Effects of siRNA-mediated knockdown of Fbw7 on Myc and cyclin E. U2OS cells were transfected with the indicated siRNAs (control [=luciferase]; Fbw7, and Myc), and Myc and cyclin E levels were determined in each cell cycle subpopulation of cells. Protein expression histograms show that Fbw7 knockdown substantially increases cyclin E levels in S and G_2 phases (rightward shift), whereas it has no significant effect on Myc profiles. (*B*) Analysis of total steady-state levels of Myc and cyclin E following Fbw7 knockdown. Western blot analysis of cells analyzed in *A*. (*C* and *D*) Quantification of relative protein levels. Fluorescence intensities for Myc (*C*) and cyclin E (*D*) were quantified by LSC in cells binned into G_1, S, or G_2/M populations, normalized to the signal for DNA content in those cells, and expressed relative to the signal from G_1 phase.

CONTROL OF TRANSCRIPTION BY THE UBIQUITIN-PROTEASOME SYSTEM

Although transcriptional activation and Ub-mediated proteolysis are two processes that have apparently very little in common, research from a number of laboratories, including our own, has revealed that these two processes come together to control gene activity.

Our efforts in this area began with the observation that within Myc, the element that signals Myc ubiquitylation overlaps *precisely* with the domain that activates transcription. Subsequent studies have revealed that the overlap of TADs and degrons occurs in most unstable transcription factors, that the relationship between these elements is intimate, and that in some cases the ability of an activator to engage the Ub-proteasome system is essential for transcriptional activation. Together with other findings demonstrating that Ub-ligases can function as transcriptional coactivators, that some general transcription factors have Ub-ligase activity, and that components of the proteasome have a nonproteolytic role in transcriptional elongation and chromatin modifications, these observations suggest a deep and mechanistic connection between the transcription and ubiquitin systems that we are anxious to explore.

Our research in this area is divided into four key areas, each of which uses the yeast *Saccharomyces cerevisiae* as a model organism. First, we are interested in how ubiquitylation and destruction control the activity of transcriptional regulators such as Myc. For these studies, we focus on the yeast transcriptional activator Gal4. We have found that Gal4 is unstable when it activates transcription, that it is destroyed by the Dsg1 Ub-ligase, and that ubiquitylation and/or destruction of Gal4 is important for its ability to productively activate transcription. We are currently investigating whether ubiquitylation and destruction are both involved in Gal4 activity or whether Ub is the activating event that is terminated by Gal4 proteolysis. Second, we are studying the ubiquitylation of histones and its role in chromatin localization and activity. Third, we are studying the effects of ubiquitylation of RNA polymerase II on the regulation of gene expression in normal and stressed states. Finally, we

are working on understanding how the proteasome interacts with chromatin and the various functions it performs during the transcriptional process.

By combining these four approaches, we hope to not only understand the scope with which Ub and the Ub-proteasome system controls gene regulation, but also reveal the underlying mechanisms. The general "rules" that we learn from studies in this streamlined eukaryote will also hopefully help guide our studies of Myc.

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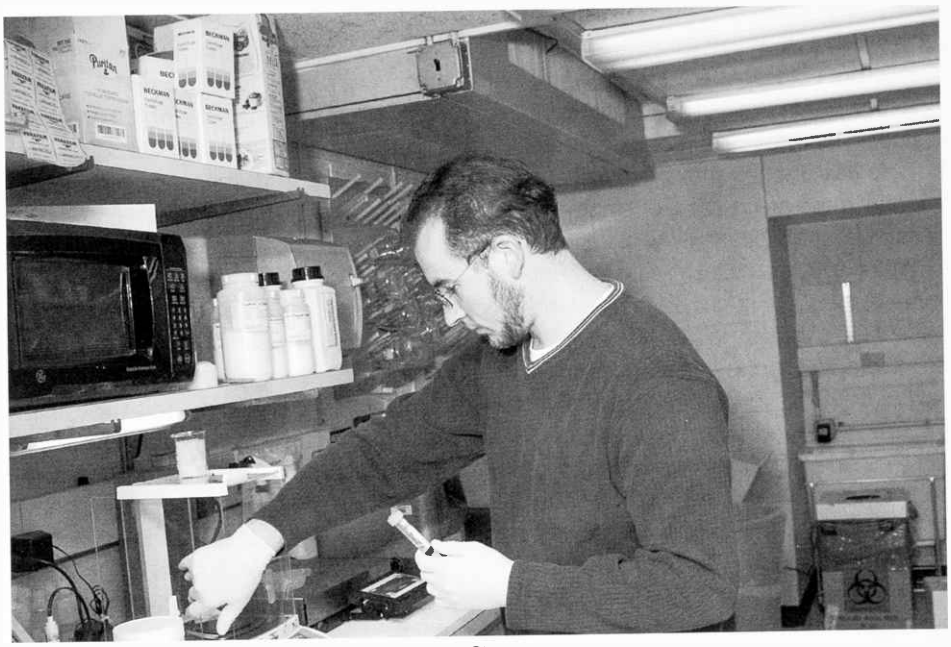

David Simpson

The discovery approximately 9 years ago of RNA interference (RNAi), a fundamental molecular process that controls gene expression in many organisms, has prompted a revolution in the biological sciences. This fundamental process has allowed the activity of nearly any gene to be manipulated at will in many biological systems including mice and humans. However, gaining these abilities has required the acquisition of a more detailed understanding of the way RNAi operates.

Greg Hannon's group continues to make many key discoveries that not only increase knowledge about RNAi as a basic and important biological process but also enable the use of RNAi ever more effectively as a tool to probe gene function. In this regard, the Hannon lab has created a genome-wide collection of RNAi-inducing tools that are being used both at the Laboratory and around the world to study a panoply of biological problems. At CSHL, the use of this library is mainly being harnessed to identify new targets for cancer therapy, an activity in which the Hannon lab is an active participant.

The mouse RNAi library is now being used by CSH Fellow Patrick Paddison to map the roles of genes in the earliest stages of mammalian development. By blocking the production of individual gene products in mouse embryonic stem cells via RNAi, Paddison aims to create a biological time line in which he can place when and in which tissues certain genes are turned on as a mouse embryo develops from its earliest stages. This research promises to reveal new insights into stem cell biology and mammalian development and should eventually provide a basis for testing stem cells in human cell replacement therapies.

Vivek Mittal studies the mechanism of blood vessel formation that is critical for tumor progression and metastasis. This year, he has shown that cells known as "blood vessel progenitors" contribute to vessel formation during the progression of small metastases into large metastases that are known to kill cancer patients. Using a type of RNAi, his group has targeted these progenitors and successfully blocked the formation of large metastases. Given the clinical importance of these progenitor cells in cancer development, the insights gained through these approaches may quickly translate into clinical applications that should ultimately lead to improved treatments for human cancer.

Scott Lowe's laboratory studies cancer gene networks and determines how genetic lesions affecting these networks contribute to tumor development and resistance to cancer therapy. This past year, Lowe and colleagues continued efforts to understand how mutations in cancer genes affect resistance to cancer therapy, and they have found a number of genetic factors that produce resistance. Lowe and his group also continued to study the process of cellular senescence, a potentially powerful mechanism for suppressing tumors. In related work, Lowe has developed an integrative approach to cancer gene discovery that combines the use of genomic information and mouse modeling to rapidly identify and characterize new cancer genes and potential therapeutic targets. These studies hold the promise to produce new insights into the genetic basis of tumor diversity between patients that can ultimately be exploited to tailor treatment strategies to individual cancer patients.

Cancer can be thought of as a disease of genetic changes—whether mutations or so-called "epigenetic" changes in gene function—and these changes are responsible for a cell developing into a tumor. To identify and characterize genes affected by mutation, Michael Wigler and Robert Lucito pioneered a technique to look to the genome of the cancer cell itself. The technique is known as representational oligonucleotide microarray analysis, or ROMA, and is based on the fact that often mutations in the genome of cancer cells can result in increased or decreased gene copy number, namely amplifications or deletions, and these changes can be used as markers for the location of oncogenes or tumor suppressor genes, respectively. Robert Lucito is using these and other leading-edge techniques to detect copy-number changes in a large set of ovarian and pancreatic cancer specimens. He and his colleagues have identified the genetic patterns of deletion and amplification in these tumor types and are currently using these alterations to identify genes involved in tumor formation.

Michael Wigler's lab uses ROMA to identify the most frequent genes mutated in breast cancer and to determine whether these genes distinguish subtypes of breast cancer. Furthermore, Wigler seeks to

understand what distinguishes breast cancer from other epithelial cancers and, in general, whether the profiles of cancers can be used to predict outcome of the disease and response to therapy. A similar program is focused on CLL (chronic lymphocytic leukemia), the most common form of adult leukemia. The Wigler lab also uses a related technology to examine the genomes of children with autism and congenital heart disease for clues into the origins of these devastating disorders of childhood.

To find more cancer genes, it is important to detect as many genomic alterations in tumor cells as possible. To this end, Scott Powers's lab continues their work on DNA copy-number analysis and forged new collaborations in 2006 with CSHL scientists Robert Martienssen and Robert Lucito to apply their methods for detecting promoter methylation, another type of genomic alteration, to the problem of cancer gene identification. The Powers lab also works with Scott Lowe's laboratory on hepatocellular carcinoma and is investigating the application of a highly effective, flexible mouse model for testing cancer genes that are operative in the liver.

Genomes are by no means fixed, immutable entities. Through genetic recombination, sections of chromosomal DNA can be lost, amplified, or rearranged, resulting in a wide variety of genomic differences among species, among individuals of a species, and within the cells and tissues of an individual organism. CSH Fellow Ira Hall is using ROMA to explore the dynamics of genome rearrangement that may underlie a variety of normal and pathological processes in biology. He is currently using mouse models to determine the genomic changes that gave rise to different strains of inbred mice, an approach that among other factors may reveal unstable or hypervariable regions of the mouse genome. As a basis for future research, Hall will also examine genome stability during normal biological processes such as tissue development ("mitotic" genome stability), reproduction ("meiotic" genome stability), and aging.

In some cases, the fusion of human cells is a normal process that leads, for instance, to the formation of muscle and bone. Seemingly innocuous infections by common viruses can also cause the cells in our bodies to fuse. Such fused cells are widely considered to be harmless because they are generally believed to die and be cleared from the body without consequences for our health. This view of cells fused by viruses as being harmless may need to be revised. A recent study of cultured cells by Yuri Lazebnik and his colleagues has revealed that cell fusion triggered by common viral infections may be a significant factor in the development of human cancer. Lazebnik's group is currently testing this possibility in animal models. Non-CSHL scientists are developing fusogenic viruses as gene-delivery tools for human gene therapy. Lazebnik's research indicates that because these viruses might cause cancer, their use for therapeutic purposes should be carefully evaluated.

Alea Mills is using a variety of mouse models that enable her to investigate the link between cancer and aging. One project involves p63, a protein similar to the well-known tumor suppressor protein p53. Work from the Mills lab has demonstrated that loss of the *p63* gene accelerates aging in mice. A second project in the Mills lab that links aging and cancer involves the *1p36* region. The Mills group is using functional approaches to try to identify the long sought after tumor suppressor that maps to this region. They have identified a genomic segment that harbors a gene regulating senescence, a process that occurs during normal aging. Remarkably, loss of this region causes cancer. Mills is now using these models to understand the delicate balance between aging and cancer, work that may lead to better anticancer therapies.

THE BIOLOGY OF SMALL RNAs

G. Hannon A. Aravin, J. Brennecke, M. Carmell, K. Chang, M. Dus, K. Fejes-Toth, T. Gingeras, A. Girard, L. He, X. He, E. Hodges, I. Ibarra, C. Malone, E. Murchison, F. Rollins, C. Schlinghyde, J. Silva, D. Siolas, O. Tam

The Hannon laboratory probes the biology of small RNAs in numerous contexts. Small RNAs represent a new gene regulatory phenomenon that has gained prominence in biology both because of its extensive impacts and because of the promise that small RNAs may provide new routes to disease therapy. Overall, work in the laboratory divides among three general topics: (1) The underlying mechanisms through which small RNAs act to regulate gene expression, (2) the biological impacts of small-RNA-based regulatory systems, and (3) the use of small RNAs to devise new cancer therapies. Descriptions of selected work from the past year appear below.

This year, we were joined by Kata Fejes-Toth, Alexei Aravin, and Julius Brennecke, all most recently from The Rockefeller University. Jidong Liu left to accept a position as an Assistant Professor at Memorial Sloan-Kettering Cancer Center, and Faby Rivas became an editor at the journal *Cell*. Michelle Carmell left for a postdoctoral position in David Page's lab at the Massachusetts Institute of Technology. Oliver Tam and Fred Rollins joined our lab as graduate students from the Watson School. Ahmet Denli left for a postdoctoral position in Rusty Gage's lab at the Salk Institute, and we were joined by Emily Hodges from Karolinska.

One of the special highlights of this last year was provided by our Partners for the Future student, Catherine Schlinghyde, who placed fourth in the National Intel Science Competition. In addition, Lin He and Jose Silva received K99 Awards from the National institutes of Health, highly competitive grants designed to bridge their transition to independent faculty positions. Lab alumnus Scott Hammond received the Gertrude Elion Award from the American Association for Cancer Research, and Despina Siolas was recognized as a Brigid G. Leventhal Scholar by the Women in Cancer Research. Finally, Tom Gingeras has returned to CSHL as a visiting scientist in the lab.

piRNAs: A New Class of Small RNAs in Mammals

A. Aravin, M. Carmell, A. Girard

Small RNAs associate with Argonaute proteins and serve as sequence-specific guides for regulation of mRNA stability, productive translation, chromatin organization, and genome structure. In animals, the Argonaute superfamily segregates into two clades. The Argonaute clade acts in RNA interference (RNAi) and in microRNA (miRNA)-mediated gene regulation in partnership with 21–22-nucleotide RNAs. Members of a distinct clade of the Argonaute family, the PIWI proteins, are usually expressed specifically in germ cells and animals, with mutations in these genes showing severe defects in gametogenesis. This year, we identified the small RNA partners of PIWI proteins, the PIWI-interacting RNA or piRNAs.

piRNAs arise from discrete locations in the genome. Although PIWI proteins and their piRNA partners have yet to be assigned definitive functions, in mice, two PIWI family members have been demonstrated to have essential roles in spermatogenesis. Recently, we examined the effects of disrupting the gene encoding the third family member, MIWI2. Miwi2-deficient mice display a meiotic progression defect in early prophase of meiosis I and a marked and progressive loss of germ cells with age.

In *Drosophila*, several studies point to a role of the PIWI subfamily in transposon silencing. We have shown that several classes of mobile elements are demethylated and reactivated in mutants for two members of the PIWI subfamily, Miwi2 and Mili, suggesting a conserved role for the PIWI subfamily in transposon silencing across metazoan clades.

piRNAs Dance Flamenco: A Small-RNA-based Immune System Protects the *Drosophila* Genome

J. Brennecke, A. Aravin, M. Dus, C. Malone, C. Schlinghyde

The genomes of essentially all living organisms are loaded to different degrees with selfish genetic elements, many of which are still active and have evolved ways to multiply in their host genome. Although several reports have demonstrated or suggested that immobile remnants of these elements are beneficial to the host genome (i.e., as essential components of heterochromatin domains), the negative effects of transposing elements have led to the development of host defense mechanisms, which prevent uncontrolled propagation of transposable elements.

Studies in *Drosophila* indicate that this mechanism is active in the female and male germ line and strongly suggest that a novel small RNA pathway constitutes the heart of the host defense system. In fact, loss of this pathway has no adverse effects on host development, except fatal consequences in germ-line development, ultimately leading to sterility.

A small RNA pathway specific for silencing selfish genetic elements elegantly offers a way to silence all highly variable types of transposons with a common strategy, but a major question is how does the host cell manage to program this defense system specifically with antisense transposon small RNAs and not small RNAs derived from other cellular transcripts?

We are studying this fascinating pathway in the fruit fly *Drosophila melanogaster* using molecular biology, biochemistry, and genetics. We initiated our research by systematically describing and identifying the core components of this pathway, which are three members of the Argonaute protein family (PIWI, Aubergine, and Argonaute3) and their thousands of different associated small RNAs that we have termed piRNAs. Our results confirm specific expression of the relevant proteins in the germ line and indicate that the three proteins are loaded with distinct sets of small RNAs, providing a molecular explanation for their genetic nonredundancy.

Our data also show that intricate correlations exist between the three ribonucleoprotein (RNP) complexes, suggesting that all three RNP complexes are interdependent of each other and are directly involved in the production on small RNAs. Interestingly, the small RNAs that we identify appear to arise from discrete genomic loci, all of which are located in the heterochromatic, pericentromeric, and telomeric regions of the four chromosomes. Two of these loci have been shown genetically to act as master control loci in the control of certain types of transposable elements. This led us to propose an "immune-system"-like defense mechanism acting in the *Drosophila* germ line, which uses distinct genomic loci loaded with remnants of transposable elements as "memory" and a linked small RNA pathway, which can respond to active transposable elements analogous to "clonal expansion."

We will continue to analyze this pathway by identifying the piRNA populations in ovaries from flies mutant in key pathway components with the aim to elucidate additional details of how this pathway processes only certain types of transcripts and not others. Furthermore, we are interested in the evolution of this system and will identify piRNA populations and their genomic origins in ten different *Drosophila* species, whose genomes have recently been sequenced.

Testing the Cancer Stem Cell Hypothesis

I. Ibarra

Recent studies have identified tumor-initiating cells in both breast and brain tumors. These findings are of therapeutic significance since drugs that fail to eliminate these rare populations of cells may permit regeneration of the tumor (relapse), although the tumor mass may initially diminish. It has been hypothesized that tumor-initiating cells are stem cells that harbor mutations in their self-renewal program. Thus, we are currently using RNAi technology in combination with mammary fat pad transplantation to screen for genes that expand or deplete the mammary epithelial stem/progenitor compartment. We are also interested in cloning small RNA molecules from mammary epithelial stem cells to determine their role in stem cell identity and cell fate.

The Role of Small dsRBD Proteins in RNAi

M. Dus

I am currently investigating the role of a novel double-stranded RNA-binding protein in viral immunity. Recent studies have highlighted the importance of the RNAi pathway in mounting an immune response against RNA viruses. In particular, when *Drosophila*

Ago2, Dcr2, and R2D2 mutants are challenged with RNA viruses such as canine parvovirus and flock house virus, they die because they are unable to respond efficiently to viral infection. I am now testing whether mutants of this protein have reduced life spans when infected with RNA viruses.

Characterization of Promoter-associated Small RNAs

K. Fejes-Toth, T. Gingeras

Small RNAs participate in the regulation of several biological processes. In yeast and plants, they have been shown to regulate chromatin structure and transcription by specifically targeting heterochromatization of homologous sequences. To date, data addressing the direct connection between small RNAs and transcription in animals are still awaited. Recently, Tom Gingeras and his group at Affymetrix have described promoter-associated small RNAs in human cell lines. They have mapped small RNAs (<200 nucleotides) to the genome using high-resolution tiling arrays and have found strong signals around the promoter region of a large proportion of the genes. We have characterized these small RNAs biochemically. Using northern blotting, we detected small RNAs of discrete sizes that mapped to several promoter regions. Specific signal was observed at around 25, 35, and 55 nucleotides with multiple probes.

Nuclear/cytoplasmic fractionation showed an enhancement of the signal in the nuclear fraction as expected for a potential transcriptional regulatory function. Subsequently, the 5′ and 3′ ends were determined using terminator and β-elimination assays, respectively. Terminator is an exonuclease that needs 5′ phosphate. The northern blot signal of the small RNAs only vanished if it was kinased prior to terminator treatment, indicating a 5′-OH end. β-elimination, on the other hand, needs free 3′-hydroxyl groups and leads to a single nucleotide shift. A shift could only be observed if the sample was dephosphorylated prior to treatment, indicating a 3′-phosphate end.

The small RNAs of the determined characteristic sizes were isolated from nuclei of HepG2 cells and cloned according to the cloning protocols established in the Hannon lab but modified to account for the end structures that we had determined. The small RNA libraries that we obtained were subsequently sequenced using high-throughput sequencing with Solexa. At the present time, the sequences are being annotated and analyzed in collaboration with Ravi Sachidanandam here at CSHL.

microRNAs and Cancer

L. He, X. He

We are interested in looking at the role of miRNA in tumorigenesis. To date, more than 200 miRNAs have been described in humans. Although there is circumstantial evidence suggesting that these regulatory noncoding RNAs have a role in tumor development, it still remains largely obscure whether an miRNA can function as an oncogene. A comparison of B-cell lymphoma samples and cell lines with normal tissues reveals that substantially increased levels of the mature miRNAs from the miR17-92 locus are often found in these cancers. Notably, the gene encoding this miRNA polycistron is present in a region of DNA amplification found in human B-cell lymphomas. Enforced expression of the miR17-92 cluster cooperates with c-*myc*, accelerating tumor development in a mouse B-cell lymphoma model. Tumors derived from hematopoietic stem cells (HSCs) expressing *mir17-92* and c-*myc* are distinguished by an absence of the apoptosis that is otherwise prevalent in c-*myc*-induced lymphomas. Our studies indicate that noncoding RNAs, specifically miRNAs, can modulate tumor formation and implicate the miR17-92 cluster as a potential human oncogene.

Critical Roles for Dicer in the Female Germ Line

E. Murchison, O. Tam

To further understand the roles of RNAi in the germ line, we have embarked on a systematic study of the roles of RNase III enzymes Dicer and Drosha in the male and female germ lines of the mouse. Toward this end, we have created Cre-*loxP* conditional alleles of Dicer and Drosha in mice. These are crossed to germline-specific Cre lines to generate mice lacking these enzymes specifically in developing germ cells.

We have found that Dicer is required for completion of meiosis in oocytes. Female mice in which Dicer has been ablated specifically in oocytes shortly after birth are sterile, and they exhibit severe defects in meiotic maturation. Although Dicer $^{-/-}$ oocytes progress to the fully grown stage, they fail to mature and develop disorganized spindles and severe chromosome congression defects. Many transcripts are misregulated in Dicer $^{-/-}$ oocytes, suggesting a role for miRNAs in regulating maternal transcripts. Our results reveal a previously unknown role for Dicer in regulating meiosis.

Future studies will focus on understanding the identity and biogenesis of small RNAs in the germ line, particularly in the female. We are developing sensitive cloning protocols for the discovery and characterization of the small RNA population in oocytes. Precise knowledge of the identity of small RNAs in the female germ line will complement studies of RNase III mutant oocytes, and we hope to identify the precise small RNA pathways that control spindle formation and chromosome segregation in oocytes.

Enhancing the Response to Targeted Cancer Therapies through RNAi

F. Rollins

I am currently using RNAi to investigate cancer biology. I am specifically interested in chemotherapeutics, the drugs that are used to treat cancer. Many of the current generation of cancer drugs have very broad activity; they cause much harm to the patient, resulting in numerous side effects. Because the side effects are so severe, the scientific community is hard at work trying to develop new drugs that target specific molecules. These targets are often chosen based on previous research that identified specific genes as important to various cancer types or to processes required for cancer development and maintenance.

One of these drugs is called Tarceva, which targets a specific cellular receptor present on cells. Tarceva is already FDA-approved for the treatment of lung cancer and for pancreatic cancer. A new drug for lung cancer is extremely important, as it is the leading cause of cancer-related deaths, and very few people who develop lung cancer survive. The only problem with Tarceva is that only about 15% of patients respond to it. So although we know that the target is expressed in patients, it does not seem to be as effective as expected in some patients. The goal of my project is to understand what makes Tarceva work and to identify the genes that cause Tarceva to be ineffective. If we could scan the entire human genome, we might be able to identify genes that make Tarceva work better. My goal is to identify these genes, which will allow us to increase the effectiveness of Tarceva in the near future.

Another important aspect of the above project is that even if I were to identify a gene that interacts with Tarceva, more work would be required to develop a drug against that gene. There is also the problem that a drug to this second gene could have the same problem as Tarceva and that only a small portion of the patients would respond. To circumvent this problem, I am developing a new drug based on RNAi. In theory, any gene could be disrupted using RNAi. My goal is to develop a drug that could be tailored to disrupt any gene in the human body. This would be a great tool for both researchers and could have tremendous clinical implications.

High-throughput RNAi Bar Code Screens as a Tool for Discovering Gene Function

D. Siolas

Recent advances in the field of RNAi have enabled researchers to conduct in-depth investigations of gene function. High-throughput screens in cultured mammalian cells can now be performed using RNAi libraries such as our own Hannon-Elledge library. Our second-generation libraries consist of more than 200,000 short hairpin RNA (shRNA) constructs targeting more than 45,000 human and mouse genes with RNAi triggers modeled after primary miRNA transcripts. Each hairpin is linked to a unique 60-nucleotide identification sequence, which serves as a bar code and allows us to virtually count the number of cells that contain a specific hairpin in a cell population. Small changes in bar code copy number can be monitored through the use of microarray technology. The bar code can be amplified from a cell's genomic DNA and fluorescently labeled to produce a probe that is hybridized to a microarray.

We have optimized our probe-labeling methods, probe size, and hybridization conditions using a plasmid DNA library in a Nimblegen platform. This optimized protocol allows us to detect 78.6% of probes within 1 standard deviation above the mean background from a complex mixture of approximately 1500 hairpins. In addition, by using two-color hybridization (Cy3 and Cy5), we can detect control subsets of hairpins known to be depleted from a sample population. We applied this RNAi bar code screening method in an in vivo screen using a complex mixture of 7500 library hairpins in HCT 116 colon cancer cells to identify genes that modify sensitivity to a common chemotherapeutic, paclitaxel. Hairpin-infected cells were treated with paclitaxel at an ineffectual dose (IC_{20}) where 80% of the cells survive. This low dose exposes genes that will increase the sensitivity of HCT 116 cells to the drug by causing an increase in cell death. Genes that synergize with a suboptimal drug treatment should make the drug more potent at a lower

dose. Cells that have increased susceptibility to paclitaxel are reflected as a loss of bar code representation on the microarray as compared to dimethylsulfoxide (DMSO)-treated control populations. This screen has resulted in a number of viable candidate genes that are currently being validated.

Genome-wide RNAi Libraries as an Engine for Gene Discovery

K. Chang

During the past year, we have expanded the availability of the shRNA libraries (human, mouse, and rat) to a lentiviral vector (pGIPz) format and continued our efforts to complete the construction of the retroviral (pSM2) libraries. For the current status of each of these libraries, see the RNAi Codex database/Web page (http://codex.cshl.edu/scripts/newmain.pl).

We have also made significant progress in building tools to harness RNAi for large-scale loss-of-function screens in mammalian cells. This has been our goal since the beginning of the RNAi library project. In the past, we have successfully carried out large-scale transfection-based screens in multiwell format, and this year, we have built a tractable screening platform that would permit us to perform screens in pools of up to 20,000 shRNAs as a complex population. Our new screening platform has several advantages over the conventional 96-well screening technology and is a significant step toward being able to perform genome-wide loss-of-function analysis in human cells without adopting robotics. The pool-based format permits monitoring of long-term effects of RNAi, as the task of creating and passaging stable cell lines in individual wells would be physically daunting. Our new method is highly parallel, and a screen can be easily carried out by one person. This is important as we have initiated a large-scale effort to screen a collection of breast cancer cell lines to identify genes that are important for breast cancer cell survival and to search for underlying targets responsible for drug resistance/sensitivity. Our goals for the breast cancer project are to find genes that when inactivated will cause cancer cells to die ("straight-lethal" analysis) and also to find ways to improve cancer therapy by discovering new drug combinations ("synthetic-lethal" analysis). In addition to the potential of improving breast cancer treatment, the cataloging of synthetic-lethal interactions can provide insight into the robustness of cancer cells to perturbations, a perspective crucial to the understanding of vulnerabilities in cancer genomes, and to overcome the "buffering" against lethal genetic interactions.

The Cytoskeleton in Tumor Suppression and Metastasis

J. Silva

I have completed an RNAi screen focused on the identification of new tumor suppressors in epithelial cancers. This screen was inspired by the extensive study of copy-number variations in cancer carried out by the Wigler, Powers, and Lucito labs here at CSHL. Beginning with a set of genes that were deleted in cancers, I found that loss of expression of key components that regulate cytoskeleton dynamics behave as putative tumor suppressors in epithelial cells. Inhibition of these genes compromises the ability of epithelial cells to establish strong interactions with neighbor cells and with their environment (extracellular matrix). In vivo, knock down of these genes cooperates with the oncogene, *ras*, to promote invasion of the stromal compartment in a mouse model of skin carcinogenesis. Finally, I have identified the presence of genetic alterations (deletions, reduced expression, and loss of heterozygosity) in a wide variety of epithelial cancers, including breast, colon and lung, the most prevalent ones. At this time, I am focusing on characterizing their involvement in metastasis and in EMT (epithelial mesenchymal transition).

Applications of Single-molecule Sequencing to Small RNA Biology

E. Hodges

Following a paradigm of Sanger sequencing, in which many high-quality whole-genome sequences have been produced, radical advances in technology have emerged that achieve DNA sequencing at a speed and ultra-high depth for a fraction of the cost. These new methods prove timely as we enter a new age in which the demand for genome resequencing is high, but funds for such projects are low. We are employing Solexa sequencing for a number of applications relevant to our work. This technology permits the sequencing of millions of single DNA molecules by in situ synthesis on arrays. Applying this method, we are interested in resequencing genomic samples selected and enriched for chromosomal regions of interest rel-

evant to diseases including cancer and neurological disorders such as autism and schizophrenia.

In addition, our work focuses primarily on the biology of RNAi mechanisms and their role in gene and transposon silencing. We have cloned populations of small RNAs from various model organisms including the fly and mouse for the purpose of deep sequencing. Deep sequencing enables us to thoroughly study small RNA content leading not only to the identification of potentially millions of new small RNAs, but also to addressing key questions regarding, for instance, changes in small RNA populations from wild-type and knockout models. It also allows us to study fluctuations over time in miRNA content between various tissues and, likewise, piRNAs during stages of germ cell development or to correlate sequence representation with expression levels providing an understanding for the tissue/disease specificity of gene targets.

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THE ORIGIN OF GENOMIC INSTABILITY IN CANCER CELLS

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A Darwinian model that can explain many features of solid cancers, especially their heterogeneity, diversity, and ability to evolve argues that these cancers are caused by genomic instability (GIN), which is defined as an abnormal and persistent change of a genome over time. According to this model, GIN generates a diverse population of abnormal cells by causing numerous genome-wide aberrations that trigger massive deregulation of gene expression. The vast majority of the cells die or fail to proliferate because of the genomic abnormalities or because they are killed or arrested by tumor suppressor mechanisms. However, some by pure chance have a combination of properties that is sufficient to bypass these mechanisms and proliferate to form cancers. Considering that the incidence of cancer relative to the number of *cells* in a human body is astronomically small, the probability of producing cancerous cells by GIN does not need to be high to be clinically significant.

In most solid cancers, GIN is manifested by chromosomal instability (CIN), a condition in which the structure of chromosomes or their assortment changes over time, making the affected cells aneuploid. Although cells of some benign tumors are also aneuploid, their number of chromosomes is usually near normal and is uniform within a tumor, indicating that the chromosomal abnormalities are CIN. In many solid cancers, however, the number of chromosomes varies among and within tumors widely, from near haploid to polyploid, indicating the presence of CIN. The incidence of CIN in solid cancers suggests that this condition either is a consequence of processes that are intrinsic to carcinogenesis or is a cause of cancer.

It is well documented that the maintenance of CIN is enabled by deficiencies in proteins that police genome integrity, such as the tumor suppressor p53, but the cause(s) of CIN in sporadic cancers remains uncertain. The primary suspects are mutations that deregulate telomere maintenance, or mitosis, yet such mutations have not been identified in the majority of sporadic cancers. Alternatively, CIN could be caused by a transient event that destabilizes the genome without permanently affecting mechanisms of mitosis or proliferation.

We previously proposed a hypothesis that several features of cancer cells, including their genomic instability, are a result of cell fusion induced by viruses. During the last year, we found that an otherwise harmless virus rapidly causes massive chromosomal instability by fusing cells whose cell cycle is deregulated by oncogenes. This synergy between fusion and oncogenes "randomizes" normal diploid human fibroblasts so extensively that each analyzed cell has a unique karyotype and some produce aggressive, highly aneuploid, heterogeneous, and transplantable epithelial cancers in mice. Because many viruses are fusogenic, our study suggested that viruses, including those that have not been linked to carcinogenesis, can cause chromosomal instability and, consequently, cancer by fusing cells.

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REGULATION OF APOPTOSIS AND SENESCENCE BY CANCER GENES

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Apoptosis is a genetically controlled form of cell death that is important for normal development and tissue homeostasis. Senescence produces "genetic death" in that the senescent cell is incapable of further propagation. Both processes are frequently disrupted in cancer cells, implying that each can limit tumor development. Moreover, radiation and many chemotherapeutic agents can induce either apoptosis or senescence, raising the possibility that the integrity of these programmed responses influences the outcome of cancer therapy in patients. The goal of our research is to understand how cancer genes control apoptosis and senescence in normal cells, and how mutations that disrupt these processes impact tumor development and therapy. Our approach emphasizes genetics, and we have increasingly relied on new types of animal models and gene manipulation technologies to study tumor development and cancer therapy in vivo.

Control of Apoptosis by Oncogenes and Tumor Suppressor Genes

A. Bric, D. Burgess, C. Chang, K. Diggins, M. McCurrach, W. Xue, L. Zender [in collaboration with former laboratory members C. Scott and J. Gil; D. Beach, Institute of Cell and Molecular Science, London; J. Teruya-Feldstein, E. Hernando, R. Benezra, and C. Cordon-Cardo, Memorial Sloan-Kettering Cancer Center]

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

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

Using a murine model of hepatocellular carcinoma (liver cancer) and representational oligonucleotide microarray analysis (ROMA) of murine and human tumors, we identified two new oncogenes that are amplified in murine and human cancers and cooperate to promote tumorigenesis (Zender et al. 2006). One encodes the yes-associated protein (Yap) and the other is the cellular inhibitor of apoptosis protein cIAP1. Interestingly, previous work from other investigators indicates that Yap can promote apoptosis, although the *Drosophila* ortholog (Yorkie) can promote proliferation. We also see that Yap can promote proliferation, whereas cIAP1 can block cell death. We are currently studying Yap function to determine how it acts as an oncogene and whether, like Myc, it can drive both proliferation and cell death.

In studying how proliferation is coupled to apoptosis, we previously showed that deregulation of the Rb-E2F pathway could also compromise mitosis by producing aberrant expression of the mitosis regulator, Mad2. These results suggested that mutations compromising Rb function could drive proliferation but also produce genomic instability, which itself could be oncogenic. Working with Robert Benezra, we examined the impact of enforced Mad2 expression, either alone or in combination with other oncogenes, on oncogenesis. Indeed, we see that deregulated Mad2 can promote tumorigenesis but, consistent with its role in promoting genomic instability, was not required for tumor maintenance (R. Sotillo et al., in press). These results support our initial suggestions that deregulated Mad2 expression is one consequence of Rb loss that can contribute to tumorigenesis.

Control of Cellular Senescence

A. Chicas, M.V. Krizhanovsky, S. Nuñez, W. Xue, L. Zender, K. Diggins [in collaboration with former laboratory members M. Narita and M. Narita; E. Hernando and C. Cordon-Cardo, Memorial Sloan-Kettering Cancer Center]

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

We previously showed that senescent cells can accumulate senescence-associated heterochromatic foci (SAHFs), which may provide a chromatin buffer that prevents activation of proliferation-associated genes by mitogenic transcription factors. This year, we showed that the high-mobility-group A (HMGA) proteins, which can promote tumorigenesis, accumulate on the chromatin of senescent fibroblasts and are essential structural components of SAHFs. HMGA proteins cooperate with the $p16^{INK4a}$ tumor suppressor to promote SAHF formation and proliferative arrest and to stabilize senescence by contributing to the repression of proliferation-associated genes (Narita et al. 2006). These antiproliferative activities are canceled by coexpression of the *HDM2* and *CDK4* oncogenes, which are often coamplified with HMGA2 in human cancers. Our results identify a component of the senescence machinery that contributes to heterochromatin formation and imply that HMGA proteins also act in tumor suppressor networks.

Our laboratory was the first to demonstrate that deregulated mitogenic oncogenes can drive cells into a senescent state thereby preventing transformation and that senescence contributes to the outcome of chemotherapy in vivo. This year, we conducted a series of experiments (discussed in more detail below) where we showed that p53-deficient liver carcinoma cells undergo senescence following p53 reactivation in vivo. Remarkably, although senescence is a cytostatic program, the tumors underwent massive regressions as a result of an attack of the immune system on the senescent cells. Thus, despite the cytostatic nature of the senescence program, senescent cells can turn over in vivo. Although it is established that chronic inflammation triggered by senescent stromal cells or other factors can promote tumorigenesis, our study illustrates how innate immune cells—when targeted against senescent tumor cells—can have antitumor effects as well. We suggest that strategies that specifically harness these processes may represent a promising therapeutic approach.

Cancer Gene Discovery and Comparative Oncogenomics

M. Spector, W. Xue, L. Zender [in collaboration with R. Lucito, D. Mu, M. Wilger, and S. Powers, Cold Spring Harbor Laboratory; C. Cordon-Cardo, Memorial Sloan-Kettering Cancer Center; J. Luk, Hong Kong University]

Hepatocellular carcinoma (HCC) represents the fifth most frequent neoplasm worldwide. However, due to the lack of treatment options, it constitutes the third leading cause of cancer death (>500,000 deaths per year). The only curative treatment options for HCC are surgical resection or liver transplantation. Unfortunately, at the time of diagnosis, the majority of HCC patients have an advanced tumor and are not candidates for surgical ther-

apy. Systemic chemotherapeutic treatment is ineffective against HCC, and thus, there is a great need for new therapies to treat this disease. To better understand this disease, we generated a new mouse model of hepatocellular carcinoma that is based on the ex vivo manipulation of liver progenitor cells, followed by the seeding of these cells into normal recipients. These methods allowed us to rapidly produce in situ liver cancers of defined genetic origin.

In an attempt to gain insights into the lesions that give rise to HCC, we used representational oligonucleotide microarray analysis (ROMA) to conduct a genome-wide analyses of tumors in this mouse model and in human hepatocellular carcinomas. Our results revealed a recurrent amplification at mouse chromosome 9qA1 and the syntenic region of human chromosome 11q22. Gene expression analyses delineated *cIAP1*, a known inhibitor of apoptosis, and *Yap*, a transcription factor as candidate oncogenes in the amplicon. In the genetic context of their amplification, both *cIAP1* and *Yap* accelerated tumorigenesis and were required to sustain rapid growth of amplicon-containing tumors. Furthermore, *cIAP1* and *Yap* cooperated to promote tumorigenesis.

Our study also suggests an integrative strategy to complement the human cancer genome project, whose goal is to catalog all of the mutations that contribute to human cancer. By integrating data from human cancer genomics with corresponding data from appropriate mouse models, it should be possible to expeditiously focus on the cancer lesions most likely to have translational potential.

First, cross-species comparisons can prioritize lesions for further study by increasing the probability that they are important, a particularly relevant issue when studying relatively low-frequency events. Second, by incorporating murine tumors into the analysis, it is possible to increase the sample size and exploit synteny to exclude candidates outside the region of overlap. Third, genetically engineered mice, by definition, develop more defined cancers than humans. Thus, the identification of a particular lesion in murine cancers immediately provides information concerning the evolutionary context in which it arose and identifies a relevant setting in which to study its oncogenic potential. Finally, mouse models provide excellent settings to test the suitability of a cancer lesion as a therapeutic target, and at the same time, they provide ideal preclinical models for subsequent drug testing. We believe that such integrative approaches will speed up the pace of discovery and help translate the promise of cancer genetics into improvements of cancer diagnosis, prognosis, and therapy.

Modulation of Gene Expression In Vivo Using RNA Interference

A. Bric, R. Dickins, B. Ma, K. McJunkin, C. Miething, P. Premsrirut, J. Simon, W. Xue, L. Zender [in collaboration with G. Hannon and L. He, Cold Spring Harbor Laboratory]

RNA interference (RNAi) is a powerful method for suppressing gene expression in mammalian cells. Stable knockdown of gene expression can be achieved by continuous expression of synthetic short hairpin RNAs (shRNAs), typically from RNA polymerase III promoters. However, primary microRNA transcripts (pri-miRNAs), which are endogenous triggers of RNAi, are normally synthesized by RNA polymerase II (pol II). In collaboration with Gregory Hannon, we previously showed that pol II promoters expressing rationally designed pri-miRNA-based shRNAs ($shRNA^{mir}$) produce potent, stable, and regulatable gene knockdown in cultured cells and in animals, even when present at a single copy in the genome. In practice, this $shRNA^{mir}$ vector system is remarkably similar to cDNA overexpression systems and should be a powerful tool for studying gene function in cells and animals. We are taking advantage of these tools to explore various aspects of tumor suppressor gene networks in vitro and in vivo (Ramiro et al. 2006; Yin et al. 2006; E.E. Bosco et al., in press).

As one example, we recently used our *tet*-regulated RNAi system to explore the role of p53 in tumor maintenance. As indicated above, p53 acts to restrict proliferation in response to DNA damage or deregulation of mitogenic oncogenes, and thus *p53* mutations increase proliferation and survival and, in some settings, promote genomic instability and resistance to certain chemotherapies. In principle, the instability associated with p53 loss could allow the tumor to evolve beyond its dependence on p53 mutations. To determine the consequences of reactivating the p53 pathway in p53-deficient tumors, we used RNAi to conditionally regulate endogenous p53 expression in a mosaic mouse model of liver carcinoma (W. Xue et al., in press). We showed that even brief reactivation of endogenous p53 in p53-deficient tumors can produce complete tumor regressions. The primary response to p53 was not apoptosis but instead involved the induction of a cellular senescence program that was associated with differentiation and the up-regulation of inflammatory cytokines. This program, although producing only cell cycle arrest in vitro, also triggered an innate immune response that targeted the tumor cells in vivo, thereby contributing to tumor clearance.

The above results indicate that p53 loss can be required for the maintenance of aggressive carcinomas and illustrates how the cellular senescence program can act together with the innate immune system to potently limit tumor growth. However, they also establish the utility of conditional shRNA technology to study tumor phenotypes in live mice. Current work is attempting to generalize this strategy with the goal of eventually enabling the spatial and temporal control of the expression of any endogenous gene.

Molecular Genetics of Drug Sensitivity and Resistance

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Our laboratory has a long-standing interest in understanding genetic determinants that influence the cellular response to conventional chemotherapy, with the goal of identifying mechanisms of drug resistance and developing new drug targets. During the last several years, we have also expanded our efforts toward understanding the action of targeted therapeutics. As one example, we studied the action of imatinib (STI-571, Gleevec) against murine cells and leukemias expressing BCR-ABL, an imatinib target and the initiating oncogene for human chronic myelogenous leukemia (CML). We showed that the tumor suppressor *p53* is selectively activated by imatinib in BCR-ABL expressing cells as a result of BCR-ABL kinase inhibition (Wendel et al. 2006). Inactivation of *p53*, which can accompany disease progression in human CML, impedes the response to imatinib in vitro and in vivo without preventing BCR-ABL kinase inhibition. Concordantly, *p53* mutations are associated with progression to imatinib resistance in some human CMLs. Our results identify *p53* as a determinant of the response to oncogene inhibition, and suggest one way in which resistance to targeted therapy can emerge during the course of tumor evolution.

We have also characterized factors that influence the sensitivity and resistance to rapamycin, a drug that targets mTOR (mammalian target of rapamycin) and is in clinical trials to treat a variety of cancers. We previously showed that rapamycin can suppress mTOR activity and synergize with chemotherapy in Akt-expressing lymphomas, leading to potent antitumor responses. Interestingly, eIF4E (eukaryotic initiation factor 4E), a translational regulator acting downstream from mTOR, accelerated lymphomagenesis and promoted drug resistance in a manner comparable to that of Akt, suggesting that a substantial portion of the Akt survival signal is transmitted through deregulated translation. This year, we showed that reduced dosage of PTEN, a negative regulator of phosphoinositide 3-kinase (PI3K) signaling, is sufficient to activate Akt, but has only a modest effect on lymphomagenesis in the same model (Wendel et al. 2006). Nonetheless, loss of even one *PTEN* allele resulted in lymphomas that were resistant to conventional chemotherapy yet sensitive to rapamycin/chemotherapy combinations. Finally, the introduction of lesions that act downstream from mTOR (*eIF4E*) or disable apoptosis (*Bcl-2* and loss of *p53*) into $PTEN^{+/-}$ lymphomas promoted resistance to rapamycin/chemotherapy combinations. Thus, whether activation of the PI3K pathway confers sensitivity or resistance to therapy depends on the therapy used as well as secondary genetic events. We believe that a better understanding of these genotype-response relationships in human tumors will be important for the effective use of rapamycin or other compounds targeting the PI3K pathway in the clinic.

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

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GENOMIC MICROARRAY ANALYSIS OF CANCER

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Mutation of the genome is central to the development and progression of cancer. Mutations occur in the genome of precancerous cells and accumulate, altering gene function until the growth of these cells goes unchecked. The genes responsible for cancer must be identified if we are to understand the cellular pathways that become subverted to allow the cell to become cancerous. We have developed a genomic microarray technique, representational oligonucleotide microarray analysis (ROMA), to identify copy-number fluctuations, which borrows the methodology of complexity reducing representations developed for RDA (representational difference analysis) to increase hybridization efficiency and increase signal to noise. A representation is a reproducible sampling of the genome, produced by first cleaving the genome with a restriction enzyme such as BglII, ligation of adaptors, and PCR (polymerase chain reaction) amplification. BglII representations of tumor and normal that are differentially labeled are compared on such an array to identify copy-number fluctuations. The array is composed of oligonucleotides based on the sequence of the human genome. We are using microarrays photochemically synthesized by Nimblegen Systems Inc. Currently, we are using arrays with 85,000 probes distributed throughout the genome and soon will be moving to an array of 390,000 probes.

We have continued surveying the genome of several cancer types to identify regions that have undergone increased or decreased gene copy number, namely, amplifications or deletions, since these changes can be used as markers for the location of oncogenes or tumor suppressor genes, respectively. Regions are being informatically searched for gene candidates, and functional analysis of these candidates is being carried out to determine which genes have oncogenic potential. Once identified, these genes functions can be investigated to understand their role in the path to tumorigenesis. In addition, by analyzing the regions alongside clinical data, we will determine if there are any regions or combination of regions that correlate to clinical outcome. The application of this type of an array spans many areas of biology, from cancer to genetic diseases. We will be using this microarray method to categorize the mutations that occur in primarily two tumor types initially, pancreatic and ovarian cancer.

PANCREATIC CANCER

There will be an estimated 30,000 cases of pancreatic cancer this year. Of those, 29,700 patients will succumb to the disease. Although the number of cases is low, in comparison to several other cancers such as colon, lung, or breast cancer, the survival rate for pancreatic cancer is one of the lowest. Treatments can extend survival or alleviate pain but seldom cure the patient. In fact, the mean survival time is approximately 6 months. Because the life span after diagnosis is very short, the number of patients that receive tumor resections is very low. This translates to few samples available for analysis. Because of this, we are collaborating with many clinicians to put together a useful tumor bank of pancreatic tissue for analysis by ROMA.

Currently, we are collaborating with Dr. Ralph Hruban of Johns Hopkins University, Dr. Daniel Von Hoff of the Arizona Cancer Center, and Dr. Vijay Yajnic of Massachusetts General Hospital, who will be providing pancreatic specimens and invaluable clinical information and expertise. To date, we have analyzed 82 samples composed of 27 primary tumors, 23 cell lines, and 32 xenografts. In addition, we have compiled published data for an additional 16 primary tumor samples into our data set to increase information content. We have confirmed that many of the known mutated regions are altered in this set, including genes such as the *INK4a-ARF* locus, *SMAD4*, *p53*, and c-*myc* to name a few. In addition, there are other less-characterized regions deleted 2p23, 3p, 4q23, 5p15, 10p15, and 12p and amplified 1q42-43, 3q26, 6p21, 7p21, 14q21, 18q11, and 19q12 frequently in this cancer, and we are continuing to study these regions to identify possible gene candidate tumor suppressors and oncogenes.

Although we are working on several regions at this time, we will summarize one region amplified on 19q12 in specifics. There are a number of tumors and cell lines that have this region amplified, but with two tumors that have the most informative amplicons, the

epicenter or common region of mutation was delimited to approximately 2 million bases. This region contains 20 gene candidates. In the samples that have gene amplification, it is highly likely that the target gene is overexpressed at the RNA level, and the nontarget genes will not be overexpressed, although they may be overexpressed as a consequence of being gene-amplified. To decrease the number of viable gene candidates, gene expression analysis was performed on the cell lines and one of the primary tumors that have the amplicon.

After this analysis was completed, the number of candidates was decreased to only two. Immunohistochemistry on one of the tumors that had gene amplification clearly demonstrated that one candidate was expressed highly in the normal as well as the tumor. The other candidate, a gene called *p21*-activated kinase or PAK4, was nearly absent in the normal but was found at extremely high levels in the tumor sample tissue. To determine if the encoded protein is active, we performed an in vitro kinase assay on extracts from cell lines that have the amplicon. In comparison to a cell line that did not have amplification of this gene, the cell lines had 10–15 more protein and 8- to 10-fold more kinase activity. Mutated or activated forms of PAK4 have been shown to increase tumorigenicity.

We are presently sequencing the PAK4 gene in these samples to determine if the gene present encodes a constitutively active protein. However, another way of activating PAK4 is to activate RAS, an extremely common event in pancreatic cancer. Codon 12 (the most commonly mutated codon) of RAS was sequenced for these samples and four of five have an oncogenic form of the RAS protein. It is possible that PAK4 itself is activated in this fifth sample, and we will dermine this shortly. In the future, we plan to use short hairpin RNA (shRNA) constructs to knock down the level of PAK4 in cells with the amplicon to determine the effect on tumorigenicity. We will also be moving this gene into an animal model for pancreatic cancer.

OVARIAN CANCER

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

At present, we have analyzed 121 tumors and have compiled the data to identify regions commonly amplified or deleted. We have identified uncharacterized regions commonly amplified (1q21, 3q26, 6p, 11q13, 12p, and 20p13) and regions that are deleted frequently (1p35, 3p26, 4p15, 4q34, 5q14, 5q34, 6q22, 9q22, 12q, 13q13, 16q, 19p, and 22q13). We have characterized these regions for gene content and have chosen several to move further for gene characterization.

One such region we are further characterizing is a deletion on chromosome 5q14. This region is relatively small and contains only seven gene candidates. Since there were so few candidates in this region, we performed quantitative PCR (Q-PCR) on the mRNA of a large panel of cell lines and primary ovarian tumors. Presently, we have removed all candidates from analysis based on the expression data (similarly to what we have done for the pancreatic region discussed above) and have three candidates to further characterize. One gene, *Centrin 3* (*CETN3*), in this region is of particular interest. *CETN3* is a component of the centrosome and takes part in the cellular duplication process. In lower organisms, there is only one centrin and experimental deletion of this gene resulted in centrosome amplification and aneuploidy, a common phenotype of cancer cells. We are presently studying whether the removal of this gene contributes to aneuploidy in cancer cells.

Methylation-specific Oligonucleotide Microarray Analysis

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In addition to genetic mutation such as amplification and deletion, there are epigenetic mechanisms used to influence the transcriptional activity of a gene. One such mechanism is methylation of the cytosines present in the DNA of the transcriptional control region, which often suppresses the expression of the gene. It has been known that methylation of DNA has been involved in the silencing of gene expression in imprinting and in cancer. Recent advances including technical and also the sequencing of the genome have

made detection of methylation at loci more reliable and accurate. However, there are few methods that can identify methylation changes over the entire genome. We have adapted ROMA to methylation detection oligonucleotide microarray analysis (MOMA). We are currently utilizing this to survey the methylation changes that occur in CpG islands in cancer, but in principle, this method would have applications for identifying methylation differences involved in imprinting or other syndromes that do not involve genetic mutation.

At the heart of this method is the use of a representation. If we wish to capture the CpG islands within a representation, we must reshape the representation to do so. We therefore set out to find which representations faithfully capture the majority of CpG islands. We determined that a restriction enzyme with a four-base recognition sequence would be required and determined that the best endonuclease would be one with a cleavage site rich in CG dinucleotides. Since an overhang is required for ligation of adaptors for subsequent PCR, the endonuclease MspI was chosen. This restriction endonuclease cleaves at both unmethylated and methylated CpGs. Therefore, we will lose some information content from the genome, but we felt that the few CpGs lost would on average not be detrimental to the data available. The ligated material is split in half, one half digested with a second endonuclease McrBC, which cleaves at (G/A)mC (N40-3000) (G/A)mC, and the other half mock-digested. The PCR amplification product of these two templates is then compared on a CpG island microarray.

We have tested the array with several samples and have demonstrated that it can discriminate when a CpG island is methylated or not methylated. We can also see, as others have reported, that when a cell becomes cancerous, there is a paradoxical decrease in global methylation and an increase in CpG island methylation. We have analyzed several cell lines and can identify methylation at common sites of several tumor suppressors such as p16, and Rassf1a. By analyzing those regions of the genome that are differentially methylated and combining the data for copy number and expression, we will obtain a more complete picture of the cancer cell. Currently, we are doing this for a set of 16 ovarian samples and another set of 83 breast samples. We expect that this information will help to identify tumor suppressors within regions where one allele has been deleted and the remaining allele has been silenced by methylation.

We have also identified a pattern of demethylation of non-gene-associated CpG islands that may be useful to identify the stage or grade of the tumor. Although grading/staging cancers is a measurement determined by a well-trained pathologist, it is still somewhat subjective since of course pathologists differ. In addition, tumor classification based on qualitative analysis of morphology, in some cases, is not necessarily predictive of clinical outcome. It has long been understood that within the genome, the CpG islands not associated with genes often proximal or contiguous with repetitive sequences become hypomethylated in cancer. This hypomethylation is thought to make the repetitive regions unstable contributing to genomic instability, with a likely consequence of the cancer becoming more aggressive. Therefore, we have surmised that the methylation content of these non-gene-associated islands could be used to judge the grade/stage of the cancer. If this hypothesis is correct, genome-wide measurements of the methylation content could be obtained and calculated, resulting in a score that could serve as a parameter utilized by pathologists to grade/stage tumors. By developing a quantitative measurement, we hope to remove some error in determining the grade/stage of tumors. If positive results are obtained, we expect to move to a larger study, hopefully in a clinical setting. The outcome will be better patient management and we hope the more accurate prescription of treatment strategies.

Mutational Analysis of Phosphatases

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The goal of this collaborative study is to integrate gene discovery technology with experimental strategies developed in Dr. Tonks's lab here at CSHL for the characterization of the protein tyrosine phosphatase (PTP) family of enzymes, to investigate how tyrosine-phosphorylation-dependent signaling pathways are disrupted in cancer.

Dr. Tonks's lab conducted a bioinformatic analysis of the human genome sequence to compile a list of all human PTPs. This list was used to identify the genomic coordinates for all PTP genes, which was then matched to the genomic copy-number data generated. This allowed us to identify those PTPs that were amplified or deleted within the tumor genomes analyzed. We identified two receptor-like PTPs, LAR and PTPsigma, that are encoded by genes amplified in these ovarian tumor specimens. These receptor-like PTPs display features of cell adhesion molecules and have been implicated in

regulating the function of adhesion complexes that control cell-cell interactions and may be disrupted in cancer.

Disruption of mitogen-activated protein kinase (MAPK)-dependent signals is known to contribute to cancer in humans. Two of the members of the PTP family that we identified as being amplified in ovarian cancer specimens are regulators of MAPK-dependent signaling modules. VHY is a dual specificity phosphatase that the Tonks lab has implicated in the activation of a signaling pathway (the JNK pathway) that has a critical role in the control of cell survival. MKP5 is another dual specificity phosphatase that functions in the inactivation of the p38 and JNK MAPKs.

Oncogenes and tumor suppressor genes are frequently altered in their expression between normal and tumor specimens. Therefore, in addition to our analysis of gene copy number, we conducted transcriptional expression analysis of tumor samples to identify any PTPs that are misexpressed. We have grown 11 ovarian and 9 pancreatic cell lines and have performed genome-wide expression analysis. Of the 76 PTPs queried in the expression data, we found that 13 displayed statistically significant altered expression in the cell lines. We have also used quantitative reverse transcriptase (RT)-Q-PCR as a complementary approach to validate these data on changes in expression.

Interestingly, in an independent project, we have identified the gene encoding Liprin as being highly amplified and displaying altered gene expression. Liprin is a binding partner for the receptor PTP LAR, which, as described above, we have also identified as being amplified in ovarian cancer. LAR has been been shown to be targeted to focal adhesions and to regulate cytoskeletal function and interactions with extracellular matrix. Liprin was shown originally to be important for targeting LAR to focal adhesions and for clustering the PTP at these sites. This observation not only highlights the potential significance of LAR in ovarian cancer, but also, and more generally, introduces an interesting extension of the primary project, i.e., an analysis of the proteins that interact with the PTPs.

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MAMMALIAN FUNCTIONAL GENOMICS

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The Mills lab (see Fig. 2) designs and utilizes novel models for elucidating the genetic and molecular basis of human disease. Research areas include (1) the functional identification of novel tumor suppressor genes at Human *1p36* and (2) investigation of the role of the p53-related protein p63 in development, cancer, and aging.

THE QUEST FOR THE *1P36* TUMOR SUPPRESSOR

Deletions encompassing *1p36*, a portion of the genome residing on human chromosome 1, were first reported in 1977. Since that time, a number of studies of late-stage tumors have indicated that *1p36* is frequently deleted in a variety of human cancers, including those of the brain and blood, as well as epithelial malignancies such as those of the breast, colon, and prostate. Although these observations suggest that an important cancer-preventing gene resides at *1p36*, this tumor suppressive gene has not been identified. We have been taking a functional genomics approach to try to identify a tumor-suppressor mapping to *1p36*. We have been using chromosome engineering to generate models with gains and losses of the region of the genome corresponding to human *1p36*. Briefly, chromosome engineering combines the power of gene targeting with Cre/*loxp* technology and allows the generation of mouse strains that harbor precise rearrangements such as deletions and duplications; these models have loss and gain of specific regions of the genome, respectively. Using this approach, we identified a 4.3-Mb region of the mouse genome with potent tumor-suppressive activity. Whereas loss of this region rendered mice tumor prone, gain of this interval triggered excessive tumor suppression (Fig. 1). Now that we have pinpointed the region containing the tumor suppressor, these models will be used to identify the gene(s) within the region that functions as a tumor suppressor. Identification of this gene is likely to have a significant impact on our understanding of the tumorigenic process.

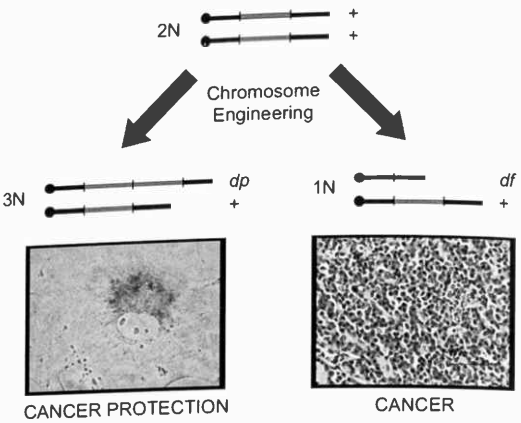

FIGURE 1 Engineerting chromosomes. We altered normal cells so that they had one extra (*left*) or one fewer (*right*) copies of a chromosome segment (representing human *1p36*) than normal cells. This caused added tumor suppression and increased cancer, respectively.

FUNCTION OF THE *p53* HOMOLOG *p63*

p63 ***Deficiency Is Tumor Protective.*** The discovery that the *p53* tumor suppressor is a member of a multigene family that also includes *p63* and *p73* has brought the *p53* field into a new era. *p63* is a transcription factor structurally and functionally similar to *p53*; in contrast to *p53*, however, *p63* is rarely inactivated in human cancers. In fact, the *3q27* region to which *p63* maps is frequently amplified, or *p63* is overexpressed in the majority of epithelial tumors, suggesting that *p63* has oncogenic potential. Several years ago, we identified *p63* and generated several *p63*-deficient mouse models. What was clear from the phenotype was that despite the striking similarities between *p63* and *p53*, they perform very different functional roles in vivo: *p63* is essential for development of stratified epithelia, whereas *p53* is dispensable during embryogenesis but functions as a potent tumor suppressor in the adult. More recently, we found that in contrast to the high incidence of spontaneous tumors in $p53^{+/-}$ mice, $p63^{+/-}$ mice were

not predisposed to cancer. In fact, *p63* heterozygosity *decreased* the high tumor incidence of $p53^{+/-}$ mice, indicating that haploid levels of p63 may even be tumor protective. Loss of the wild-type *p63* allele did not occur and *p63* expression was maintained in the rare tumors that did develop in the $p63^{+/-}$ cohort. $p63^{+/-}$ mice were not even susceptible to chemical carcinogens. This indicated that *p63* does not perform a role equivalent to that of *p53* in tumor suppression and that reduced *p63* may provide a novel tumor-suppressive mechanism, suggesting that modulation of p63-mediated pathways could offer an effective strategy for anticancer therapy. We are currently working on how the six different p63 proteins modulate cancer.

p63 Links Cellular Senescence and Aging. During the course of the tumor study outlined above, we discovered that $p63^{+/-}$ mice had a significant reduction in life span and developed age-related pathology. Using a conditional system to shut down p63 function specifically in proliferating cells of stratified epithelia such as the skin, we discovered an unanticipated link between *p63*, cellular senescence, and aging. Remarkably, *p63* deficiency activates a program of cellular senescence and leads to accelerated aging. This finding suggests a mechanism for the low tumor incidence of $p63^{+/-}$ mice: Cellular senescence effectively removes aberrantly proliferating cells from the proliferative pool, thus providing a tumor-suppressive mechanism. A further understanding of how p63-mediated pathways modulate the senescent process and which of the different p63 isoforms modulates this process is currently under way. This work will provide a clearer understanding of cellular senescence that will likely impact our ability to design more effective anticancer regimens in the future.

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ANGIOGENESIS-MEDIATED TUMOR GROWTH AND METASTASIS

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D. Gao D.J. Nolan
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In addition to malignant cancer cells, the tumor microenvironment comprises nonmalignant cells that regulate tumor progression, invasion, and metastasis. Of the nonmalignant cells, the bone marrow (BM)-derived hematopoietic and endothelial progenitor cells (EPCs) offer significant proangiogenic activity. Although the hematopoietic cells promote tumor angiogenesis through the release of proangiogenic factors or by creating permissive conditions in the tumor microenvironment that favor the growth of locally derived blood vessels, the EPCs are believed to provide an alternative source of endothelial cells that directly incorporate into nascent blood vessels as bona fide endothelial cells. Despite the general importance of these cells, the precise composition of specific individual lineages and their relative contribution to tumor angiogenesis remain poorly understood. However, the existence of a BM reservoir of EPCs, and their selective involvement in tumor neovascularization, has attracted considerable interest because these cells represent novel targets for therapeutic intervention.

Our lab is using a multidisciplinary approach to investigate the cellular and molecular mechanisms governing blood vessel formation in tumors by de novo recruitment of BM-derived EPCs. Our results demonstrate that EPC-mediated neovascularization is critical for the progression of both primary tumors and metastatic lesions and suggest that EPC targeting may offer a promising approach for cancer therapy. In the last 3 years, we have systematically addressed these issues by using a powerful combination of mouse genetic models, functional genomics, and BM transplantation (BMT) approaches. Using this strategy, we have demonstrated the precise spatial and temporal contribution of EPCs to the neovascularization of growing transplanted and spontaneous tumors in vivo. We have demonstrated that early tumors recruit BM-derived EPCs that differentiate into mature BM-derived endothelial cells and luminally incorporate into a subset of sprouting tumor neovessels. Notably, in later tumors, these BM-derived vessels are diluted by an increase in non-BM-derived vessels from the periphery, which accounts for purported differences in previously published reports. Importantly, specific and selective ablation of EPCs in vivo by an α-emitting radioimmunoconjugate, which targets an EPC-specific receptor, significantly reduced tumor growth as a consequence of reduced EPC contribution and decreased vessel density. These results not only have demonstrated the biological role of EPCs in angiogenesis-mediated tumor growth, but have also suggested an approach for preventing tumor growth by ablation of one critical and specific component of the tumor microenvironment. We have extended our analysis of primary tumors toward elucidating the progression of metastatic lesions. Although metastasis remains the main cause of death in cancer patients, little is known about the progression of micrometastases to macrometastases in secondary organs. We have demonstrated that a second angiogenic switch underlies the progression of avascular lung micrometastases to vascular macrometastases. De novo contribution of EPCs was observed in the neovascularization of these progressing lesions. Importantly, ablation of EPCs did not affect initial colonization by metastatic cells but markedly impaired their progression into macroscopic lesions as a consequence of reduced neovascularization. Our progress in the last year is elucidated below.

Investigating the EPC Lineages and Vascular Niche in the BM Compartment

D.J. Nolan, K. Bambino

In last year's Annual Report, we described our progress in understanding the spatial and temporal contribution and the functional role of EPCs in the neovascularization of a variety of tumors including xenografts (B6RV2 lymphomas and Lewis lung carcinoma) and an orthotopic tumor (B16F0 melanoma). More recently, this analysis has been extended to spontaneous breast tumor mouse models. Detection of BM-derived EPCs in these models highlights their general relevance in the process of tumor angiogenesis. The

biological function of EPC was discerned by ablating them with an anti-VE-cadherin antibody that specifically recognized the monomeric VE-cadherin present on EPCs, but not the homodimerized form present on mature ECs. Cytotoxicity was enhanced by coupling this antibody with radioisotope actinium (^{225}Ac). Ablation of EPCs reduced tumor mass, associated with decreased vascular density, underscoring the critical role of EPCs in angiogenesis-mediated tumor progression.

In the last year, we have put our efforts in unraveling hierarchies of the endothelial lineage in the BM compartment. Elucidation of the endothelial lineages is likely to provide insights into EPC biogenesis and fate determination and may also lead to the identification of endothelial "stem" cells capable of preferentially repopulating the vascular compartment. We have begun to identify endothelial lineages within the BM compartment by analyzing the Lin$^-$ c-Kit$^+$ Sca1$^+$ (LKS) population. Since the LKS population is known to contain both the long-term and short-term hematopoietic progenitors, we believe that it may also contain EPCs with similar potential to reconstitute the endothelial lineage. Further investigation of the LKS population with respect to EPC markers (VEGFR2, VE-cadherin) and progenitor markers (c-Kit, ScaI) has revealed three distinct populations in resting animals. Kinetic analyses of these populations posttumor challenge have begun to define hierarchical lineages and have unraveled VEGFR2$^-$ VE-cadherin$^-$ ScaI$^-$ Kit1$^+$ cells as the mobilizing population. The observation that the mobilizing cells are VEGFR2$^-$ is paradoxical because cell surface VEGFR2 has been demonstrated to have a role in mobilization in reports by other investigators. Further investigation of the recycling endosome pathway determined that the VEGFR2 receptor was internalized in the mobilizing EPCs.

We have also determined that the VE-cadherin$^+$ cells in the BM compartment give rise to mature endothelial cells. Flow-sorted VE-cadherin$^+$ Lin$^-$ cells (which also include ~80% of VEGFR2$^+$ cells) were cocultured with mature endothelial cells in vitro. VE-cadherin$^+$ cells rapidly differentiated into endothelial cells and incorporated into endothelial networks, confirming that they are part of a BM-derived endothelial lineage. We are currently using flow sorted green fluorescent protein (GFP$^+$) EPC populations to determine whether any of these EPC lineages can repopulate the vascular niche and give rise to endothelial lineages.

Our future plans are to determine the long-term reconstitution potential of these individual lineages through serial transplantation experiments. We will also investigate the EPC niche in the context of the hematopoietic and osteoblastic niches within the BM compartment and determine how the niche microenvironment regulates the balance between cellular self-renewal and differentiation and, importantly, how tumors influence migration of EPCs out of the niche.

BM-derived EPCs Regulate the Progression of Micrometastases to Macrometastases

D. Gao

In cancer patients, malignant tumor cells disseminate from their site of origin, metastasize, and colonize distant organs. Following initial colonization, these metastatic colonies usually remain microscopic or dormant and may eventually progress into macrometastases, leading to organ dysfunction and resulting in death of the cancer patient. However, despite the clinical importance of metastasis, the cellular and molecular mechanisms that govern macrometastases formation remain poorly understood. We have demonstrated that the progression of micrometastaes to macrometastases is mediated by an angiogenic switch, associated with the de novo contribution of BM-derived EPCs. EPCs are recruited to the sites of active neovascularization via stromal-derived factor 1 (SDF1)–chemo-kine receptor 4 (CXCR4) and VLA (integrin $\alpha4\beta1$)–VCAM interactions. Generation of a loss of function in EPCs with inducible RNA interference (RNAi)-mediated suppression of Id1 transcription factor did not effect formation of pulmonary micrometastases but impaired their progression into macrometastases and increased overall survival of animals (Fig. 1). Our results demonstrate that EPC-mediated neovascularization is critical for metastasis progression, and suggest that therapeutic targeting of EPCs may be promising in situations where metastatic colonization has already occurred.

To Determine Transcriptional and Posttranscriptional Events Regulating Mobilization of EPCs

A. Mellick

Last year, we began to delineate molecular pathways with respect to differentially regulated genes and microRNAs (miRNAs) that are responsible for tumor-induced EPC mobilization/differentiation. Candidate

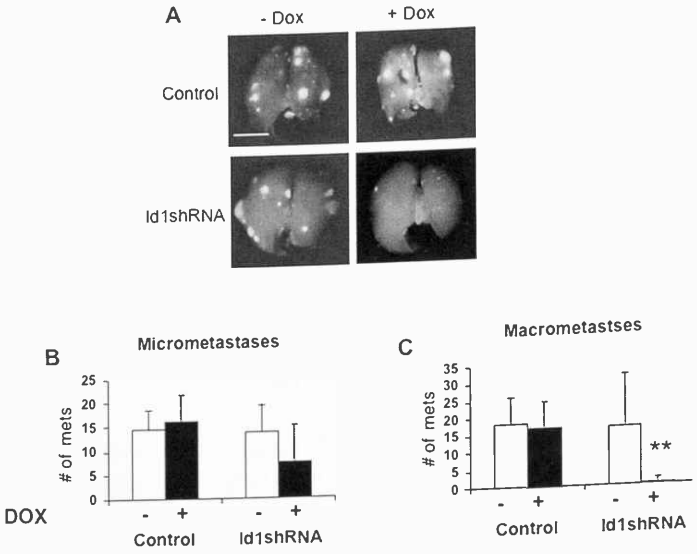

FIGURE 1 Inducible RNAi-mediated suppression of Id1 in the BM progenitors impaired macrometastases formation in the lungs. (*A*) A representative fluorescent stereomicroscopic image depicting pulmonary metastases (day 28) in a nonspecific shRNA (short hairpin RNA) BMT mice (*upper panel*) and Id1-shRNA BMT mice (*lower panel*) in the absence of Dox (–Dox) and presence of Dox (+Dox). Bar, 20 μM. (*B*) Quantitation of the total number of micrometastases in the lungs of inducible control nonspecific shRNA and Id1 shRNA BMT mice at day 28 in the absence of Dox (–Dox) and presence of Dox (+Dox). (*C*) Quantitation of macrometastases in the lungs of inducible control nonspecific shRNA and Id1shRNA BMT mice at day 28 in the absence of Dox (–Dox) and presence of Dox (+Dox).

genes such as helix-loop-helix transcription factor Id1, chemokine receptor CXCR4, stromal-derived factor SDF1, VE-cadherin, flk1/VEGFR2, GATA2, and Brn3a were identified. The expression of some of these candidate genes was localized to the Lin^- VE-$cadherin^+$ EPCs. To carry out functional analysis of EPCs in vivo, we have begun to investigate the promoters of these genes (Id1, VEGFR2, and VE-cadherin) for their ability to mark EPCs in vivo. The proximal promoters were cloned and used to drive two fluorescent markers (red fluorescent protein and GFP). Of these, the Id1 promoter was found to specifically mark EPCs in the early BM, peripheral blood, and the tumor.

We next investigated whether the exquisite property of the Id1 promoter to mark EPCs can be exploited for cell-specific ablation or generating loss of EPC function. The Id1 promoter was used to drive a suicide gene, herpes simplex virus–thymidine kinase (HSV-TK). Administration of ganciclovir specifically ablated EPCs in TK BMT mice. EPC ablation impaired tumor growth associated with reduced vessel density.

In another powerful approach, we have used RNAi for suppressing the expression of EPC-specific genes (e.g., Id1 and VEGFR2) in vivo. Suppression of Id1 or VEGFR2 in EPCs resulted in mobilization defects and impaired angiogenesis-mediated growth of tumors. Collectively, our results suggest that these promoters are useful reagents for investigating EPC function in vivo.

In last year's Annual Report, we also described our efforts in determining microRNA changes that may regulate EPC mobilization. We identified 53 microRNAs as differentially regulated (at least twofold) in the Lin^- BM following tumor challenge. Detailed analysis of these candidate microRNAs is being conducted in conjunction with Exiqon, to localize microRNAs to EPCs in the BM, blood, and tumor. We believe that the EPC-specific promoter we have recently identified will allow us to more closely understand which microRNAs might be critical in EPC biology.

To Determine the Contribution of Individual Oncogenes and Tumor Suppressor Genes in the Angiogenesis-mediated Progression of Hepatocellular Carcinoma

K. McDonnell [in collaboration with S. Lowe, Cold Spring Harbor Laboratory]

Oncogenes and tumor suppressors have been implicated in angiogenesis-mediated expansion of tumor mass. For example, loss of certain tumor suppressors,

including p53, or oncogene activation, such as Myc, is associated with robust angiogenesis resulting from down-regulation of a negative angiogenesis regulator thrombospondin 1 (TSP1), possibly via microRNA 17-92. Some activated oncogenes like *EGFR* or *Ras* stimulate signaling pathways in cancer cells that cause increased expression of the angiogenic factor VEGF (vascular endothelial growth factor).

This project seeks to determine how oncogenes and tumor suppressor genes impact tumor angiogenesis, particularly via recruitment of BM-derived progenitor cells. As a mouse model of cancer, we are using the hepatocellular carcinoma (HCC) model developed in the Lowe laboratory. This model has allowed us to rapidly develop spontaneous liver tumors driven by an oncogene of choice, including Ras, Myc, Akt, or β-catenin. We are determining the windows of the "angiogenic switch" in each of these tumors in order to assess the spatial and temporal contribution of BM-derived EPCs. The mechanisms by which these oncogenes recruit BM-derived EPCs will be explored further. The expansion and activation of BM cellular elements occur within BM stem cell niches. Presently, the biology of these niches remains incompletely characterized. We are anticipating that delineation of the oncogene and tumor suppressor genes associated with BM cellular mobilization will define molecular pathways through which to further investigate the physiology of these niches.

The elucidation of the oncogene and tumor suppressor pathways associated with mobilization and recruitment of BM cellular elements and the unveiling of the biology of BM stem cells niches may identify novel molecular and cellular targets for the development of effective therapeutic strategies directed against tumor angiogenesis.

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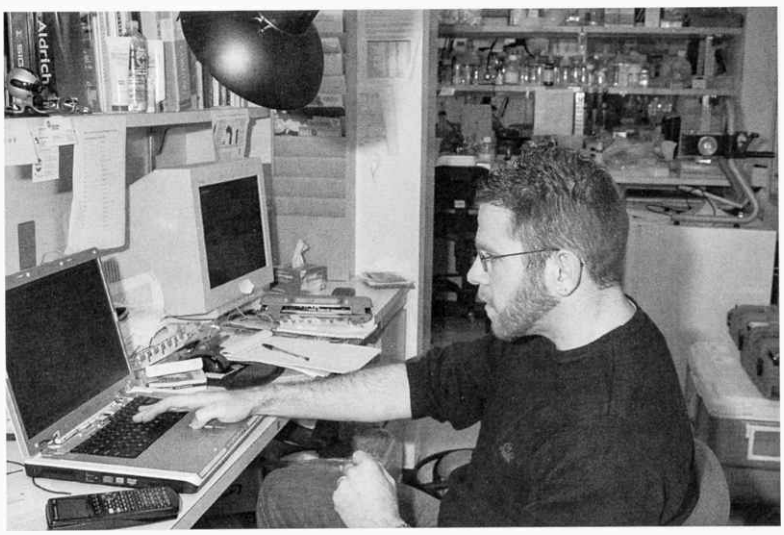

Daniel J. Nolan

CANCER GENES

S. Powers A. Bakleh K. Nguyen
R. Kohnz J. Zhang
D. Mu

Cancer is caused by the accumulation of genomic damage that affects a subset of human genes casually involved in the cancer process. Genomic damage is random, but the cancer process is not. Starting with a slightly altered premalignant cell, fully malignant tumors will evolve only if additional genomic alterations provide distinct proliferative or survival advantages. This Darwinian process, which mixes random genomic alterations with selection for increasingly malignant cells, results in tumors that contain different configurations of genomically altered cancer genes. Although this genomic heterogeneity is responsible for the wide variation in cancer outcomes and therapeutic responses, some of the cancer genes are commonly altered in a significant percentage of tumors. A select few of these commonly altered cancer genes—*EGFR*, *BCR-ABL*, *HER2*—have provided the starting point for several new highly effective cancer therapies.

How can we find more of these select cancer genes? Clearly, it is important to detect as many genomic alterations in tumor cells as possible. Toward this end, our laboratory has in the past successfully exploited the genome-wide DNA copy-number alteration techniques developed by Mike Wigler and Rob Lucito here at CSHL to detect new cancer genes. This year, in addition to continuing DNA copy-number analysis, we have forged new collaborations with CSHL scientists Rob Martiensen and Rob Lucito to apply their methods for detecting promoter methylation, another type of genomic alteration, to the problem of cancer gene identification.

Is comprehensive detection of genomic alterations enough to find these select cancer genes? Unfortunately, no. Genomic damage frequently extends over regions containing several genes, making it impossible to pinpoint the true culprit by detection methods alone. Fortunately, experiments that address the functional importance of candidates can identify bona fide cancer genes. Here, the rate-limiting factor is the experimental approach itself, and we are very fortunate to collaborate with Scott Lowe's laboratory on hepatocellular carcinoma, as they have developed a highly effective, flexible mouse model for testing cancer genes that are operative in the liver. We are also fortunate that Greg Hannon's laboratory has developed an extraordinarily useful resource in the genome-wide RNA interference (RNAi) libraries that contain ready-made RNAi molecules to virtually every candidate cancer gene. Currently, these systems are being used in a large-scale systematic evaluation of hundreds of candidate cancer genes culled from the list of genes that are deleted or amplified in human liver cancer. We are extending this type of systematic analysis to other tumor types as well.

Previously, we had used ROMA (representational oligonucleotide microarray analysis) to discover a new type of genomic instability in colon cancer (the deletion syndrome). We continued our study of the gene that is most frequently deleted in colon cancer and determined that its deletion was a by-product of the deletion syndrome and that it did not have any cancer-related effect on the properties of the cell. Other genes that are deleted are already known to be functionally and clinically important, but this gene is very frequently deleted without phenotypic consequence. It is possible that this deletion may be useful diagnostically. We have drafted our manuscript on our findings on this deletion syndrome, and this coming year, we will publish our findings.

This year, we concluded a preliminary effort with Dr. Sandy Markowitz at the Cleveland Clinic to use ROMA to find genomic DNA copy-number alterations which could distinguish primary colon cancer that would not metastasize from colon cancer with clear metastatic potential. Although we did find DNA copy-number alterations that were more significantly prevalent in metastatic colon cancers, they coincided exactly with what had previously been discovered using lower-resolution techniques (e.g., 8p loss and 8q gain). We may go back to revisit this important diagnostic issue with newer genomic methodologies, but our current primary focus is on functional studies of genomically altered genes of high-interest. These include a group of secreted proteins operative in liver cancer and a set of what appear to be lineage-specific oncogenes in lung cancer. Our goals in the next year are to determine the degree to which these factors are

involved in their respective tumor types and whether they do or do not provide a good starting point for developing new cancer therapies.

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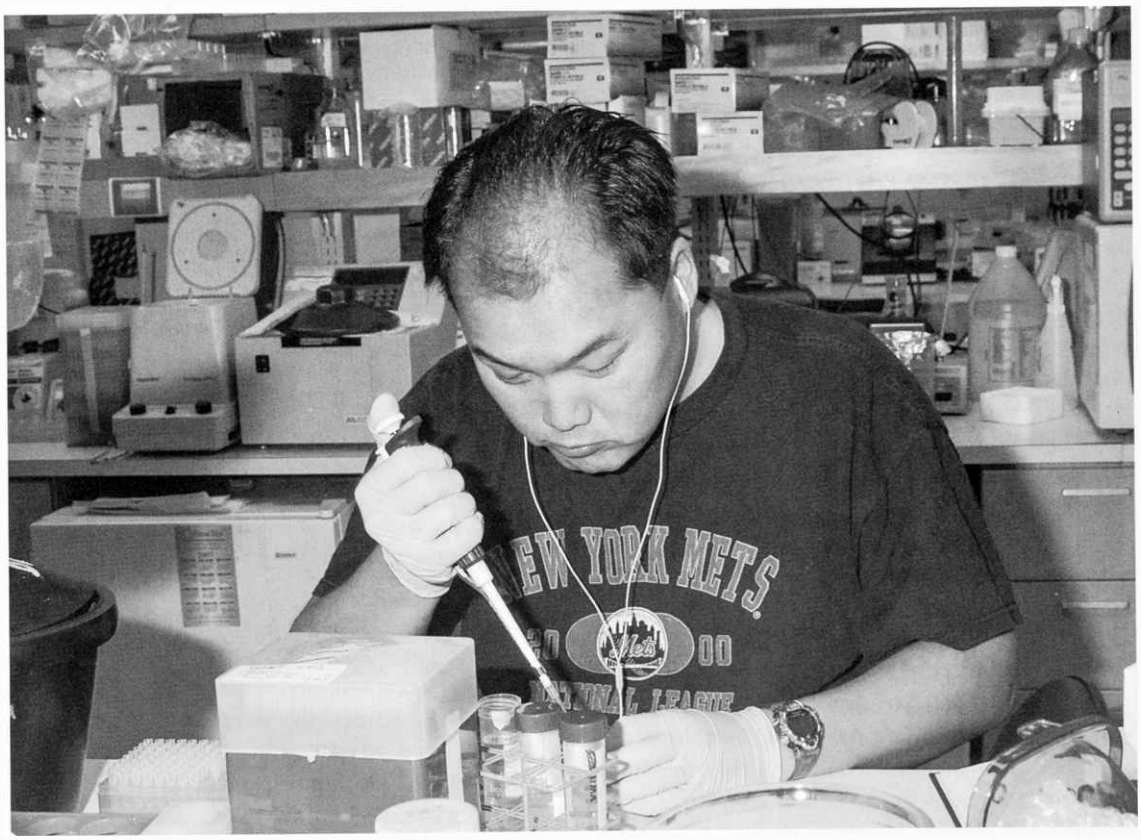

David Mu

COPY-NUMBER ANALYSIS AND HUMAN DISEASE

M. Wigler M. Chi, D. Esposito, V. Grubor, J. Hicks, J. Hicks, L. Hufnagel, J. Kendall, A. Krasnitz, Y-H. Lee, E. Leibu, A. Leotta, J. Meth, L. Muthuswamy, N. Navin, D. Pai, M. Riggs, L. Rodgers, J. Troge, B. Yamrom, S. Yoon

MAMMALIAN GENETICS

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

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

CANCER

Our studies of breast cancer have largely drawn upon samples from two Scandinavian collaborations: Anders Zetterberg at the Karolinska Institute, Sweden, and Anne-Lisa Borresen-Dale at the Radium Hospital, Norway, and with Larry Norton at the Memorial Sloan-Kettering Cancer Center (MSKCC), New York. These studies have elucidated a set of loci, called epicenters, which are the recurrent sites for genome amplification and deletion in breast cancer. The set of breast cancer epicenters overlap with epicenters from lung cancer (data from Scott Powers and David MU, CSHL) but are clearly a distinct set. In fact, we can distinguish breast cancers from lung cancers largely by the loci involved in amplification and deletion, a method that may be useful in a clinical setting. The epicenters are locations where reside many of the genes that will make good tumor markers or therapeutic targets. Work in progress suggests that by dissecting tumors, we may be able to determine the time sequence of the amplifications and deletions, enabling us to determine the earliest genomic events. In a collaboration with the laboratories of Scott Lowe, Scott Powers, and Rob Lucito (all of CSHL), we have found that animal models will be a further source of information about these epicenters (Zender et al. 2006)

We documented a new form of genomic instability that is very common in breast cancer. There is a good correlation between the number of genomic events, and the type of genomic instability, with survival (Hicks et al. 2006). In working with Dr Zetterberg and pathologist Kiki Tan at MSKCC, we have shown an excellent correlation between CGH and fluorescent in situ hybridization (FISH), a mainstay of clinical pathology (see also Navin et al. 2006). CGH is in many ways more reliable than FISH, and we anticipate that FISH will be replaced by CGH, particularly for tests that oncologists use to decide on treatment.

In collaboration with David Botstein and Robert Pelham of Princeton Univesity, New Jersey, we have used mouse ROMA (Lakshmi et al. 2006) to determine how many of the stromal cells of human tumors transplanted in mice are likely to be both mutant and clonal (Pelham et al. 2006). We do not know whether these mutant stroma are selected by the tumor from

circulating cells or whether they become mutated during stromal growth. But the results open up a new window on host-cancer interactions.

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

Utilizing a new microarray designed by Robert Lucito here at CSHL, we have begun to assess the methylation status of CpG islands in the genomes of cancer and leukemia cells. These islands are rich in sites for DNA methylation, and the status of methylation at these sites may be clues to cell lineage and cancer progression. Although still preliminary, the results suggest that the vast majority of CpG islands in all cells are partially methylated, and we can readily distinguish cell-specific patterns by the small number of recurrent sites that have become hypo- or hypermethylated. Only a few of the islands change status as the tumors progress. The clinical significance of these changes remains to be evaluated. Nevertheless, we expect that determination of methylation state in the DNA from a biopsy can be used to determine the origins of a tumor, as well as determine the cellular composition of a biopsy, both useful objectives in a clinical setting.

GENETIC DISORDERS

Since the seminal discovery that CNV is common in the human gene pool (Sebat et al., *Science 305:* 525 [2004]; Iafrate et al., *Nat. Genet. 36:* 949 [2004]), in collaboration with the laboratory of Jonathan Sebat here at CSHL we have focused on the role of CNVs in human disease and, in particular, on the role of spontaneous or de novo CNVs (see, e.g., Jobanputra et al., *Genet. Med. 7:* 111 [2005]). We tested the hypothesis that de novo CNV is associated with autism spectrum disorders (ASD). We performed CGH on the genomic DNA of patients and unaffected subjects to detect copy-number variants not present in their respective parents. Candidate genomic regions were validated by higher-resolution CGH, paternity testing, cytogenetics, FISH, and microsatellite genotyping. Confirmed de novo CNVs were significantly associated with autism ($P = 0.0005$). Such CNVs were identified in 12/118 (10%) of patients with sporadic autism, in 2/77 (2%) of patients with an affected first-degree relative, and in 2/196 (1.0%) of controls. Most de novo CNVs were smaller than microscopic resolution. Affected genomic regions were highly heterogeneous and included mutations of single genes. These findings establish de novo germ-line mutation as a more significant risk factor for ASD than previously recognized and clearly point to a new and potentially rich avenue of approach for the further study of the genetic basis of the disorder (for details, see Sebat's report in this volume).

Consonant with the finding of spontaneous CNVs in humans, studies in mice (a collaboration with Ira Hall at CSHL) have demonstrated that de novo CNVs occur frequently in mice lineages (for details, see Hall's report in this volume).

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Before a cell divides, it must first make an exact copy of its DNA. Bruce Stillman's group studies the process of DNA replication, focusing on how it is regulated and how it is coordinated with other events such as chromosome segregation during mitosis. First, the cell must "know" where to begin copying. DNA contains start sites called origins that direct the initiation of DNA replication. Binding to these origins are large complexes of proteins that have a role in the process. Recent work in the Stillman lab has revealed that one such protein—Orc1—is involved in preparing the mass of condensed DNA and proteins known as chromatin for DNA replication.

Tatsuya Hirano studies how cells assemble and organize their chromosomes in preparation for cell division. His research focuses on two protein complexes, known as cohesin and condensin, that are structurally related to each other but have different functions in this process. During the past year, Hirano identified a new cohesin-binding protein, termed Wapl, and studied how it regulates the dynamic behavior of cohesin during cell division. His group showed that Wapl helps remove cohesin from chromosomes, thereby allowing two copies of each chromosome to split properly before they are transported into daughter cells.

David Spector and his colleagues have identified a messenger RNA that lingers uncharacteristically in the cell nucleus but can be rapidly released into the cytoplasm in response to cellular stress (or other signals) such as viral infection. The "cut-and-run" mechanism revealed by this study is a new paradigm of gene regulation that is likely to be broadly relevant to biology and biomedical research. It establishes a role for the cell nucleus in harboring mRNA molecules that are not immediately needed to produce proteins, but that are ready at a moment's notice to produce protein in response to stress or other cellular signals. Ongoing studies are focused on identifying and characterizing additional nucleus-retained RNAs and elucidating their roles in regulating gene expression.

Cells register external signals and react with an internal response. Understanding this process of "signal transduction" is key to understanding such fundamental cellular processes as growth, proliferation, differentiation, migration, and metabolism. Nicholas Tonks studies the coordinating and competing actions of proteins called protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs) that are crucial for the regulation of signal transduction pathways. Furthermore, disturbance of this delicate balance between the activity of PTKs and PTPs has been shown to be a cause of human diseases including cancer, diabetes, and inflammation. With this in mind, the focus of the Tonks lab is to characterize the structure, modes of regulation, and physiological function of members of the PTP family of enzymes. Tonks and his team are making progress in defining the functional links between particular PTPs and specific signaling pathways, with a view to establishing whether disruption of such functions underlies disease and whether individual PTPs show potential as therapeutic targets.

Some clusters of abnormal or precancerous cells in the breast progress to malignancy, while others remain benign. Senthil Muthuswamy studies why some precancerous lesions develop the loss of tissue organization and increased rate of cell division that are the hallmarks of cancer. The goal of Muthuswamy's lab is to identify new molecules and signaling pathways that are crucial for genetic changes in breast tissue organization during the initiation and progression of cancer. He has developed a three-dimensional tissue culture system that mimics the basic glandular structure of the breast. Using this system, his group has identified a direct relationship between *ErbB2*, a gene known to be misregulated in nearly half of all breast cancers, and molecules that regulate cell architecture. *ErbB2* appears to inactivate proteins that help establish normal breast tissue structure. This ability of *ErbB2* to disrupt breast tissue organization may contribute to the observed disruption of normal tissue organization in breast cancer.

Linda Van Aelst's research is focused on signal transduction pathways involving members of the family of proteins known as Ras and Rho GTPases and the physiological processes they regulate. These proteins have key roles in cellular activities controlling cell growth control, differentiation, and

morphogenesis. Alterations that affect normal Ras and Rho function result in the development of several diseases including cancer and neuropathologies. Van Aelst and her team made progress in 2006 in defining the role and mechanisms by which Ras and Rho family members exert their effects on tumorigenesis and on neuronal development.

Arne Stenlund is using the papillomavirus replication system to gain a better understanding of the crucial first steps of how DNA is copied in human cells. He has managed to recapitulate the assembly of the viral "helicase," providing the first description of how such a protein-DNA complex is formed. The helicase is the enzyme that unwinds DNA in preparation for DNA replication. Until now, little has been known about precisely how this complex forms in human cells. Stenlund's efforts to dissect the helicase assembly process provide an opportunity to achieve a detailed understanding of the initiation of DNA replication in mammalian systems.

HIGHER-ORDER CHROMOSOME DYNAMICS

T. Hirano N. Aono I. Onn
R. Gandhi K. Shintomi
M. Hirano

The long-term goal of our research program is to understand the molecular basis of chromosome segregation during mitosis and meiosis. Central to this process are two multiprotein complexes, known as condensin and cohesin, that regulate chromosome condensation and cohesion, respectively. The two complexes are structurally related to each other and contain members of a large family of chromosomal ATPases, known as structural maintenance of chromosomes (SMC) proteins. Mutations in the subunits of condensin and cohesin lead to genome instability in many model organisms, and recent lines of evidence implicate that subtle perturbation of condensin and cohesin functions might be linked to developmental diseases in humans. Our laboratory takes multidisciplinary approaches to understand how condensin, cohesin and SMC proteins might work at a mechanistic level both in vivo and in vitro.

Assembly and Subunit Geometry of Human Condensin Complexes

I. Onn, N. Aono, M. Hirano, T. Hirano

Vertebrate cells possess two types of condensin complexes, termed condensin I and condensin II, that have essential yet distinct roles in chromosome condensation and segregation. Both complexes share the same pair of SMC2 and SMC4 subunits as their core subunits, but they have different sets of three non-SMC regulatory subunits (i.e., two HEAT-repeat subunits and a kleisin subunit). To better understand their molecular architecture and mechanism of action, we have reconstituted human condensin complexes from their recombinant subunits. A reconstituted human condensin I fraction functionally complements a *Xenopus* egg extract that has been depleted of endogenous condensin I. Subunit-subunit interaction assays reveal that both condensin I and condensin II have a pseudo-symmetrical structure, in which the amino-terminal half of kleisin links the first HEAT subunit to SMC2, whereas its carboxy-terminal half links the second HEAT subunit to SMC4. No direct interactions are detectable between the SMC dimer and the HEAT subunits, indicating that the kleisin subunit acts as the linchpin in holocomplex assembly. Construction and characterization of a panel of SMC mutant proteins allow us to conclude that the assembly and integrity of the complexes are ATP-independent. Furthermore, a limited proteolysis assay suggests that SMC2 may undergo large conformational changes upon its binding to ATP. This study represents the first step in our long-time effort to dissect the detailed mechanism of action of the chromosome condensation machinery.

Specialized Functions of Condensin II in DNA Replication

N. Aono, T. Hirano

A previous genetic study in fission yeast suggested that condensin I (the sole condensin complex in this organism) may have a role in replication checkpoint responses. In vertebrate cells, condensin I is sequestered in the cytoplasm, whereas condensin II is located within the nucleus during interphase. We therefore hypothesize that it is condensin II, not condensin I, that may have an interphase function, if any, in vertebrate cells. To test this hypothesis, we have investigated the possible contribution of condensin II to DNA replication in *Xenopus* egg-cell-free extracts. We show that condensin II is not required for replication under unperturbed conditions but becomes essential under stressed conditions. For instance, DNA replication in the extracts is blocked in the presence of aphidicolin, but such a block is suppressed by addition of caffeine, an inhibitor of checkpoint kinases. It is thought that the so-called "dormant" origins become activated under this stressed condition to complete DNA replication. When condensin II, but not condensin I, is depleted from the extracts, the replication from the dormant origins becomes incomplete. This requirement for condensin II in dormant

origin replication is bypassed when the checkpoint kinase (chk1) is depleted from the extracts. This is the first indication that condensin II may have a specialized role in DNA replication in vertebrate cells. Further studies will help unravel how condensin II might regulate replication from dormant origins and how the two condensin complexes might contribute to maintaining the genome stability in distinct manners.

Role of Microcephalin in Condensin Regulation

N. Aono, R. Gandhi, T. Hirano

Primary autosomal recessive microcephaly is a neurodevelopmental disorder characterized by marked reduction in brain size and mental retardation. The *MCPH1* gene, one of the genes responsible for microcephaly, encodes microcephalin, a protein containing multiple BRCT (BRCA1 carboxy-terminal) domains. Cells from *MCPH1* patient cells display a unique cellular phenotype with premature chromosome condensation in early G_2 phase and delayed decondensation postmitosis. Our previous study showed that this phenotype is caused by misregulation of condensin II, but not of condensin I. To further understand how microcephalin might regulate condensin II, we have raised antibodies specific to human microcephalin. We show that microcephalin coimmunoprecipitates with condensin II from a HeLa cell lysate. Less efficient coprecipitation is observed in a mitotically arrested cell lysate, indicating that the interaction between condensin II and microcephalin is cell-cycle dependent. We are now in the process of expressing recombinant microcephalin to test whether the interaction between the two components is direct or indirect.

We also show that small interfering RNA (siRNA)-mediated depletion of micocephalin in HeLa cells causes premature chromosome condensation, a phenotype reminiscent of that observed in the *MCPH1* patient cells. Although the cells depleted of microcephalin undergo apparently normal mitosis, the morphology of metaphase chromosomes prepared from these cells is highly abnormal: They display curly chromatid axes (reminiscent of condensing-II-depleted chromosomes) and loosened centromeric cohesion (reminiscent of condensing-I-depleted chromosomes). These observations support the idea that an intricate balance of the actions of condensin I and condensin II determines the final architecture of metaphase chromosomes. We anticipate that further studies of microcephalin will help dissect the differential regulation of condensin I and condensin II during the cell cycle.

Molecular Basis of Sister-chromatid Cohesion

K. Shintomi, T. Hirano

The cohesin complex is composed of two SMC core subunits (SMC1 and SMC3) and two non-SMC regulatory subunits (Scc1 and SA1/SA2). Although it has been demonstrated that this complex has a central role in sister-chromatid cohesion from yeast to humans, very little is known about how it might work at a mechanistic level. To fully understand the molecular basis of sister-chromatid cohesion, we have reconstituted sub- and holocomplexes of cohesin from its recombinant subunits. A panel of SMC mutant proteins defective in their ATPase cycle has also been constructed. Limited proteolysis assays using the wild-type and mutant forms of the SMC1-SMC3 dimer suggests that the core subunits of cohesin undergo large conformational changes upon binding to ATP or DNA. We will extend this assay to the holocomplex and address how the non-SMC subunits might modulate the ATP- and DNA-dependent conformational changes of the SMC dimer. To test the functional relevance of the conformational changes, we also plan to examine the behavior of the wild-type and mutant forms of cohesin in *Xenopus* egg extracts, which recapitulate the whole process of sister-chromatid cohesion in vitro.

Another yet related aim of this project is to understand how the establishment of cohesion might be coupled to DNA replication. Our effort is now focused on Eco1, a class of replication-coupled regulator of cohesion that was originally identified in yeast. Vertebrate cells have two different Eco1 proteins, known as Eco1A/ESCO2 and Eco1B/ESCO1, and mutations in the human *ESCO2* gene cause Roberts syndrome, a disorder characterized by growth retardation and craniofacial anomalies. Preliminary results show that Eco1A is the predominant form in *Xenopus* egg extracts and that its association with chromatin is regulated during the cell cycle. We are now exploring the functional contribution of Eco1A to sister-chromatid cohesion in this system by immunological and biochemical approaches.

Wapl Regulates Sister-chromatid Cohesion

R. Gandhi, T. Hirano

In vertebrate cells, most of the cohesin complex is removed from chromosome arms during mitotic prophase in a manner dependent on two mitotic kinases: Polo-like kinase 1 (Plk1)- and Aurora B. A small amount of cohesin, which is concentrated at centromeres, escapes this removal and holds sister centromeres together until it is proteolytically cleaved at the onset of anaphase by a specialized protease known as separase. During the past year, we have focused on the human ortholog of wings apart-like (Wapl), a gene product originally identified as a potential regulator of heterochromatin organization in *Drosophila melanogaster*, and we have found that human Wapl is a new cohesin-binding protein that promotes cohesin removal during prophase. siRNA-mediated depletion of Wapl in HeLa cells causes transient accumulation of prometaphase cells. Chromosomes in these prometaphase cells retain a high level of cohesin on their arms, and their sister chromatids are poorly resolved. This phenotype is relieved when the level of cohesin is reduced in the Wapl-depleted cells. Conversely, overexpression of Wapl causes premature separation of sister chromatids. The Wapl protein coimmunoprecipitates with cohesin from HeLa cell nuclear extracts. As judged by in vitro reconstitution experiments, Wapl directly interacts with the regulatory subunits of cohesin in a stoichiometric manner, implicating its direct and noncatalytic contribution to cohesin removal. Because premature separation of sister chromatids is a potential cause for aneuploidy, a hallmark of many types of cancer and human genetic diseases, future studies on this class of cohesin regulators will enhance our understanding of how genomes are stably maintained throughout the cell cycle and transmitted across generations.

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EPITHELIAL CELL BIOLOGY AND CANCER

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

Breast cancer is a leading cause of cancer death among women. Current therapeutic strategies are mostly aimed at controlling malignant breast cancers. Although this strategy is helping patients by extending their life span, in the end, the patients succumb to the disease. I believe that there are alternative strategies that can be exploited. If we can treat or control precancerous lesions so that they never progress to form malignant cancers, the patients can be maintained tumor-free. The bottleneck in accomplishing this goal is the almost complete lack of understanding of the molecular mechanisms that regulate development and progression of precancerous lesions. Our long-term goal is to understand the molecular mechanisms that are important for development of precancer and its progression to cancer.

Increased rates of cell proliferation and disruption of normal organization of epithelial cells are among the principal changes observed in precancerous lesions. Although changes in proliferation rates can occur under normal physiological states such as pregnancy, disruption of epithelial organization is unique to disease states. Thus, epithelial cells must possess mechanisms that actively maintain normal tissue organization under normal physiological conditions, and oncogenes must have gained ways to disrupt them. Although significant inroads have been made toward understanding how oncogenes increase cell proliferation, we do not understand how they disrupt epithelial organization/architecture. I believe that a detailed understanding of the mechanism by which oncogenes disrupt cell organization/architecture will provide new insights into understanding precancerous lesions.

Human-breast-derived epithelial cells grown as monolayer cultures on plastic culture dishes are poor models for investigating changes in epithelial organization, because they do not recreate the three-dimensional organization observed in vivo. To overcome this, we use a three-dimensional organotypic cell culture model using the nontumorigenic immortalized human mammary epithelial cell line, MCF-10A. MCF-10A cells cultured on a bed of basement-membrane matrix resemble breast acini in vivo. Thus, the cultured three-dimensional structures can serve as an excellent platform to interrogate the molecular mechanisms by which oncogenes induce disruption of epithelial organization.

Among the oncogenes implicated in breast cancer, amplification of ErbB2/HER2, a receptor tyrosine kinase, is observed in 25–30% of breast cancer and correlates with poor clinical outcome. Expression of ErbB2 in mouse models results in development of invasive mammary tumors. Thus, ErbB2 serves as a model for an oncogene, which can induce initiation and progression of tumors that end as an aggressive disease. Since there is no known ligand for ErbB2, we use a chimeric receptor that can be inducibly activated using a small-molecule FKBP-dimerizing ligand (AP1510, Ariad Pharmaceuticals). Activation of ErbB2 homodimers in MCF-10A three-dimensional structures induced uncontrolled proliferation, prevented apoptosis, and disrupted epithelial organization, resulting in formation of large multiple acini-like (multiacinar), noninvasive structures that resemble early lesions with atypia. This phenotype contrasts with other oncogenes such as cyclin D1 that induce uncontrolled proliferation but fail to induce multiacinar outgrowths, suggesting that ErbB2 must engage mechanisms to disrupt epithelial organization/architecture.

RESEARCH PROGRESS

During the past year, we have made significant progress. We discovered a new pathway used by ErbB2 to disrupt epithelial organization. In doing so, we have defined a role for proteins that regulate apical-basal polarity during transformation of epithelial cells organized as three-dimensional acini (Fig. 1). The polarity proteins have now occupied a central place in our research program because, as outlined below, they not only regulate changes in epithelial organization, but also regulate apoptosis and metastatic progression. Thus, it is likely that either polarity proteins them-

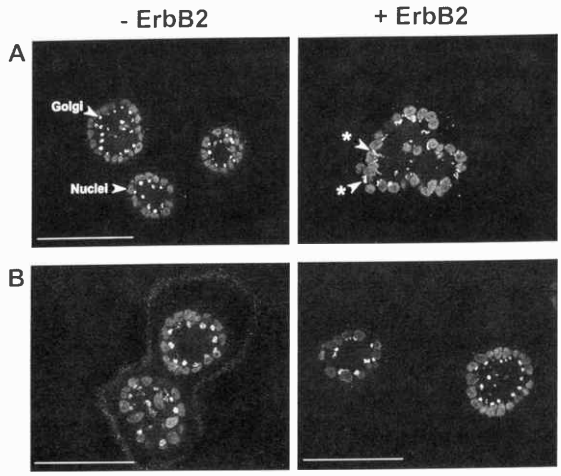

FIGURE 1 Par6-aPKC is required for ErbB2-induced disruption of apical-basal axis of polarity in MCF10A three-dimensional acini. Day-20 acinar structures stimulated with dimerizer for 4 days were immunostained for a *cis*-Golgi matrix protein, GM130 (Golgi). Nuclei costained with DAPI (nuclei). Arrows show mislocalization of GM130 to the lateral or basal (*) surfaces. Bar, 50 μm. The images demonstrate that whereas activation of ErbB2 in the presence of active Par6-aPKC interaction disrupted apical-basal localization of GM130 (*A*), absence of active ErbB2-Par6-aPKC interaction blocked ErbB2–induced mislocalization of GM130 (*B*).

selves, or pathways that regulate polarity proteins, have a critical role during development and progression of precancerous lesions.

Polarity proteins and disruption of epithelial organization: We reported that ErbB2 interacts with components of the Par epithelial cell polarity protein complex during disruption of epithelial organization. Interfering with the ErbB2-Par complex interaction blocked the ability of ErbB2 to induce formation of multiacinar structures but did not affect ErbB2-induced cell proliferation. Thus, we identified polarity proteins as mediators required for oncogene-induced disruption of epithelial organization but was dispensable for oncogene-induced proliferation.

Polarity proteins and apoptosis: During the course of the above study, we made a surprising observation—the ErbB2-Par pathway was not only required for disruption of three-dimensional epithelial organization, but also required for ErbB2 to protect cells from apoptosis. Thus, regulators of cell polarity may provide a survival advantage during cancer initiation. We followed up on this observation using Myc, an oncogene that can induce proliferation but has the ability neither to disrupt epithelial cell polarity nor to block apoptosis. Forced disruption of polarity by RNA interference (RNAi)-mediated down-regulation of a polarity gene, *Scribble*, protected cells from Myc-induced apoptosis. To determine the in vivo relevance of this observation, we used a mammary-fat-pad transplantation model system to rapidly study genetic interactions during mammary tumorigenesis. Loss of *scribble* protected cells from Myc-induced apoptosis in vivo, demonstrating that polarity genes have important roles during tumorigenesis. The mosaic mouse model allows us to establish the importance of the observations we make using three-dimensional culture for mammary tumorigenesis in vivo.

Polarity proteins and invasive progression: Increasing loss of cell architecture is thought to be associated with progression of precancerous lesions to invasive cancers. It is possible that progression of precancerous lesions can be triggered by inducing changes in cell architecture. To test this possibility, we performed a genetic screen using a library of RNAi vectors targeting expression of polarity genes to identify genes that can confer an invasive property to the noninvasive ErbB2-induced multiacinar structures. Loss of AF-6 was sufficient to promote invasion of ErbB2-induced multiacinar structures. We are pursuing this observation to understand the mechanistic basis of this cooperation. Thus, pathways that regulate polarity may have a critical role during both initiation and progression of precancerous lesions.

Pathways and interactions specific to three-dimensional organized epithelia: None of the above-mentioned relationships between ErbB2 and polarity genes were observed in cells cultured as two-dimensional monolayers. This prompted us to identify pathways and interactions that are unique to cells within a three-dimensional context, because such analysis can aid in identification of novel targets that were not realized by experiments in two dimensions. Microarray analysis of changes in gene expression led to identification of several gene expression changes unique to three-dimensional culture. Our initial studies have identified two novel interactions, one involving a protein tyrosine phosphatase, PTPRO, and another involving a receptor tyrosine kinase, EphA2. How these molecules, and their targets, regulate ErbB2-induced disruption of three-dimensional structures is currently being pursued.

Thus, using a unique set of approaches we have identified novel molecular pathways that critically regulate development and progression of precancerous lesions. I believe that developing ways to treat and control precancerous lesions will be an excellent complement or an alternative to the current approaches to control late-stage cancer.

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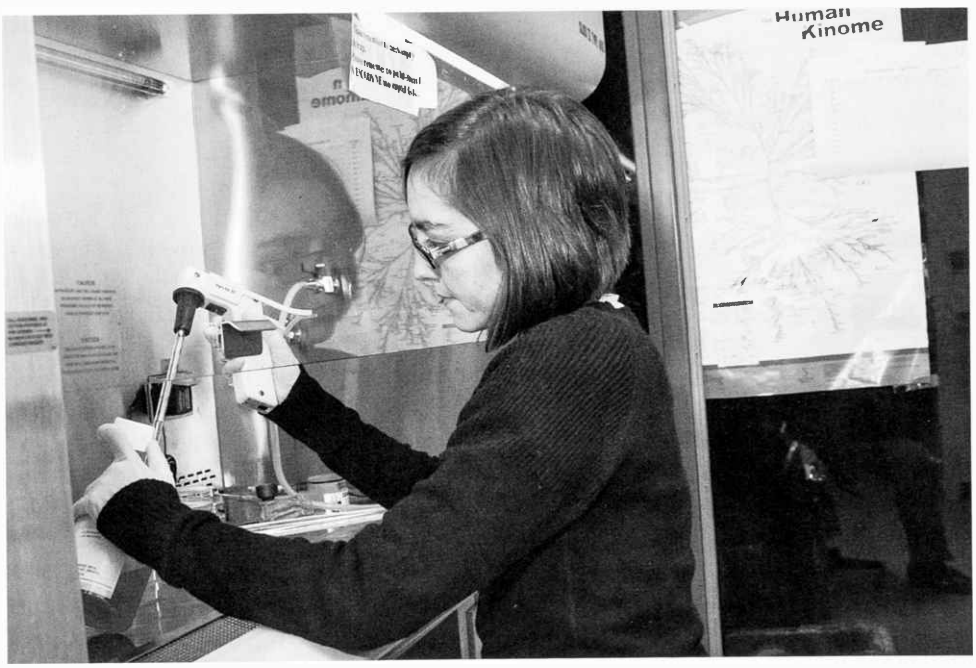

Victoria Aranda Calleja

CELL BIOLOGY OF THE NUCLEUS

D.L. Spector M. Camiolo, Y.-C.M. Chen, L. Denis, Y. Fang, S. Hearn, M. Hübner, N. Hübner, I. Kumaran, T. Nakamura, K.V. Prasanth, H. Sunwoo, R. Thakar, J. Wilusz, M. Yuan, R. Zhao

Most cellular processes can trace their beginnings to the nucleus where a gene is activated, resulting in the production of an RNA molecule that must get processed and transported to the cytoplasm. Although much biochemical information is available regarding many of the factors involved in gene expression, the spatial and temporal aspects of gene expression and the dynamics of the nuclear domains that the gene expression machinery occupies are less well understood. During the past year, we have focused a significant amount of our efforts on two main areas: (1) examining the mechanism by which epigenetic marks are transmitted to daughter cells through the cell cycle and (2) characterizing the dynamics of nuclear bodies in mammalian cells and in living *Arabidopsis* plants. In addition, we have continued our studies focused on characterizing the role of noncoding RNAs in regulating gene expression.

Polycomb Bodies and Gene Regulation

M. Hübner, N. Hübner

The expression of the approximately 25,000 different protein coding genes in the human genome needs to be tightly regulated in a spatial and temporal manner that is also cell-type specific. This regulation must be both flexible in order to allow for plasticity during development and differentiation and stable in order to maintain cell identity during the lifetime of an organism. Genes encoding proteins that are not required in a certain cell type become inactivated through epigenetic mechanisms. These mechanisms comprise chromatin modifications such as histone methylation, ubiquitylation, and acetylation, as well as DNA methylation. Polycomb group (PcG) proteins are essential components of the epigenetic silencing mechanism. PcG proteins are found in at least two complexes called polycomb repressive complexes PRC-1 and PRC-2. The PRC-2 complex is thought to be involved in the establishment of silencing, whereas the PRC-1 complex is thought to be involved in the maintenance of silencing. Members of the PRC-1 complex localize in nuclear structures called polycomb bodies. To date, little is known about the dynamics and function of these bodies and how they contribute to the inherited epigenetic state of a gene. Using a human cancer cell line stably expressing the yellow fluorescent protein (YFP)-tagged PRC-1 member Bmi1, we have shown that PcG bodies are remarkably static structures that show little movement and remain inside very confined spaces in interphase nuclei. Interestingly, PcG bodies remain associated with chromatin throughout mitosis (Fig. 1). Starting in prophase, PcG bodies appear as doublets, presumably occupying corresponding positions on sister chromatids. The doublets align on the metaphase plate and during anaphase move with the chromatids to the spindle poles. Consequently, PcG bodies occupy the same genes in the daughter cells, thereby providing a means for the mitotically stable inheritance of the silenced state of genes. We demonstrate that throughout mitosis, PcG bodies colocalize with histone H3K27 dimethylation and trimethylation but not K27 monomethylation or K9 trimethylation. In S phase, PcG bodies colocalize with dimethylated and trimethylated H3K27 and occupy mid-to-late replicating chromatin. We sought to induce the formation of a new PcG body at a defined genetic locus using our previously established cell line (Janicki et al., *Cell 116:* 683–698 [2004]). Recruitment to that locus of a LacI fusion of the PRC-2 histone methyltransferase EZH2, but not a catalytically inactive mutant, leads to H3K27 di- and trimethylation of the locus. Importantly, H3K27 trimethylation leads to the colocalization of the PcG body component Bmi1 with the locus. We are currently investigating the functional consequences that PcG body formation has on gene expression and the epigenetically inheritable nature of gene silencing by PcG proteins.

Our goal is to understand the mechanisms leading to the induction and maintenance of the epigenetic regulation of gene expression. A loss of regulation results in the aberrant expression of genes and is often

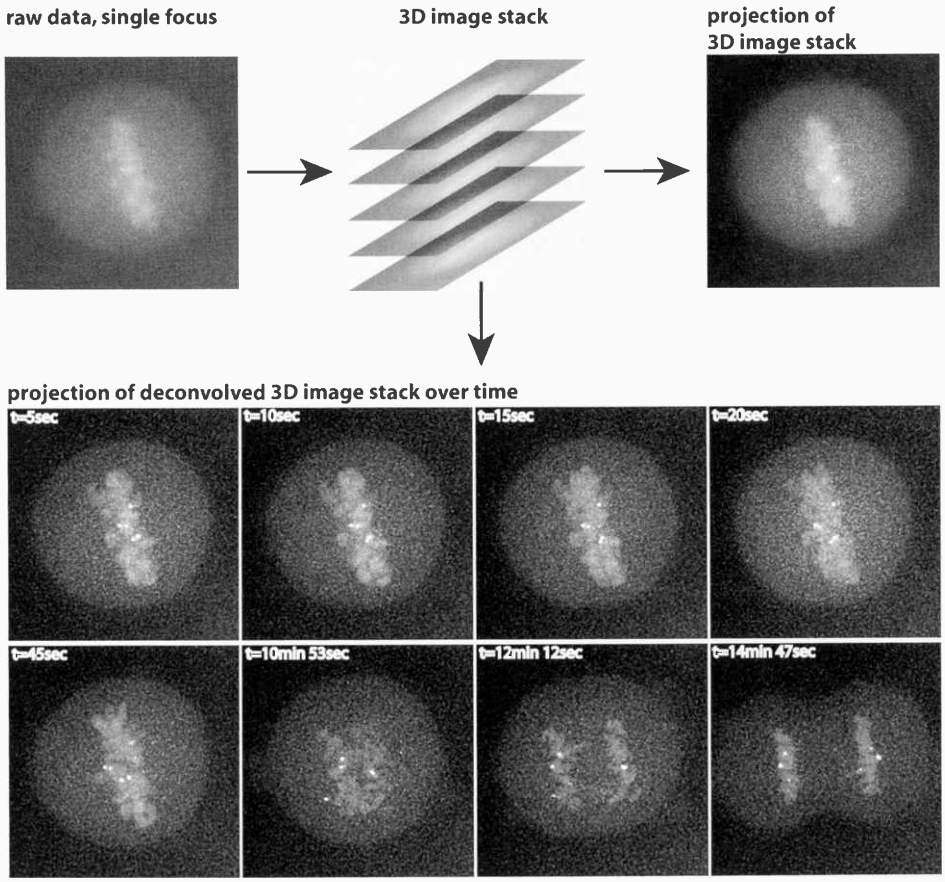

FIGURE 1 Live-cell imaging of a mitotic human U2OS osteosarcoma cell expressing YFP-tagged polycomb protein Bmi1 (*dots*). Chromatin is visualized by mRFPcherry-H2B. A three-dimensional image stack (five images, 1 μm spacing) was acquired every 5 seconds and images from each time point are shown as projections. (From Michael Hübner.)

the cause of uncontrollable cell proliferation. In fact, a number of mutations in polycomb group proteins have been associated with human cancers. Therefore, our work will help to elucidate the basic underlying principles of gene expression with the ultimate goal of developing approaches to regulate genes whose expression has been altered in disease.

Dynamics of PML Nuclear Bodies during the Cell Cycle

Y.-C.M. Chen [in collaboration with C. Kappel and R. Eils, DKFZ, Heidelberg, Germany]

Although the cell nucleus was once thought of as an amorphous milieu containing little organization or functional compartmentalization, more contemporary studies have identified an increasing number of specialized domains or subnuclear organelles. In some cases, these domains are dynamic structures, exhibiting rapid protein exchange between the domain and the nucleoplasm. To date, more than 12 different nuclear domains have been identified. Ongoing studies in the laboratory are focusing on two of these nuclear domains, promelocytic leukemia (PML) nuclear bodies (PML NBs) and a newly identified nuclear body in plant cells (see next section).

PML NBs have received much attention because they display a more dispersed intranuclear pattern in blast cells from individuals with acute PML carrying a t(15,17) translocation involving a fusion of the PML protein and the retinoic acid receptor-α. Retinoic acid or arsenic trioxide treatment can induce complete remission of the disease and results in reformation of the PML bodies. In addition, PML NBs are modulated by interferon or heat shock treatments, and they are associated with the sites of initial DNA tumor virus

transcription/replication in infected cells and are subsequently disrupted at later stages in the infectious cycle. A clear function for these bodies has as yet not been established; however, roles in transcriptional regulation, as storage sites regulating the levels of active proteins within the nucleus or as sites of active proteolysis, have been pursued.

To reveal the function of these NBs, we have been particularly interested in their dynamics and how they are segregated to daughter cells during the process of cell division. We developed a U2OS cell line stably expressing two different proteins that localize to PML NBs: ECFP-PML1 (PML-V) and EYFP-Sp100. Live-cell imaging (4D) revealed that 40% of PML NBs exhibit rapid directed movement in prophase cells as compared to only 12% in interphase nuclei. During this period of time, and prior to nuclear envelope breakdown, the dynamic PML NBs move freely within the interchromatin space and Sp100 is still present within the PML NBs. At later time points, PML NBs fuse to form mitotic PML NBs, which contain significant amounts of PML protein and low levels of Sp100. From prometaphase to early G_1, rapid directed movements are significantly reduced and 90% of the PML NBs move by diffusion. After cytokinesis, Sp100, Daxx, and Hp1-α enter the daughter nuclei, prior to the entry of PML protein; however, the formation of PML NBs is initiated only after entry and multimerization of the PML protein. Newly assembled PML NBs appear only after a functional nuclear membrane has been reformed. These studies suggest that chromatin condensation, upon entry into prophase of mitosis, results in a loss of tethering between regions of chromatin and PML NBs, thereby resulting in their increased dynamics. Upon chromatin decondensation at the end of mitosis, PML NB formation initiates in association with HP1-α. Future studies will examine the molecular interactions necessary for the formation of PML NBs.

NBs Involved in Pri-microRNA Processing in *Arabidopsis thaliana*

Y. Fang

microRNAs (miRNAs) are important for regulating gene expression in muticellular organisms. miRNA processing is a two-step process; in animal cells, the first step is nuclear and the second step is cytoplasmic, whereas in plant cells both steps occur in the nucleus via the enzyme Dicer-like1 (DCL1). The miRNA processing pathway in *Arabidopsis* involves several proteins including a zinc finger domain protein Serrate (SE), DCL1, a double-stranded RNA (dsRNA)-binding domain protein Hyponastic Leaves1 (HYL1), a methyltransferase and dsRNA-binding domain protein Hua Enhancer (HEN1), and the slicer Agronate1 (AGO1). However, little is known about the cellular basis of miRNA biogenesis. To determine the subnuclear organization of proteins involved in miRNA biogenesis, we generated transgenic *Arabidopsis* plants expressing YFP- or cyan fluorescent protein (CFP)-tagged SE, DCL1, and HYL1, each under the control of their endogenous promoters. The functionality of each fusion protein was determined by complementation of mutant phenotypes. Using live-cell imaging, we found that DCL1 and HYL1 colocalize in discrete nuclear bodies measuring 0.2–0.8 μm in diameter, as well as being diffusely distributed throughout the nucleoplasm. The number of bodies per nucleus ranged from one to four. A population of DCL1 bodies (~60%) localizes in close proximity to, but not within, nucleoli. These bodies, which we refer to as nuclear dicing bodies (D-bodies), differ from Cajal bodies which contain components involved in small interfering RNA (siRNA)-directed cytosine methylation at endogenous DNA repeats. Importantly, mutated DCL1 (DCL1-9), in which the carboxy-terminal 73 amino acids of DCL1 (second dsRNA-binding domain) were truncated, resulting in impaired function in miRNA processing, failed to target to D-bodies. If the D-bodies represent sites of miRNA biogenesis, primary (pri)-miRNAs should be recruited to these sites. To directly track a pri-miRNA in the nucleus, an amplified 245-bp genomic DNA fragment flanking mi173 was fused upstream to 24 tandem MS2 translational operators (MS2 repeats) and introduced into tobacco cells by coinfiltration with MS2 coat protein–YFP (MS2-YFP) and DCL1-CFP fusion proteins. Upon induced expression of MS2-YFP, its binding with the MS2 repeats allows the pri-miRNA to be localized in the cells, whereas DCL1-CFP under the control of its endogenous promoter was used as a marker for D-bodies. pri-mi173 was observed to be enriched in D-bodies, indicating that pri-miRNAs are recruited to D-bodies where the machinery for their processing is enriched. If D-bodies are the nuclear sites for the dicing reaction of DCL1, protein-protein interaction of the respective proteins (DCL1 and HYL1) should occur within D-bodies. We used bimolecular fluorescence complementation (BiFC) to determine the locations in the cell nucleus where these proteins interact. BiFC signals between DCL1, HYL1,

and SE were observed in D-bodies, while the signal in the nucleoplasm was very low, suggesting that these proteins predominantly interact in the nuclear D-bodies. In contrast, mutant DCL1-9 showed no interaction with SE, HYL1, or DCL1. On the basis of these data, we propose that D-bodies are crucial for orchestrating pri-miRNA processing in the nuclei of plant cells. Our results provide significant insight into the function of this nuclear body and to our understanding of how the pri-miRNA processing machinery is organized in the cell nucleus of living plants.

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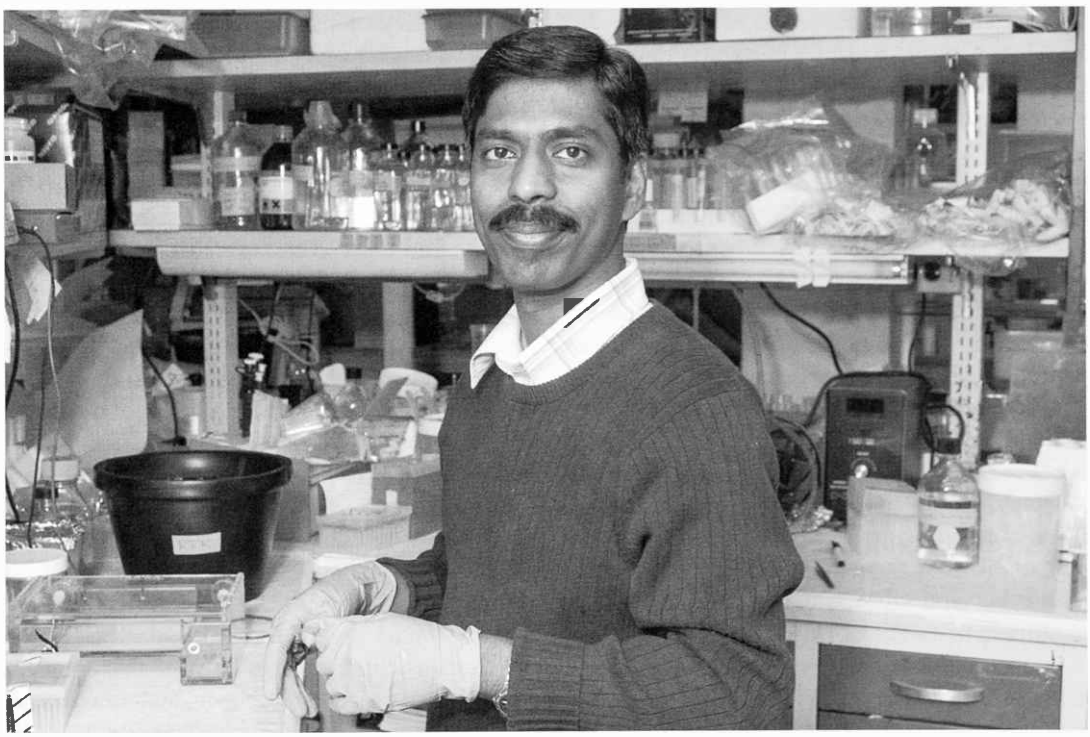

Ileng Kumaran

MOLECULAR BIOLOGY OF PAPILLOMAVIRUSES

A. Stenlund X. Liu
S. Schuck

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

An impediment to the study of papillomaviruses has been the inability to define simple in vitro cell culture systems for analysis of the viral life cycle. These viruses normally require specialized differentiating cells that only with difficulty can be generated in cell culture. However, for bovine papillomavirus type 1 (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.

MUTATIONAL ANALYSIS OF THE E1 DBD SURFACE

The DNA-binding domain (DBD) of the E1 protein clearly is responsible for the site-specific DNA binding of the E1 protein. This site-specific DNA binding is responsible for recognition of the origin of DNA replication in the viral genome. Furthermore, on the basis of both structural and biochemical studies of the E1 DBD, the DBD also provides the dimerization surface for the E1 protein and also contains an interaction surface for the DBD from the E2 protein. In addition, imaging analysis of, for example, SV40 T antigen indicates that the E1 DBD likely takes an active part in formation of large complexes such as the double hexamer, which appear to be the entity that unwinds the origin of DNA replication.

To identify additional functions in the DBD we have performed a complete surface mutagenesis of the E1 DBD. On the basis of the high-resolution structure

of the E1 DBD, we have substituted 63 surface residues for alanine, avoiding areas known from the structural analysis to be involved in DNA binding and dimerization. After screening these E1 mutants for expression and for in vivo DNA replication activity, we were able to identify 16 mutants with defects in DNA replication. Four of these mutants were defective for expression in vivo, whereas the remaining 12 gave rise to wild-type levels of full-length protein.

To determine which biochemical functions might be defective in these mutants, we expressed the 12 mutant E1 proteins in *Escherichia coli* and purified them. We tested these mutants in different in vitro assays related to DNA replication. From the behavior of the mutants in these assays, we could group these mutants into four categories depending on which aspect of DNA replication was affected by the mutation. The results from in vitro DNA replication were particularly informative. Of the 12 mutants, 8 had severe defects for in vitro DNA replication. These mutants clearly had biochemical defects for replication-related processes. The remaining four mutants (Group I) still had activity for replication in vitro. These mutants are therefore likely to affect processes that are only required for DNA replication in vivo, such as regulation of viral DNA replication, for example, by cell cycle mechanisms.

Despite our efforts to avoid residues involved in DNA binding, four substitutions (Group II) had slight defects for DNA binding, most likely because these mutants affected the structure of the E1 DBD. Although these four mutants may also affect other activities, such effects would be obscured by the defect for DNA binding. A third group (III) consists of two mutants that have wild-type activity for all of the biochemical activities that we can measure, with the exception of in vitro DNA replication. These two mutants are likely to affect a function required only for DNA synthesis, such as the interaction with cellular DNA replication factors. The final and fourth group consists of two mutants that have specific defects in the formation of the double trimer and double hexamer. These mutants also show the expected defects for unwinding activity.

It is apparent that there are many ways to disable the DNA replication activity of the E1 protein, which is a testimony to the multitude of diverse functions that this class of protein encodes. It is surprising, however, that so many of these activities appear to depend to some extent on the E1 DBD. This provides good evidence that the E1 DBD takes part in many biochemical activities that previously have been considered to reside in other domains.

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

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

To determine the specific role of this residue, we substituted the histidine with a wide range of residues, including A, V, L, R, N, M, F, and Y, and tested these mutant E1 proteins for DNA replication in vivo and in vitro. In vivo DNA replication assays demonstrated that only H507F had significant replication activity. DNA replication assays in vitro, however, showed that both H507F and H507Y had activity similar to that of the wild-type protein, whereas the rest of the substitutions lacked activity. To determine at which step in initiation of DNA replication the H507 has an essential role, we tested the H507 substitutions in a variety of assays that we have developed. By using EMSA (electrophoretic mobility-shift assay) we determined that the wild-type E1, H507F, and H507Y all could form the DT complex. However, only the wild-type E1 and H507F could form the DH helicase. Because of the location of the β-hairpin on the inside surface of the hexameric ring, we also tested all of the mutants for DNA helicase activity.

Although we could observe slight (less than twofold) differences in helicase activity of some H507 substitutions compared to wild-type E1, a helicase defect is not responsible for the replication defect of these substitutions, since H507F, which is the only mutant with wild-type replication activity, had among the lowest activities. In contrast, mutation of the neighboring residue, K506, resulted in complete loss of DNA helicase activity, demonstrating that this particular residue is required for the helicase.

These results provide a clear demonstration that the β-hairpin is directly involved in both local ori melting and helicase activity of E1 and that mutation of neighboring residues distinguishes between these two activities. H507 is required specifically for local

ori melting, whereas the adjacent residue, K506, is required for both local melting and DNA helicase activity. Consistent with these results, an alignment of known SF3 helicases demonstrates that K506 is well conserved in both papovaviruses and parvoviruses, in agreement with the importance of this residue for the helicase activity of these proteins (Fig. 1A). In contrast, H507 is only conserved in the papovavirus group. This distinction between the papovavirus and parvovirus groups is consistent with the fact that papovaviruses have double-stranded DNA genomes, whereas the parvoviruses have single-stranded DNA genomes and therefore do not require a local ori melting activity.

These results now provide an explanation for how the local melting activity and the helicase activity are linked (Fig. 1B). In the DT, which initially melts the double-stranded DNA, the β-hairpin, and specifically H507, is involved in melting the double-stranded DNA, most likely through a direct interaction with the DNA. As the DNA is melted, additional E1 molecules are recruited to the complex and as the helicase is formed, K506 in the β-hairpin now contacts the single-stranded DNA in the helicase. This scheme provides an explanation for the transition between the local ori melting activity and the helicase activity and provides a novel mode of helicase loading in that the E1 helicase, in essence, loads itself through the DT precursor, which melts the DNA template.

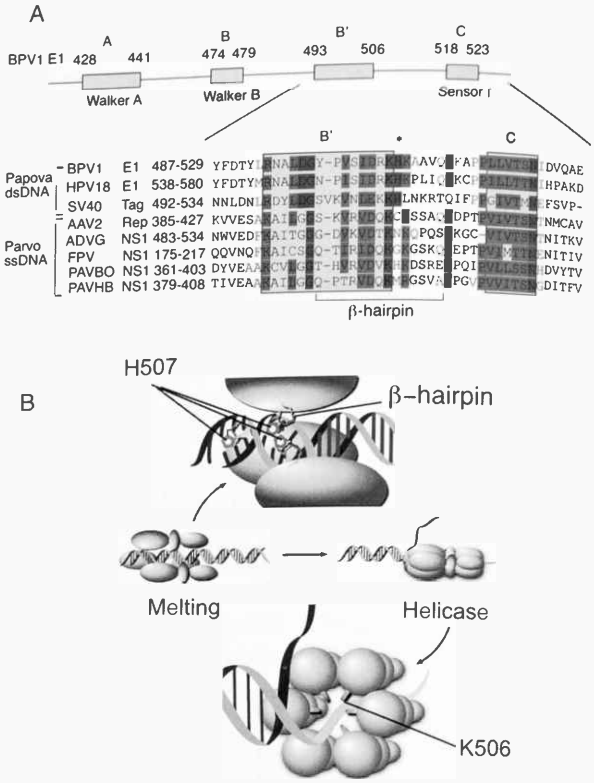

FIGURE 1 (*A*) The β-hairpin generates the substrate for helicase loading. Sequence alignment of the B′ and C motifs of representative members of the SF3 helicase family. Shown above the alignment is the relative location of SF3 signature motifs in BPV E1. The number above each motif corresponds to the starting and end residue number of each motif in BPV E1. The residue at the tip of the β-hairpin (H507) is marked with an asterisk. Included in the comparison are BPV-1 E1 (bovine papillomavirus type-1 E1 protein), HPV-18 E1 (human papillomavirus type-18 E1 protein), SV40 TAg (simian virus 40 large T antigen), AAV-2 Rep (adeno-associated virus type-2 Rep 40 protein), ADVG NS1 (Aleutian mink disease parvovirus [strain G] NS1 protein), FPV NS1 (feline panleukopenia virus NS1 protein), PAVBO NS1 (bovine parvovirus NS1 protein), and PAHVB NS1 (human parvovirus B19 NS1 protein). (*B*) Model for the transition between the E1 DT complex, which melts DNA, and the E1 DH, which unwinds DNA. E1 forms a DT and H507 at the tip of the β-hairpin intercalates into the DNA in the minor groove. The DT-DNA complex is the substrate for formation of the DH, which forms on one of the melted strands, and K506 at the tip of the hairpin interacts with single-stranded DNA. For simplicity, only one trimer and one hexamer is shown.

MECHANISM OF LOCAL ORI MELTING IN PREPARATION FOR INITIATION OF DNA REPLICATION

Although local ori melting is an essential process in all organisms, surprisingly little information is available about the process and the types of activities that perform the melting. For many years, it has been known that in prokaryotes, the protein DnaA is responsible for ori melting, although the mechanism is not understood. In eukaryotes, no activity has been identified that melts the ori in preparation for DNA replication. Viral initiator proteins such as the E1 protein from papillomaviruses have long been known to be responsible for local ori melting, as detected by oxidation of unbase-paired T residues in the ori, although the mechanism is not known.

To define the local ori melting process, we have examined this process in detail, including the sequence dependence for local melting. We have determined that local melting is not a single event but consists of several events that happen in sequence. The earliest event is carried out by a DT of E1. The helicase domain in the E1 protein specifically recognizes a stretch of six T-A base pairs that flank the E1-binding site.

Surprisingly, this recognition occurs in the minor groove and results in local melting of the six T-A base pairs. As expected from this result, the six T-A base

pairs are also essential for formation of the DH, for unwinding of the ori, and for DNA replication in vivo. In addition to melting of the six T-A base pairs, which likely results from a direct interaction between the E1 β-hairpin and the six T-A base pairs, the six T-A base pairs also direct melting 5–6 bp away from the T-A base pairs, toward the E1 binding site (BS). This melting is likely generated by interaction of the oligomerization domain in E1 and the DNA. These events account for melting of about 15 bp on each side of the E1 BS. The E1 BS is clearly melted by a different mechanism, most likely by untwisting of the DNA by the two trimers.

Together, these data suggest a simple model for how E1 may be able to melt large regions of DNA using simple means. One consequence of the dual interactions of each E1 molecule with DNA is that each interaction need only be responsible for melting of a few base pairs since a total of six E1 molecules and 12 interactions are involved in melting in the E1 DT.

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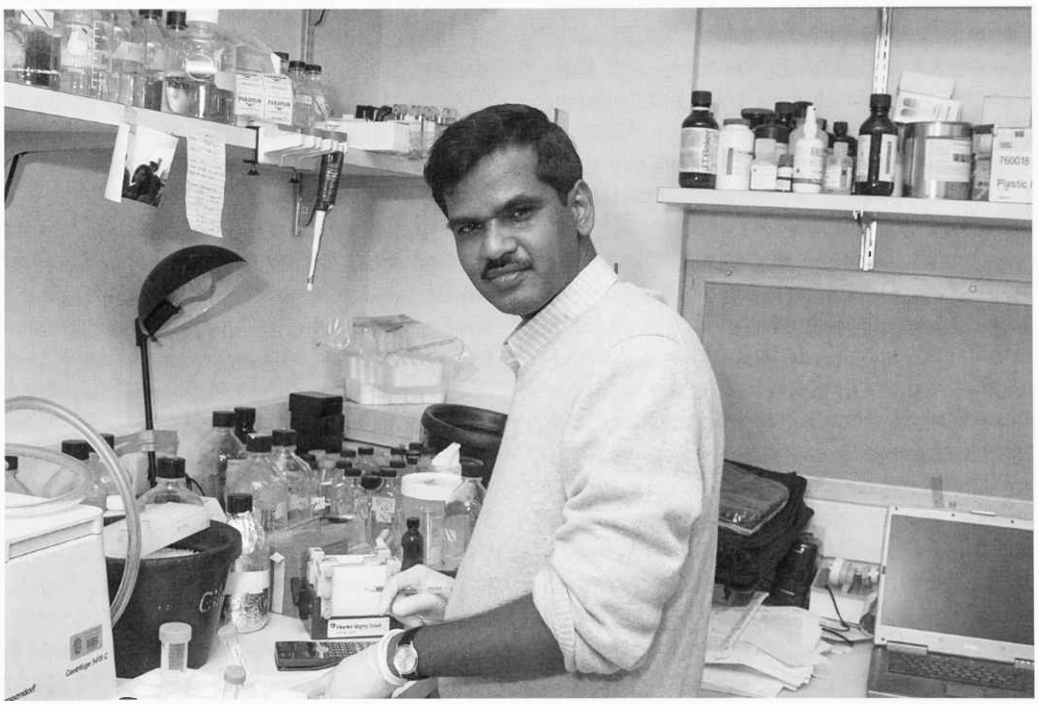

Mayilvahanan Shanmugam

DNA REPLICATION AND CHROMATIN INHERITANCE

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Chromosomal DNA replication in eukaryotes is a highly regulated process that involves the licensing of chromosomes prior to S phase for subsequent initiation of DNA replication at origins. The licensing occurs at specific sites on DNA by the formation of prereplicative complexes (pre-RCs). A central component of the pre-RC is the origin recognition complex (ORC) that recruits Cdc6, Cdt1, and MCM proteins to form a stable pre-RC at each origin. This assembly occurs in telophase of mitosis in rapidly proliferating cells or in G_1 phase in cells stimulated to replicate from a period of quiescence. Later in G_1 phase, upon commitment to cell division, cyclin-dependent kinase (CDK) and Cdc7-Dbf4 protein kinase (DDK) are activated and stimulate assembly of preinitiation complexes that then recruit the DNA polymerases and their accessory factors to replicate the DNA from each origin. Origin activation, or firing, occurs in a predetermined temporal order that is specific to each cell type. Moreover, DNA replication is spatially regulated in the S-phase nucleus.

Previous genetic studies have shown that the Cdc7-Dbf4 protein kinase (called DDK for Dbf4-dependent protein kinase) is required for the initiation of DNA replication at each origin that is licensed by a pre-RC. The action of DDK is triggered by the synthesis of the Dbf4 subunit in late G_1 phase of the cell division cycle. From our previous research, and that of other investigators, it was known that DDK can phosphorylate the MCM (minichromosome maintenance) proteins in vitro. The six different, but sequence-related, MCM subunits form a hexamer structure that is similar to the virus-encoded DNA helicases such as SV40 T antigen or the papillomavirus E1 (see reports by A. Stenlund and L. Joshua-Tor). Genetic studies by other investigators had demonstrated weak suppression of a *cdc7* null allele by a mutation in the *MCM5* gene (*mcm5*^{bob1}), suggesting that activation of the MCM hexameric complex is a downstream consequence of activation of DDK. The problem was that we had demonstrated that of the six MCM subunits, Mcm5 was not a substrate for DDK in vitro, whereas Mcm2, Mcm4, Mcm6, and Mcm7 were good substrates in vitro. Prior attempts to map the DDK phosphorylation sites and demonstrate that they were important DDK phosphorylation sites in vivo had not resulted in conclusive evidence that MCM subunits were the key DDK targets. Our experiments during the past year have addressed this issue and have elucidated a mechanism for how DDK recognizes some of its key substrates.

One of the proteins that binds the MCM hexamer is Cdc45, a protein that we had previously shown to be loaded onto origins of DNA replication only after activation of both CDK and DDK kinases in late G_1 phase of the cell cycle. Immunoprecipitation experiments showed that Cdc45 associated tightly with the MCM hexamer in S phase and only on chromatin. Soluble Cdc45 did not associate with the MCM complex. More interestingly, the Mcm4 subunit in the Cdc45-MCM complex was hypophosphorylated. The appearance of the phosphorylated form of Mcm4 was dependent on DDK activity in vivo. The Mcm4 subunit of MCM was a substrate for DDK phosphorylation in vitro, and we mapped the phosphoacceptor sites to the amino-terminal 174 amino acids of the Mcm4 subunit. This region is predicted to be unstructured and is rich in serine (S) and threonine (T) residues (S/T constitute 29% of the first 174 amino acids in Mcm4 in *Saccharomyces cerevisiae*) and is also rich in acidic amino acid residues. This 174-amino-terminal fragment was sufficient as a substrate for DDK, but the phosphorylation was very weak compared to the full-length Mcm4 protein, and multiple phosphorylation sites were not observed. A small region of the Mcm4 protein, lying immediately colinear to the amino-terminal region, was found to be a binding site for the DDK, and when this binding site was combined with the S/T-rich region, the phosphorylation by DDK was as efficient as the full-length Mcm4 protein. Moreover, phosphorylation on multiple sites was

shown to be processive, with the DDK enzyme remaining bound to the substrate while phosphorylating multiple sites in the adjacent amino-terminal 174 amino acids. Thus, phosphorylation of Mcm4 requires the binding of DDK to a region of Mcm4 that is itself not a phosphorylation target, but which promoted phosphorylation of an adjacent S/T-rich sequence. Foreign S/T-rich amino acid sequences could substitute in vitro and in vivo for the S/T-rich domain of Mcm4, as long as the DDK-binding site was intact.

Mutations in the Mcm4 amino terminus were defective for loading Cdc45 onto chromatin, and this phenotype could be complemented by expression of Mcm4 derivatives containing artificial phosphoacceptor sites or by conversion of S/T residues to aspartic (D) residues, thereby mimicking phosphorylation. These results demonstrated that Mcm4 (and possibly Mcm2 and Mcm6) phosphorylation is a functional target of DDK in vivo and that this phosphorylation was key to recruitment of Cdc45 to the MCM complex. Activation of MCM protein as a DNA helicase has recently been shown by other investigators to require Cdc45 and the GINS protein complex, and thus, we have suggested that activation of MCM helicase requires DDK. These studies have uncovered a key target of DDK, but there are almost certainly other substrates that are important for cell cycle progression. We have used the biochemical information gained by studying the Mcm4 target to search for other DDK targets in a biochemical screen. To date, a number of interesting candidates have emerged.

In addition to regulation at the G_1/S-phase transition of the proteins that are involved in DNA replication, the production of the building block precursors for DNA synthesis is also highly regulated. These deoxyribonucleoside triphosphates (dNTPs) that are used by DNA polymerases are synthesized from ribonucleoside diphosphates (NDPs) by the enzyme riboncleotide reductase (RNR) to produce dNDPs that are rapidly phosphorylated to dNTP. RNR in eukaryotes is one of the most highly regulated enzymes known. Production of the RNR1 subunit is controlled by transcription of the *RNR1* gene only in G_1 phase, and the protein itself is proteolytically degraded after S phase is completed. Furthermore, a protein inhibitor of RNR called Sml1 blocks any RNR activity present in G_1 phase. Finally, the RNR enzyme is controlled by feedback inhibition by one of the end products of the reaction, dATP. Thus, cells go to extraordinary lengths to make sure that dNTPs are only produced in S phase when the synthesis of chromosomal DNA occurs. This raised the obvious question of why was all of this regulation necessary?

We investigated the consequences of deregulation of RNR so that it could produce high levels of dNTP throughout the cell division cycle in the yeast *S. cerevisiae*. This was achieved by the controlled overexpression of an Rnr1 subunit that had a mutation in the dATP feedback control site (rnrD57R). When this protein was induced, the high levels overcame the Sml1 inhibition and sufficient enzyme was active to produce 30-fold higher dNTPs than present in normal cells. This high dNTP level dramatically slowed entry into S phase and inactivated the checkpoint regulation in response to DNA damage. Checkpoint control ensures that cells do not progress through S phase when DNA damage is present in cells. If this regulatory pathway is blocked, DNA damage is copied, thereby promoting genome instability.

Moderately high, constitutive dNTP levels in yeast cells were lethal when the number of active origins of DNA replication was reduced by a mutation in one of the origin recognition complex (ORC) subunits. The particular ORC subunit mutants used reduce the number of origins that are active in any given cell, but normally, cells survive because of the redundancy of origins of DNA replication in chromosomes. When, however, dNTP levels were moderately overexpressed, these cells died.

These results suggest that dNTP levels regulate the initiation of DNA replication and progression through S phase. DNA-damage checkpoint pathways normally monitor the progression of chromosome replication through S phase. Therefore, one possibility is that dNTP directly regulates the activity of these checkpoint pathways. Alternatively, dNTP levels could directly regulate pre-RC proteins to control the process of initiation of DNA replication. We have shown that both ORC and Cdc6 are ATPases required for assembly of pre-RCs and for the subsequent steps of initiation of DNA replication. dNTP activity could control the ATPase activity of one or more of the pre-RC proteins, thereby linking DNA synthesis to production of the building blocks used by DNA polymerases. A final possibility is that the dNTP may control one of the regulators of progression into or though S phase, such as DDK. Since DDK regulates the initiation of DNA replication at each origin of DNA replication and also monitors progression through S phase by its association with the checkpoint protein Rad53, it is possible that the activity of DDK may be controlled by dNTP levels. Such possibilities are under investigation.

In addition to studying the biochemistry and control of DNA replication in yeast, we have continued our studies on the role of ORC and other pre-RC proteins in human cells. We have shown that unlike the yeast ORC, the human complex is dynamically regulated throughout the cell division cycle. Of particular interest is the role of human Orc1 in the process of assembly and control of ORC localization during the cell cycle. In human cells, ORC subunits also participate in DNA replication, centrosome duplication, and the function of centromeres and kinetochores during mitosis and during cytokinesis. The human cell ORC is a dynamic complex during the cell division cycle. For example, the Orc1 subunit is degraded by a Skp2-dependent, ubiquitin-mediated process at the G_1/S-phase transition. Interestingly, Orc1 localization in the nucleus of G_1-phase cells varies as cells progress toward commitment to cell division and S phase. The spatial and temporal patterns of Orc1 localization in the nucleus anticipate the pattern of MCM localization and DNA replication in S phase after activation of the CDK and DDK. These studies show that ORC participates in spatial-temporal patterning of the nucleus in newly born cells.

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Yi-Jun Sheu

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 and differentiation. Protein phosphorylation is a reversible, dynamic process in which the net level of phosphate observed in a target substrate reflects the 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. We are integrating a variety of experimental strategies to characterize the physiological function of members of the PTP family. Disruption of normal patterns of tyrosine phosphorylation has been implicated in several human diseases. Therefore, insights into the mechanisms involved in modulating PTP function may ultimately yield important information to help counter such diseases.

During the last year, Jannik Andersen left the lab to take up a Project Leader/Senior Scientist position at Merck Research Labs, and Zhong Yao joined the University of British Columbia as a Research Associate. Li Li joined the lab as a postdoctoral fellow from Albert Einstein School of Medicine.

DEFINING THE "PTPOME"

Unlike the protein kinases, which are derived from a common ancestor, the protein phosphatases have evolved in separate families that are structurally and mechanistically distinct. During the past couple of years, we defined the composition of the PTP family in humans and other organisms. The PTPs, which are characterized by the active-site signature motif $HC(X)_5R$, are divided into the classical pTyr-specific phosphatases and the dual specificity phosphatases (DSPs). There are 38 classical *ptp* genes encoding receptor-like proteins, which have the potential to regulate signaling directly through ligand-controlled protein dephosphorylation, as well as nontransmembrane cytoplasmic enzymes. There are approximately 65 genes encoding a heterogeneous group that are broadly described as DSPs. In general, they share the same catalytic mechanism as the classical PTPs, but the construction of the DSP active site allows them to accommodate pSer/pThr residues as well as pTyr residues in proteins. Nonetheless, in terms of physiological function, the "DSPs" may actually show preference for either Tyr or Ser/Thr residues. Some even target nonprotein substrates such as inositol phospholipids and RNA.

Overall, there are about 100 human *ptp* genes, compared to 90 human protein tyrosine kinase (PTK) genes, suggesting similar levels of complexity between the two families. However, the number of genes only illustrates the minimal level of complexity in the family, with additional diversity introduced through use of alternative promoters, alternative mRNA splicing, and posttranslational modification. This structural diversity is indicative of the functional importance of the PTPs in the control of cell signaling. It is now apparent that the PTPs have the capacity to function both positively and negatively in the regulation of signal transduction. Furthermore, the PTPs have the potential to display exquisite substrate, and functional, specificity in vivo. Therefore, the definition of the "PTPome" provides a foundation for detailed analyses of the structure, regulation, and physiological function of the members of the PTP superfamily as critical modulators of signal transduction.

RNA interference is a powerful approach for conducting loss-of-function analyses as a method for examining the physiological function of particular proteins in mammalian cells. Having defined the composition of the PTP superfamily in humans during the past year, we have now designed and constructed a library of short hairpin RNA (shRNA) constructs that will allow us to examine systematically the function of individual PTPs in a variety of cell systems. We are using the pMLP retroviral expression vector produced in Scott Lowe's lab here at CSHL. The construct utilizes a murine stem cell virus (MSCV) backbone. The hairpin is incorporated into the sequence of the human

microRNA-30 (miR30), which facilitates processing of the expressed hairpins. A puromycin resistance marker and green fluorescent protein (GFP) are also expressed from an internal ribosome entry site (IRES), to enrich small interfering RNA (siRNA) expressing cells and generate stable lines. The construct we will use, in which the modified miR30 is expressed from the viral long terminal repeat (LTR), has been shown to work effectively for both transient and stable expression. In future follow-up studies, we may also use a construct from which the shRNA is expressed conditionally.

We are working in collaboration with Senthil Muthuswamy, also here at CSHL, in our first application of this library. We are using his three-dimensional culture system to explore the effect of attenuating PTP expression on MCF10A mammary epithelial cell differentiation. This cell culture system recapitulates several aspects of mammary gland architecture in vivo and has been used to model the biological activities of cancer genes. Our preliminary results reveal that attenuation of expression of receptor PTPs LAR and PTPRO leads to abnormal development of acini. In the latter case, the effects of disruption of PTPRO were accentuated in MCF10A cells that overexpress the oncoprotein tyrosine kinase ErbB2 (HER2), which is overexpressed in approximately 25% of breast tumors. Thus, PTPRO may have an as yet undefined role in down-regulating signaling pathways triggered by ErbB2. Currently, we are completing a screen in which we are testing the effects of attenuating expression of each of the PTPs on acinar development in the three-dimensional (3D) culture of MCF10A cells. I anticipate that this approach will serve as a first step to shed new light on potential roles of PTPs in the etiology of breast cancer.

REGULATION OF PTP FUNCTION BY REVERSIBLE OXIDATION

As might be anticipated for a family of enzymes that have such critical roles in the regulation of cell signaling, the activity of PTPs is tightly controlled in vivo. Recently, the production of reactive oxygen species (ROS), such as hydrogen peroxide, and the resulting posttranslational modification of PTPs by reversible oxidation has been implicated in the regulation of tyrosine-phosphorylation-dependent signaling pathways initiated by a wide array of stimuli, including growth factors, hormones, cytokines, and cellular stresses. The signature motif of the PTP family, [I/V]HCxxGxxR[S/T], contains an invariant Cys residue, which, due to the unique environment of the active site, is characterized by an extremely low pKα. The low pKα promotes the function of this Cys residue as a nucleophile in catalysis but renders it highly susceptible to oxidation with concomitant abrogation of nucleophilic function and inhibition of PTP activity. Work from several labs has now established that multiple PTPs are transiently oxidized in response to various cellular stimuli. This represents a novel tier of control of tyrosine-phosphorylation-dependent signaling and is a major empasis of the research efforts in our lab.

In our initial investigations of PTP oxidation in response to cell stimulation by growth factors and hormones, we had applied a modified in-gel PTP assay that we had developed to measure reversible PTP oxidation in a cellular context. Although this has proven to be a powerful approach and has allowed us to characterize the oxidation of several different PTPs in a variety of signaling contexts, there are certain technical challenges that limit its application. In particular, this assay does not detect the oxidation of receptor PTPs.

Recent efforts have focused on developing new assays that allow us to measure oxidation of all members of the PTP family in cells. We have been optimizing a protocol that uses a chemical label to tag the oxidized PTPs. Cells are lysed under anaerobic conditions in the presence of an alkylating agent, which irreversibly inactivates any PTPs that are present in the reduced, active state. Those PTPs that are in an oxidized state are protected from alkylation. Lysates were then subjected to buffer exchange on a size-exclusion chromatography column. In this key step, the alkylating agent was removed and a reducing buffer allowed the oxidized PTPs to be reduced back to the active state, in which they may then react with biotinylated active-site-directed compounds. The two biotinylated compounds used were a bromobenzylphosphonate (BBP) activity-based probe (in collaboration with Zhong-Yin Zhang, Department of Biochemistry and Molecular Biology, Indiana University) or a sulfydryl-reactive polyethylene oxide (PEO)-iodoacetyl probe. Subsequent purification by streptavidin pull-down and immunoblotting allowed us to identify those PTPs that were susceptible to oxidation. Initially, we have applied the method to measurement of PTP oxidation in angiomyolipoma cells, which are transformed by expression of platelet-derived growth factor, PDGF-BB. We have identified several PTPs as targets of PDGF-receptor-induced oxidation. Oxidation of these PTPs was shown to be elevated in PDGF-BB-transformed cells and to be dependent on NADPH oxidase activity. Interestingly, a similar pattern of immunoreactive bands was observed with both BBP- and PEO-labeled probes, suggesting that both probes are labeling

a similar pool of oxidized PTPs. Finally, in addition to detecting nontransmembrane PTPs, this method also revealed receptor PTP oxidation. This improved method for detecting reversible oxidation of the entire PTP superfamily is now being applied to other cell systems and should help delineate new signaling cascades and identify potential therapeutic targets.

In collaboration with T.C. Meng, Kay-Hooi Khoo, and their colleagues at the Academia Sinica in Taiwan, we have been developing mass-spectrometry-based approaches to characterize the various oxidative modifications of Cys residues in PTPs. In HepG2 and A431 human cancer cells, which produce high levels of ROS constitutively, we detected the reversible oxidation of three PTPs, TC-PTP, PTP-PEST, and PTP1B. We have focused our attention on PTP1B and have used mass spectrometry to determine the oxidation status of each Cys residue in the protein as isolated by immunoprecipitation from these cancer cells. Oxidation was only detected on the active-site Cys residue, whereas the other Cys residues in the PTP were unaffected, consistent with the unique properties of this residue conferred by the architecture of the active site. We observed that up to 50% of PTP1B was reversibly oxidized in HepG2 and A431 cells. Furthermore, due to the presence of the high ROS levels produced in these cells, we observed that up to 40% of PTP1B was irreversibly oxidized. Inhibition of NADPH oxidases by treatment with diphenyleneiodonium attenuated ROS production and inhibited PTP oxidation, concomitant with a decrease in tyrosine phosphorylation of cellular proteins and inhibition of anchorage-independent cell growth. These data suggest that the high level of ROS produced by HepG2 and A431 cells may promote the transformed phenotype by inactivation of cellular PTPs. Furthermore, it may now be possible to apply this approach to determining the stoichiometry of oxidation of specific PTPs in various signaling contexts.

JSP1

Multiple DSPs have been identified that have the ability to dephosphorylate and inactivate various mitogen-activated protein kinases (MAPKs), constituting a complex response network for attenuation of MAPK-dependent signals. We identified a novel DSP and observed, contrary to expectation, that it had the capacity to *activate* JNK specifically, exerting its effects upstream of MKK-4 in the JNK signaling cascade, hence the name JSP1 (JNK stimulatory phosphatase 1). This study illustrates a new potential tier of control of the JNK signaling pathway and a novel aspect of the role of protein phosphatases in the control of MAPK signaling. This raised the possibility that JSP1 may offer a new perspective to the study of various disorders associated with aberrant JNK signaling.

We have been characterizing JSP1 knockout mice, which contain a targeted deletion at exon 3 resulting in the production of a truncated, inactive protein of approximately 20 amino acids. These mice reproduce at normal Mendelian ratios, display no obvious physical abnormalities, and have a life expectancy similar to that of their wild-type controls. We are characterizing two aspects of their phenotype, one indicating that JSP1 may have a role in the neurodegenerative processes that lead to Parkinson's disease and the other implicating JSP1 in the regulation of innate and adaptive immunity.

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

Considering the ability of JSP1 to regulate JNK activity in cell transfection systems, we investigated JNK signaling in the JSP1 knockout mice. On the basis of previous studies that have shown that basal JNK activity is high in brain tissue, we examined JNK phosphorylation in the cortex, cerebellum, and hippocampus of wild-type and J*sp1*$^{-/-}$ mice and found no alterations in JNK activity due to JSP1 ablation. In addition these mice do not display any outwardly apparent neuronal phenotype. To investigate the possibility that JSP1 may regulate JNK signaling in the context of neuronal stress, we injected wild-type and *jsp1*$^{-/-}$ mice with MPTP and examined c-JUN phos-

phorylation in the SNpc following MPTP treatment. These studies are being performed with Serge Przedborski and his colleagues at Columbia University. Using confocal fluorescence microscopy, we observed significant phosphorylation of c-JUN in the dopaminergic neurons of wild-type mice at 6 hours post-MPTP treatment. Interestingly, the level of MPTP-induced c-JUN phosphorylation in *jsp1*$^{-/-}$ mice was dramatically lower than in their wild-type counterparts. This indicates that JSP1 may act to regulate MPTP-mediated JNK signaling in dopaminergic neurons. We are currently using laser-scanning cytometry (iCys®) to quantitate the effects of ablation of JSP1 on both MPTP-induced JNK signaling and degeneration of dopaminergic neurons and are focusing on identifying the substrates of the phosphatase that underlie these effects.

Modulation of Innate and Adaptive Immunity by JSP1. Preliminary analysis of JSP1 knockout mice has revealed interesting immunological phenotypes. We have observed that ablation of JSP1 leads to attenuation of the inflammatory response to Gram-negative bacterial endotoxin. Production of tumor necrosis factor-α (TNF-α) in response to sublethal lipopolysaccharide (LPS) administration is significantly decreased in JSP1 knockout mice compared to their wild-type counterparts; this appears to be dose-dependent for JSP1, as the heterozygous animals produce an intermediate TNF-α response. Interestingly, interleukin-6 (IL-6) production is unaffected, indicating that JSP1 acts at a point downstream from Toll receptors, where the signals mediating TNF-α and IL-6 production diverge. To pursue the mechanistic basis for this effect, we have been trying to identify a suitable cell type from the mouse which, in culture, would recapitulate the in vivo response (decreased LPS-induced production of TNF-α) and which could be cultured in sufficient numbers for biochemical analysis of signaling downstream from TLR4, the Toll receptor that mediates the effects of LPS. Splenocytes isolated from JSP1 knockout mice display decreased TNF-α secretion in response to LPS compared to wild type; however, the low numbers and short life span of these cells make it difficult to perform biochemical analysis of the signaling pathways responsible for this difference. Similar results were also obtained with bone-marrow-derived dendritic cells, but again, this system does not yield sufficient cells for a comprehensive biochemical characterization. We did not observe a difference in LPS-induced TNF-α production between resident macrophages from the JSP1 wild-type and KO mice; however, we observed a reduction of approximately 50% in TNF-α levels produced by bone-marrow-derived macrophages from knockout compared to wild-type animals. At this time, we have not observed a difference in LPS-induced activation of MAPK signaling pathways, or in IκBα degradation, between wild-type and JSP1 KO bone-marrow-derived macrophages.

Analysis of aged JSP1 knockout animals (>1 year) revealed that these mice develop moderate splenomegaly and that this is associated with lymphoid hyperplasia and an expansion of memory T cells. No differences were observed in the proliferative capacity of memory T cells isolated from wild-type and JSP1 KO mice; however, analysis of naïve CD4$^+$ T-cell proliferation has revealed an interesting phenotype. At an early age (1-month-old mice), JSP1 KO naïve CD4$^+$ T cells display a reduced proliferative capacity compared to wild type, as measured by thymidine incorporation and IL-2 production. However, at 4 months, JSP KO naïve CD4$^+$ T cells display a moderately increased proliferative capacity compared to wild type. This apparent disparity can be explained by the observation that wild-type CD4$^+$ T cells undergo a dramatic decline in proliferative capacity as they age from 1 to 4 months. In contrast, although JSP1 KO CD4$^+$ T cells from young mice do not display as robust a response to T-cell activation as their wild-type counterparts, they display no significant age-dependent decline in proliferation. These data, and the observation that aged JSP1 KO mice possess an increased memory T-cell pool, indicate that JSP1 may have important roles in aging of the adaptive immune system. Current efforts are focused on defining the effects of ablating JSP1 on signaling in these cells.

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RAS AND RHO GTPases AND THE CONTROL OF SIGNAL TRANSDUCTION

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K. John Y.-T. Yang

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

ROLE OF RAP1 SIGNALING IN MORPHOGENETIC PROCESSES

The Rap1 protein, a member of the Ras family, was initially identified as an antagonist of oncogenic Ras proteins; however, more recent studies indicate that the function of Rap1 is largely Ras-independent. Increasing evidence points to a critical role for Rap1 in the control of epithelial morphogenesis, and recent studies suggest a dysregulation of Rap1 signaling in malignant processes. How Rap1 proteins exert their effects in vivo has remained largely elusive. We previously described the identification of the mammalian junctional protein AF-6 as a Rap1-interacting protein. To demonstrate the physiological relevance of this interaction, we decided to carry out functional analyses of their orthologs (dRap1 and Canoe) in the more genetically tractable *Drosophila* system. We found that both dRap1 and Canoe are required for epithelial migration events in the embryo and that Canoe acts as a downstream effector of dRap1 in these processes.

More recently, we identified a *Drosophila* Rap1-specific exchange factor, dPDZGef, that is responsible for Rap1 activation in migrating embryonic epithelia. *Canoe* acts downstream from *dRap1* and *dPDZGef* in this event. Interestingly, we found a striking loss in cell tension in the proximity of junctional complexes in *dPDZGef* mutant cells generated in a mosaic analysis.

Surrounding wild-type cells often compensate this loss by increasing tension and display ectopic concentrations of myosin II (zip). In addition, overexpression of dPDZGef in a defined epithelial compartment results in a striking decline of cell and tissue tension that is partially compensated for by wild-type cells in the adjacent compartment. Thus, changes in tissue tension can be signaled and integrated across clone and compartment boundaries. Furthermore, we established that dPDZGef activity is genetically linked to myosin II/zip and thereby provide first insight into the molecular machinery targeted by Rap signaling to modulate cell plasticity. We propose that dPDZGef-dependent signaling functions as a rheostat that links Rap1 activity to the regulation of cell tension in epithelial morphogenesis and homeostasis at different stages of development.

ROLE OF DOK PROTEINS IN MITOGENIC AND ONCOGENIC SIGNALING

Dok-1 (also called $p62^{dok}$) was initially identified as a tyrosine-phosporylated 62-kD protein associated with Ras-GAP in Ph^+ chronic myeloid leukemia (CML) blasts and in v-Abl–transformed B cells. This protein was termed Dok (downstream of kinases), since it was also found to be a common substrate of many receptor and cytoplasmic tyrosine kinases. Subsequently, four additional Dok family members have been identified. Among them, Dok-1 and Dok-2 share the ability to bind to a negative regulator of Ras, Ras-GAP. We described previously that Dok-1 acts as a negative regulator of growth-factor-induced cell proliferation and that Dok-1 inactivation in mice causes a significant shortening of the latency of the fatal myeloproliferative disease induced by $p210^{bcr\text{-}abl}$. Strikingly, in collaboration with Dr. Pandolfi's group (Memorial Sloan-

Kettering Cancer Center), we also found that mice lacking both *Dok-1* and *Dok-2* spontaneously develop a CML-like myeloproliferative disease, likely resulting from increased cellular proliferation and reduced apoptosis. Thus, Dok proteins function as negative regulators of mitogenic and oncogenic signaling.

More recently, we obtained insight into the molecular mechanism by which Dok-1 exerts its negative effect on mitogenesis and oncogenic transformation. Using *Dok-1* knockout cells and *Dok-1* mutants deficient in binding to specific Dok-1-interacting proteins, we found that Dok-1 interferes with platelet-derived growth factor (PDGF)-stimulated c-*myc* induction and Ras/MAPK (mitogen-activated protein kinase) activation by tethering different signaling components to the cell membrane. Specifically, Dok-1 attenuates PDGF-elicited c-*myc* induction by recruiting Csk to active Src kinases, whereupon their activities and consequent c-*myc* induction are diminished. On the other hand, Dok-1 negatively regulates PDGF-induced MAPK activation by acting on RasGAP and at least one other Dok-1-interacting protein. Most importantly, we demonstrated that the actions of Dok-1 on both of these signaling pathways contribute to its inhibitory effect on mitogenesis. Our data suggest a mechanistic basis for the inhibitory effect of Dok-1 on growth-factor-induced mitogenesis and its role as a tumor suppressor.

ROLE OF RHO REGULATORS IN NEURONAL DEVELOPMENT

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

We obtained insight into the function of *oligophrenin-1* (*OPHN1*), a gene located on chromosome Xq12 that codes for a negative regulator of Rho GTPases. Mutations in *OPHN1* (which result in OPHN1 loss of function) have been reported in a family with nonsyndromic X-linked mental retardation (MR) and in families with MR associated with epilepsy and/or cerebellar hypoplasia. We demonstrated that OPHN1 is present in major regions of the brain, including the hippocampus, cerebellum, and cortex, and provided evidence for the requirement of OPHN1 in dendritic spine morphogenesis of hippocampal neurons. Using RNA interference and antisense approaches, we showed that knockdown of OPHN1 levels in CA1 pyramidal neurons in hippocampal slices results in a significant decrease in dendritic spine length and that this spine length phenotype is mediated by the Rho/Rho-kinase pathway. In addition, we recently demonstrated a biochemical interaction between OPHN1 and Homer, a postsynaptic adaptor molecule involved in spine morphogenesis and synaptic transmission. The interaction between OPHN1 and Homer raises the intriguing possibility that OPHN1 acts downstream from glutamatergic receptors to regulate RhoA activity in spines, and thus spine morphogenesis. Our future experiments are geared toward addressing these interactions and their functional roles in dendritic spine morphology, calcium signaling, and synaptic plasticity.

More recently, we identified a novel activator of the Rac GTPases, termed DOCK7, and demonstrated a critical role for this protein in axon development and axon-dendrite polarization. DOCK7 belongs to the DOCK180-related superfamily of proteins, which has recently emerged as a group of distinct regulators of the Rho proteins. We found that DOCK7 is highly expressed in major regions of the brain during early stages of development and, importantly, that the protein is asymmetrically distributed in unpolarized hippocampal neurons and becomes selectively expressed in the axon. We obtained evidence that DOCK7 has a critical role in the early steps of axon formation. Knockdown of DOCK7 expression prevents axon formation, whereas overexpression induces the formation of multiple axons. We further demonstrated that DOCK7 and Rac activation lead to phosphorylation and inactivation of the microtubule-destabilizing protein stathmin/Op18 in the nascent axon and that this event is important for axon development. Our findings unveil a novel pathway linking the Rac activator DOCK7 to a microtubule regulatory protein and highlight the contribution of microtubule dynamics to axon development. Together, our studies contribute to defining the molecular mechanisms by which neurons acquire their polarity and may also shed light on axon regenerating processes.

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Yu-Ting Yang

Neuroscientists at CSHL are continuing to make tremendous strides in understanding many aspects of normal brain structure and function, as well as what goes wrong in several major diseases including Alzheimer's, Parkinson's, schizophrenia, and autism.

Understanding brain function seems impossible without a comprehensive description of its structure on the level of individual synapses. To this end, Dmitri Chklovskii's lab is developing technologies for high-throughput reconstructions of brain circuits using serial section electron microscopy. His group was able to reconstruct a 1000-m^3 neuropil volume entirely, including every synapse, axon, and dendrite. Such reconstructions already provide invaluable information, necessary to construct a theoretical description of brain design based on engineering principles. Full reconstruction of "interesting" circuits requires scaling up reconstruction capabilities at least hundredfold, thus posing interesting computational challenges.

New neurons are constantly produced in the adult brain. The birth of new neurons is a dynamic process and it changes in response to a wide range of stimuli. The role of this constant supply of new neurons is still unclear; however, there is increasing evidence that adult neurogenesis may be important for learning, memory, and mood. Grigori Enikolopov's lab studies signals that regulate distinct steps in the cascade converting stem cells into neurons. Enikolopov also studies whether similar signals regulate stem cells in other tissues, and he is particularly interested in how neurogenesis may be linked to mood regulation and may underlie the action of antidepressant therapies. He and his team have developed a new approach for identifying and quantifying cellular targets of neurogenic stimuli and have used it to determine cell populations targeted by antidepressants in the adult brain. They are now using this approach to examine whether different types of treatments that regulate mood have a similar effect on the neuronal differentiation cascade and to identify signaling molecules that translate the treatment into an increased number of new neurons.

Karel Svoboda explores how information gathered from our senses is converted first into electrical activity and then transferred into memories through changes in synapses, neurons, and neural circuits. He combines a variety of powerful imaging techniques with rodent models to probe the brain's circuitry and how it changes over time. Many synaptic connections in the brain persist for months or years. When synaptic connections break down, memory is destroyed. Svoboda's group has discovered that the protein components that form synapses are remarkably short-lived. They recently helped resolve the paradox posed by persistent synapses and labile proteins by discovering intricate mechanisms that control the comings and goings of proteins at synapses.

Holly Cline studies how specific patterns of connections among brain neurons arise and how these patterns can change over time. By using time-lapse imaging of developing tadpoles, Cline's group is revealing the fundamental molecular and cellular mechanisms that are likely to underlie brain plasticity across species, including in humans.

Even fruit flies need to learn and remember in order to survive. Moreover, by comparing the mechanisms of learning and memory in flies, humans, and other organisms, Tim Tully has discovered ancient, fundamental molecular pathways of memory that are shared from flies to man. Tully and his colleagues have uncovered some 170 candidate memory genes (CMGs) in fruit flies and are in the process of elucidating the roles of these genes in learning and memory.

Josh Dubnau is using a combination of approaches to identify the molecular and anatomical pathways that underlie learning and memory in flies. His group has identified several genes that the researchers believe are involved in a mechanism that stores memories in response to external experiences. They are also using a genetic technique to map the neuroanatomical circuitry involved in memory processing in flies. Finally, Dubnau is pioneering an "artificial evolution" strategy with the goal of uncovering the network of gene interactions that govern learning and memory.

Partha Mitra is one of a number of CSHL neuroscientists that combine theoretical, computational, and experimental approaches to exploring brain function. His group is continuing to develop mathe-

matical algorithms and powerful software necessary for making sense of large volumes of neurobiological data. He participates in a number of experimental collaborations, on and off campus. With Tim Tully and Josh Dubnau, he is performing integrative analysis of memory formation in the fruit fly, combining genetic, neurobiological, and behavioral approaches. Collaborative efforts include the study of songbirds, with Ofer Tchernichovski of City College of New York, and studies of cognition and consciousness with Niko Schiff and Keith Purpura at the Weill Medical School of Cornell University.

Within the Neuroscience department, the Center for the Neural Mechanisms of Cognition (CNMC) continued its success in 2006. The CNMC consists of the research groups of Carlos Brody, Zach Mainen, and Anthony Zador. The goal of the Center is to develop rodent models that combine behavioral training with electrophysiological recordings to understand the neurological basis of several complex brain functions including learning, memory, time perception, motivation, and decision-making.

Carlos Brody is interested in how neurons interact with one another to form the neural networks that underlie rapid decision-making and short-term or "working" memory. His group is also exploring how time and temporal patterns are sensed and represented in the brain.

Zach Mainen studies how odors are detected, represented in the brain, and transformed into decision-making and other behaviors. A few years ago, his group discovered that rats (and probably sommeliers and other humans) can respond quickly and accurately to odors in a single sniff. Mainen and his colleagues continue to study the implications of this finding. From a "neuroeconomic" perspective, Mainen is studying how the brain deals with the biological "costs" of decision-making (e.g., uncertainty, effort, and delay). His group is also investigating the effects of psychoactive drugs in rodents to uncover neural circuits that might be targets of improved therapies for schizophrenia, Parkinson's disease, and other disorders.

The brain is able to solve hard computations that remain far beyond the reach of the fastest computers. Anthony Zador uses a combination of theoretical and experimental approaches to study how the brain computes, particularly in the realm of processing auditory input. Most of what is known about the auditory cortex comes from studies of the anaesthetized cortex. But in order to understand the way the brain can direct attention to a single sound among many ("selective attention"), it is necessary to study the cortex of unanaesthetized animals. Zador and colleagues have therefore focused on developing experimental techniques for studying the activity of neurons in the auditory cortex of awake animals. The techniques they use reveal that the diversity in neural responses seen in awake animals is much greater than those in anesthetized animals, and they are currently trying to elucidate the causes of this diversity.

Writers and poets know that memory is suffused with pain, ecstasy, fear, and desire. Science now supports the notion that intense emotions drive the changes in our brains that encode learning and memory. As part of his groundbreaking studies of the molecular basis of learning and memory, Roberto Malinow (in collaboration with Anthony Zador) recently discovered that fear conditioning—a form of learning and memory—is encoded by the movement of proteins called AMPA (α-amino-3-hydroxy-5-methyl-3-isoazole) receptors into synapses in a portion of the brain known to be responsible for basic instinctual learned responses.

Jonathan Sebat is using representational oligonucleotide microarray analysis (ROMA) to identify genetic changes that cause neuropsychiatric disorders. His initial study of 200 families with sporadic autism has found a very high frequency of deletions that occur spontaneously in affected children. These findings suggest that specific genes are involved in the pathology of autism, including a hormone known as oxytocin and a regulator of splicing called ataxin-2-binding protein 1. Sebat's group has taken a similar approach to studying psychosis. ROMA analysis of 400 patients with schizophrenia has identified genomic imbalances at several interesting loci. The most frequent mutations found in schizophrenia involve genomic regions that are similar between the X and Y chromosomes.

One of the central questions of biology is how changes in gene activity transform one type of cell into other types. Cold Spring Harbor Fellow Lee Henry studies this process, called cellular differentiation, by using taste bud development as a model system. Taste buds comprise unspecialized "progenitor stem cells" at their periphery. These cells become specialized taste receptor cells within the center of the bud. Different taste receptor cells respond to different tastes (sweet, sour, salty, and bitter).

Henry is working to define taste at the level of individual genes. To do this, he and his colleagues have developed a method that allows the gene products of individual cells to be attached to magnetic beads, which are then "read" using a series of chemical modifications. By comparing specialized taste receptors to unspecialized stem cells, these researchers aim to understand how individual taste receptor cells tell our brains, for example, "this is sweet."

Josh Huang is deciphering how the brain develops neural networks that respond to GABA, the primary inhibitory neurotransmitter in the brain. Huang's group discovered that a cell adhesion protein called CHL1 has a major role in directing how neurons controlled by GABA connect to other neurons. These findings may have significant implications for understanding schizophrenia and other common neurodevelopmental and psychiatric disorders.

Alexei Koulakov uses mathematical methods to explore how real-world neurons form functional networks in the brain. Some of his work has generated robust theoretical models of visual and olfactory neural circuits—models that match experimental observations and, importantly, make testable predictions that are likely to reveal new clues to brain structure and function.

Memories can be destroyed when synaptic connections in the brain break down. Yi Zhong studies this breakdown, which underlies neurodegenerative disorders such as Alzheimer's disease. In his lab, researchers use the fruit fly brain as a model system for studying corresponding human genes that affect brain function in Alzheimer's patients. This analysis is revealing new clues about the disease and is providing insight into the general molecular mechanisms of learning and memory. Zhong's recent work has focused on neurofibromatosis 1 (*NF1*) and other genes implicated in Alzheimer's. His group has uncovered the pathways by which *NF1* controls learning and memory and continues to explore the mechanisms that underlie β-amyloid-induced neurodegeneration and memory loss in Alzheimer's disease.

COMPUTATIONAL SYSTEMS

C. Brody S. Chakraborty, S. Chow, E. Glushenkova, S. Lima, C. Machens, M. Nikichenko, S. Pai

Our lab is interested in how neurons interact with each other to form networks that underlie flexible cognitive acts, such as decision-making and short-term memory. We are also interested in how time and temporal patterns are sensed and represented in the brain. Our approach to these questions is both experimental and computational. During 2006, several projects either came to fruition or began to produce final results.

Shraddha Pai successfully completed the development of training protocols to teach rats to discriminate sound durations or discriminate sound frequencies. Using the latter task as a control for the former, she has begun lesioning brain structures in order to identify structures uniquely necessary for duration discrimination. Christian Machens published an elegant mathematical analysis of a large family of computational models that support continuous attractors for short-term memory. Stephanie Chow showed, first, that contextual modulation of neural responses exists in secondary somatosensory cortex and, second, that this is sufficient to radically change readout of such responses.

Finally, after a nearly two-year-long effort frequently marked by both signs of success and subsequent dashing of hopes, Carlos Brody succeeded in reliably training rats to perform a two-stimulus-interval discrimination task. This task had previously been performed only by monkeys and had formed the behavioral basis for much of the laboratory's data analysis and modeling efforts. In December 2006, the laboratory moved to Princeton University and Brody became an Adjunct Professor at Cold Spring Harbor Laboratory.

Determining the Neuroanatomical Loci of Sound Duration Discrimination in the Rat

S. Pai

Interval timing (IT) is the ability to estimate time, and it is used in decision-making in tasks where timing is important; e.g., in foraging, the renewability of food sources must be timed to maximize food intake. Identifying the sources and electrophysiological correlates of IT have been challenging due to the lack of a controlled behavioral paradigm in a model organism amenable to lesion studies and electrophysiology.

Discrimination of pure-tone durations using the two-alternative forced-choice (2AFC) paradigm in rats is a powerful yet simple model to study relative IT. We have developed a behavioural paradigm in which rats discriminate pure-tone durations that are longer than a standard interval from those that are shorter (temporal bisection). We have also trained the rats to discriminate sound frequencies (higher than a standard vs. lower than the standard). The 2AFC paradigm is extremely well-controlled, allowing us to precisely measure the accuracy and extent of discrimination in both tasks using psychophysical analysis. The same stimuli and the same responses are involved in the two tasks: What differs is what aspect of the sound stimulus the rat uses to make its discrimination. We are lesioning different auditory structures—specifically, the medial geniculate body of the thalamus, the auditory cortex, and auditory striatum—using a combination of reversible and permanent lesioning techniques to determine the brain regions that lead to the ability to time auditory stimuli. We specifically seek areas that, when lesioned, cause an impairment in one discrimination task but not the other. The unimpaired task will then provide a control showing that the impairment is neither sensory (rat can still hear) nor motor (rat can still move to report result) nor general cognitive (rat can still make a discrimination) but is instead specific to either duration or frequency discrimination.

Two-stimulus-interval Discrimination Tasks in Rodents

C. Brody

Much work in the Brody group has been based on data collected by R. Romo's group in Mexico, recording from monkeys trained in a two-stimulus-interval dis-

crimination task. In such tasks, a first stimulus (f1) is delivered and then there is a delay, typically lasting several seconds. A second stimulus (f2) is then delivered, and the subject must compare the two stimuli and make a two-alternative forced choice as to which of the two is the larger (f1 > f2? Yes or No). The task therefore requires short-term memory to remember f1 in the delay between f1 and f2, a comparison computation, and decision-making.

Together with Zachary Mainen's and Anthony Zador's labs here at CSHL, one of our goals is to bring such high-level tasks, whose neurophysiology is often explored with monkeys, into the rodent domain. This would provide a far more tractable model system for data collection and manipulation of brain activity. Initial training attempts, although at first apparently successful, proved unstable, and for unknown reasons, the rats did not maintain high performance. Thanks to the numerous training rigs that the Center for the Neural Mechanisms of Cognition obtained, we were able to explore a very large set of approaches and were able to find an approach that produced the desired results. On Friday October 28, 2006, we obtained our first rat to successfully complete training and then maintain subsequent stable performance. Other rats have since successfully completed this training procedure.

Design of Continuous Attractors with Monotonic Tuning Using a Symmetry Principle

C. Machens

Neurons that sustain elevated firing in the absence of stimuli have been found in many neural systems. In "graded persistent activity," neurons can sustain firing at many levels, suggesting a widely found type of network dynamics in which networks can relax to any one of a continuum of stationary states. The reproduction of these findings in model networks of nonlinear neurons has turned out to be nontrivial. A particularly insightful model has been the "bump attractor" in which a continuous attractor emerges through an underlying symmetry in the network connectivity matrix. This model, however, cannot account for data in which the persistent firing of neurons is a monotonic—rather than a bell-shaped—function of a stored variable. Here, we show that the symmetry used in the bump attractor network can be employed to create a whole family of continuous attractor networks, including those with monotonic tuning. Our design is based on tuning the external inputs to networks that have a connectivity matrix with Toeplitz symmetry. In particular, we provide a complete analytical solution of a line attractor network with monotonic tuning and show that for many other networks, the numerical tuning of synaptic weights reduces to the computation of a single parameter.

Context-dependent Modulation of Functional Connectivity: S2 to PFC Connections in Two-stimulus-interval Discrimination Tasks

S. Chow

Processing of signals from the environment must be flexible: A sensory cue may prompt different actions in different contexts. Our goal is to use context-dependent modulations of firing rates in the secondary somatosensory cortex (S2) as a basis for a biologically plausible model that achieves a context-dependent inversion of the inputs to the prefrontal cortex (PFC) without requiring changes in connectivity. The sign change is required by our laboratory's recently proposed network model of processing in the PFC (see Machens et al., *Science 307:* 1121 [2005]). Many neurons in PFC and S2 exhibit firing rates that are functions of stimulus frequency and can be classified into two groups by their firing-rate response to the first stimulus "f1." "Plus" neurons have firing rates that increase with increasing stimulus frequency, whereas "minus" neurons have firing rates that decrease with stimulus frequency. PFC neurons may switch the sign of their frequency dependence in response to the second stimulus "f2," whereas S2 neurons do not. Salinas showed that a simple two-layer network model is sufficient to implement drastic changes in functional connectivity, enabling context switching without rewiring. We combined the insights of Salinas' model with the extensive neurophysiological data from Ranulfo Romo's laboratory to develop a biologically plausible model of context switching in the signal transformation from S2 to PFC. The resulting static network solves the sign-change problem and is robust to variations in model parameters.

In Press

Machens C.K. and Brody C.D. 2007. Design of continuous attractors with montonic tuning using a symmetry principle. *Neural Computation* (in press).

FROM NEURONAL CIRCUIT RECONSTRUCTIONS TO PRINCIPLES OF BRAIN DESIGN

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

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

STATISTICAL CIRCUIT RECONSTRUCTIONS FROM LIGHT MICROSCOPY AND ELECTROPHYSIOLOGY

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

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

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

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

COMPLETE CIRCUIT RECONSTRUCTIONS FROM ELECTRON MICROSCOPY

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

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

To reconstruct more complex circuits, we are using techniques from image processing and machine learning to develop automated tracing algorithms. So

far, we have reconstructed a neuropil volume of 1000 cubic microns. We intend to scale up our automated algorithms to fully reconstruct circuits of wide interest among neurobiologists, such as the fly brain, the vertebrate retina, and the cortical column.

FROM WIRING DIAGRAMS TO BEHAVIOR

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

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

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

DESIGN PRINCIPLES BASED ON OPTIMIZATION THEORY

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

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

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

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

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BRAIN DEVELOPMENT AND PLASTICITY

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

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

During the past 15 years, we have demonstrated a role for afferent coactivity, postsynaptic *N*-methyl-D-aspartate receptor (NMDAR) activity, and downstream activation of calcium-dependent enzymes including calcium/calmodulin-dependent protein kinase II (CaMKII) in controlling retinotectal synaptic maturation, optic tectal cell structural plasticity, and topographic map formation (Wu et al., *Science 274:* 972 [1996]; Zou and Cline, *Neuron 16:* 529 [1996]; Wu and Cline, *Science 279:* 222 [1998]; Zou and Cline, *J. Neurosci. 19:* 8909 [1999]; Ruthazer et al., *Science 301:* 66 [2003]; Haas et al. 2006; Ruthizer et al. 2006). More recently, we have demonstrated that visual experience has multiple effects on visual system development. A relatively brief period, 4 hours, of visual experience enhances the growth rate of tectal cell dendritic arbors through a mechanism that requires glutamatergic transmission and the RhoA GTPases (Li et al., *Nature Neurosci. 3:* 217 [2000]; Li et al., *Neuron 33:* 741 [2002]; Sin et al., *Nature 419:* 475 [2002]). This same brief period of visual experience increases the excitability of tectal neurons and their sensitivity to visual stimuli, through a mechanism that requires intracellular polyamines, the modulation of α-amino-3-hydroxy-5-methyl-3-isoazole receptor (AMPAR) function, and compensatory changes in sodium channel activity (Aizenman et al., *Neuron 34:* 623 [2002]; Aizenman et al., *Neuron 39:* 831 [2003]).

The finding that we can use visual stimulation to modify the development and properties of the retinotectal system has spurred our interest in determining the function of activity-induced genes on visual system plasticity. For instance, our studies of *Homer*, *Arc*, and *cpg15* (candidate plasticity gene 15) demonstrate that each has distinct roles in controlling neuronal plasticity. Homer is a widely expressed scaffold protein, which affects calcium signaling and metabotropic glutamate receptor (mGluR) signaling. In addition to finding a role in axon guidance (Foa et al., *Nature Neurosci. 4:* 499 [2001]; Foa et al., *J. Comp. Neurol. 487:* 42 [2005]), our more recent work indicates that experience-dependent changes in postsynaptic Homer expression regulates mGluR-mediated plasticity of retinotectal transmission (Van Keuren-Jensen and Cline 2006).

This is particularly interesting in light of recent work suggesting that mGluR-mediated potentiation and depression of synaptic transmission may have a role in developmental neurological disorders such as Fragile X. CPG15, another activity-induced protein, is noteworthy because it is a GPI-linked signaling molecule whose expression results in a large increase in dendritic arbor development, coupled with an increase in glutamatergic retinotectal synaptic maturation and a coordinated elaboration of presynaptic retinal axon arbors (Nedivi et al., *Science 281:* 1863 [1998]; Cantallops et al., *Nat. Neurosci. 3:* 1004 [2000]; Nedivi et al., *J. Comp. Neurol. 435:* 464 [2001]). We have recently shown that CPG15 mediates these changes by promoting synapse formation, which in turn enhances axonal arbor growth (Javaherian and Cline, *Neuron 45:* 505 [2005]). These data suggest that CPG15 is akin to an activity-induced targeted growth factor.

It now appears that many activity-induced genes function in a homeostatic manner to maintain synaptic strength within a functional operating range, despite experience-dependent increases or decreases in synaptic strength. This is the case for *Arc* (Rial Verde et al. 2006) and *Homer* (Van Keuren-Jensen and Cline 2006), as well as ornithine decarboxylase, which generates polyamines and thereby regulates neuronal excitability and the strength of glutamatergic synaptic transmission.

The goal of this body of work is to generate a comprehensive understanding of the role of experience in shaping brain development. We have taken a multidisciplinary approach to this question which has successfully revealed the complexity of brain development. Our experiments use a combination of molecular/ genetic manipulation and quantitative observations of structural and functional plasticity in response to visual stimulation. Our experiments have demonstrated a diverse range of effects of visual activity on the development and plasticity of the visual system and have the potential to reveal both direct and homeostatic mechanisms of circuit development. We are now poised to move beyond analysis of individual neurons within the retinotectal system and to address questions more related to circuit development and function.

Activity-induced Genes *Homer1a* and *Arc* Mediate Homeostatic Changes in the Strength of Neuronal Connections

K. Van Keuren-Jensen, E. Rial Verde

A fundamental adaptive feature of neurons within a functional network is their ability to respond to afferent activity by changing the strength of synaptic connections. At most excitatory synapses, changes in ionotropic AMPAR transmission underlie changes in synaptic strength, whereas the ligand and voltage-dependent NMDARs and mGluRs are thought to modulate AMPAR synaptic plasticity and downstream signaling events. In two independent sets of experiments, we find that activity-induced neuronal genes, *Homer1a* and *Arc*, function to regulate synaptic strength in a homeostatic manner.

These data suggest that activity-induced genes have two distinct types of functions with respect to circuit plasticity: One function is to more or less directly affect synaptic strength, for instance, as we have shown that CPG15 enhances synaptic strength and increases dendritic and axonal arbor elaboration (Nedivi et al., *Science 281:* 1863 [1998]; Cantallops et al., *Nat. Neurosci. 3:* 1004 [2000]; Javaherian and Cline, *Neuron 45:* 505 [2005]). The second function of activity-induced genes is to control the magnitude of the changes in synaptic strength through homeostatic mechanisms, so that plasticity can occur despite stimuli that drive synaptic strength toward extreme high or low values.

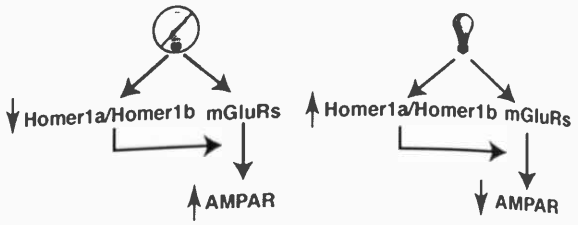

FIGURE 1 Model of *Homer1a/Homer1b* regulation of mGluR-mediated changes in AMPAR synaptic transmission. After 12–15 hr dark, *Homer1a/Homer1b* is low and mGluR activation enhances AMPA-mediated transmission. With visual stimulation *Homer1a/Homer1b* increases, reducing the mGluR-mediated increase in AMPAR synaptic transmission.

Homer proteins are integral components of postsynaptic density and are thought to function in synaptogenesis and plasticity, possibly through their interaction with mGluRs. Brief mGluR activation leads to plasticity of AMPAR synaptic transmission. To test whether mGluR-mediated plasticity of AMPAR transmission is influenced by recent neuronal activity, we manipulated visual activity in *Xenopus laevis* tadpoles in vivo. We compared mGluR-mediated plasticity of AMPAR transmission in optic tectal cells of tadpoles with low levels of previous synaptic activity (overnight in the dark) to transmission in neurons from animals following 4 hours of constant visual stimulation. mGluR-mediated plasticity of AMPA transmission was significantly decreased in neurons with recent activity. By changing the ratios of *Homer1a* to *Homer1b* in vivo, either by induction of endogenous *Homer1a* by visual activity, or by ectopic expression of *Homer1a* or *Homer1b*, we could change the direction of mGluR-mediated plasticity (Fig. 1). This is the first evidence that mGluR-mediated changes in AMPA transmission can be regulated by Homer proteins in response to physiologically relevant stimuli.

Arc is an immediate-early gene whose expression levels are increased by strong synaptic activation, including synapse-strengthening activity patterns. *Arc* mRNA is transported to activated dendritic regions, conferring the distribution of ARC protein both temporal correlation with the inducing stimulus and spatial specificity. We found that increased ARC levels unexpectedly reduce the amplitude of synaptic currents mediated by AMPARs. This effect is prevented by RNA interference (RNAi) knockdown of ARC, by deleting a region of ARC known to interact with endophilin 3 or by blocking clathrin-coated endocytosis of AMPARs. Our results demonstrate that ARC reduces the number of synaptic AMPARs leading to a

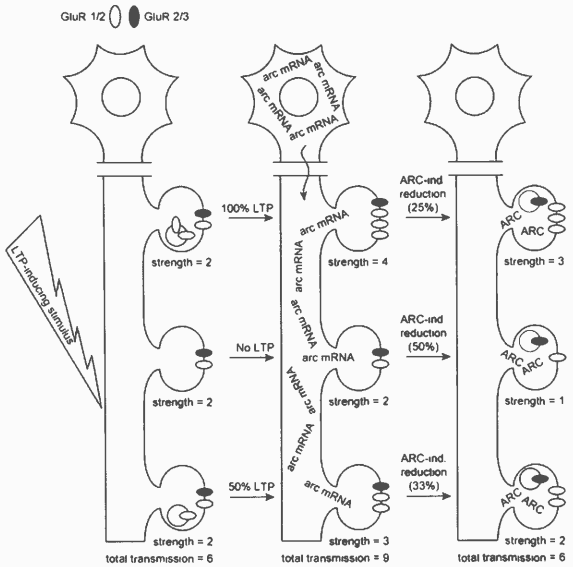

FIGURE 2 *Arc* mediates homeostatic control of glutamatergic transmission. The schematic illustrates the scenario in which a region of the dendrite receives a synapse-strengthening stimulus that causes different amounts of potentiation in three neighboring synapses and induces *Arc* mRNA expression and localization to that region. Synapse 1 is 100% potentiated, synapse 2 is not potentiated, and synapse 3 is 50% potentiated. ARC-induced depression preferentially affects synapses with relatively more GluR2/3 content. Consequently, the potentiated-to-non-potentiated synaptic strength ratio is increased by ARC-induced GluR2/3 removal, e.g., synapse 1-to-synapse 2 ratio, initially 1, becomes 2 after long-term potentiation (LTP) and increases to 3 after ARC has acted. In addition, total synaptic strength for that dendritic region is homeostatically regulated (initially 6, becomes 9 after LTP, returning to 6 after ARC's action).

decrease in synaptic currents, consistent with a role in the homeostatic regulation of synaptic strength (Fig. 2).

Regulation of Retinal Axon Arbor Elaboration and Branch Dynamics by Synaptic Contacts

E. Ruthazer, J. Li

Patterned neural activity and synaptic transmission guide the remodeling of axonal arbors in the developing central nervous system by regulating the addition, stabilization, and elimination of branches as arbors grow. In the retinotectal projection of frogs and fish, the dynamic rearrangement of axonal branches actively refines and maintains the retinotopic map. Previous work in our lab had demonstrated a critical role for

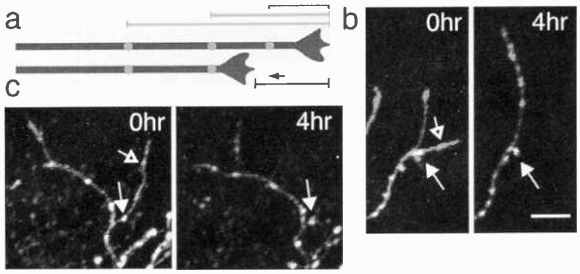

FIGURE 3 Branch retractions halted by mature SYP puncta. (*a*) Schematic of axon branch retractions relative to the positions of synapses. (*b*,*c*) Examples of branch retractions over 4 hr that stop at mature SYP puncta (*closed arrow*). Faint puncta do not prevent branch retraction (*open arrow*). For a detailed explanation, see Ruthazer et al. (2006).

activity-dependent Hebbian mechanisms in controlling axon branch retraction. We showed that the cellular mechanisms involved retrograde signaling downstream from activation of postsynaptic tectal NMDARs, which effectively function as correlation detectors. These and other studies, including our studies on motor neuron axon development (Javaherian and Cline, *Neuron 45:* 505 [2005]), suggest a central role for synaptic connections in axon arbor remodeling and raise the intriguing possibility that as synapses mature and strengthen, they may serve double duty as structural sites for axon branch stabilization.

To examine the role of synaptogenesis and synaptic maturation in the structural development of axonal projections during the formation of the topographic retinotectal projection, we coexpressed cytosolic fluorescent protein (FP) and FP-tagged synaptophysin (SYP) in small numbers of retinal ganglion cells in living albino *X. laevis* tadpoles to reveal the distribution and dynamics of presynaptic sites within labeled retinotectal axons. Two-photon time-lapse observations followed by quantitative analysis of tagged SYP levels at individual synapses demonstrated the time course of synaptogenesis: Increases in presynaptic punctum intensity are detectible within minutes of punctum emergence and continue over many hours.

Puncta lifetimes correlate with their intensities. Furthermore, we found that axon arbor dynamics are affected by synaptic contacts. Axon branches retract past faintly labeled puncta but are locally stabilized by mature synapses with intensely labeled SYP puncta (Fig. 3). Visual stimulation for 4 hours enhanced the stability of the arbor at intense presynaptic puncta while concurrently inducing the retraction of exploratory branches with only faintly labeled or no synaptic sites.

Depolarizing GABAergic Conductances Regulate the Balance of Excitation to Inhibition in the Developing Retinotectal Circuit in Vivo

C. Akerman

Neurotransmission during development regulates synaptic maturation in neural circuits, but the contribution of different neurotransmitter systems is unclear. We investigated the role of $GABA_A$ receptor-mediated Cl^- conductances in the development of synaptic responses in the *Xenopus* visual system. Intracellular Cl^- concentration ($[Cl^-]_i$) was found to be high in immature tectal neurons and then falls over a period of several weeks. GABAergic synapses are present at early stages of tectal development and, when activated by optic nerve stimulation or visual stimuli, induce sustained depolarizing Cl^- conductances that facilitate retinotectal transmission by NMDARs.

To test whether depolarizing GABAergic inputs cooperate with NMDARs during activity-dependent maturation of glutamatergic synapses, we prematurely reduced $[Cl^-]_i$ in tectal neurons in vivo, by expressing the Cl^- transporter KCC2. This blocked the normal developmental increase in AMPAR-mediated retinotectal transmission and increased GABAergic synaptic input to tectal neurons. Therefore, depolarizing GABAergic transmission has a pivotal role in the maturation of excitatory transmission and controls the balance of excitation and inhibition in the developing retinotectal circuit. Control cells, expressing GFP or a mutant form of the transporter Y1087D are comparable to untransfected cells (Fig. 4). For details, see Akerman and Cline 2006.

AMPARs Regulate Experience-dependent Dendritic Arbor Growth In Vivo

K. Haas, J. Li

The size and shape of neuronal dendritic arbors affect the number and type of synaptic inputs, as well as the complexity and function of brain circuits. In the intact brain, dendritic arbor growth and the development of excitatory glutamatergic synapse are concurrent. Consequently, it has been difficult to resolve whether synaptic inputs drive dendritic arbor development.

Here, we test the role of AMPAR-mediated glutamatergic transmission in dendrite growth by expressing peptides corresponding to the intracellular carboxy-terminal domains of AMPAR subunits GluR1 (GluR1Ct) and GluR2 (GluR2Ct) in optic tectal neu-

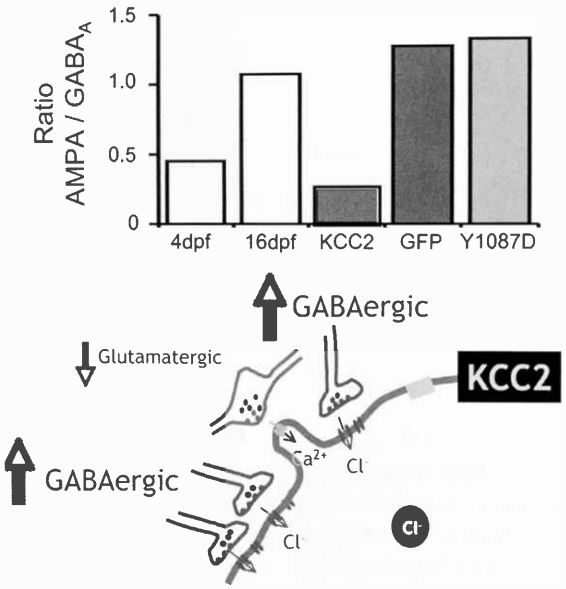

FIGURE 4. Low $[Cl^-]_i$ impairs the developmental balance of glutamatergic and GABAergic synaptic inputs in the retinotectal circuit in vivo. (*a*) The ratio of AMPA input to GABA input increases with development but was significantly reduced by premature expression of KCC2, the Cl^- transporter. Control cells, expressing green fluorescent protein (GFP) or a mutant form of the transporter,Y1087D, are comparable to untransfected cells. For details, see Akerman and Cline (2006).

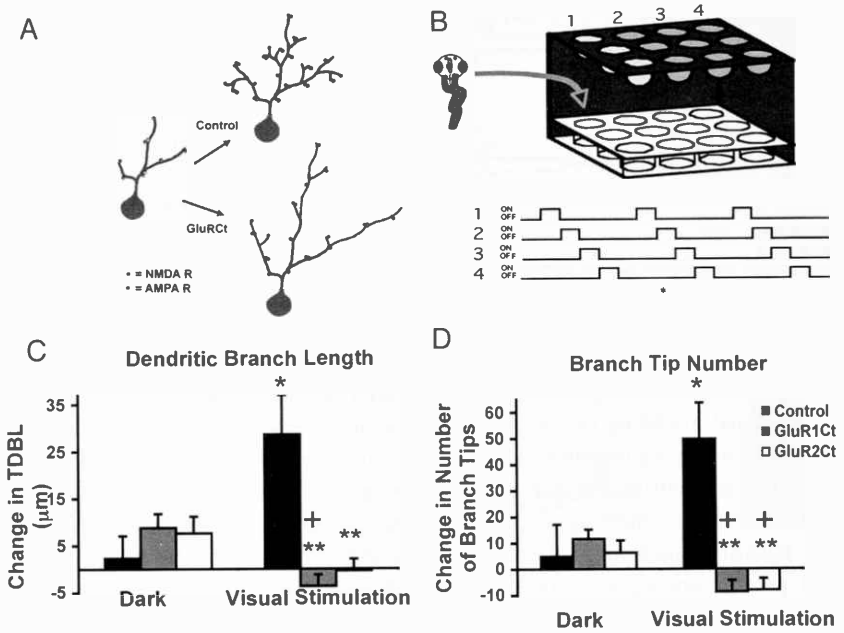

FIGURE 5 Maturation of glutamatergic synapses is required for normal dendritic arbor growth and experience-dependent dendrite arbor plasticity. (*A*) Schematic of the effect of expressing the carboxy-terminal peptides of AMPARs on dendritic arbor development. (*B*) Retinal input activity to the optic tectum was increased by exposure of freely swimming tadpoles to moving-bar visual stimulation for 4 hr following 4 hr in the absence of visual stimulation. (*C*) Relative changes in tectal neuron total dendritic arbor length and (*D*) branch tip number over each 4-hr period for control neurons and neurons expressing GluR1 or GluR2 carboxy-terminal peptides. GluR1Ct and GluR2Ct neurons retracted branches in response to visual experience, whereas control neurons increase arbor growth with experience. (*) Significant difference compared to controls within same time period, $p < 0.05$; (+) significant difference within same group between dark and visual stimulation periods, $p < 0.05$.

rons of the *Xenopus* retinotectal system. These peptides significantly reduce AMPAR synaptic transmission in transfected neurons while leaving visual system circuitry intact. Daily in vivo imaging over 5 days revealed that GluR1Ct or GluR2Ct expression dramatically impaired dendrite growth, resulting in less complex arbors than controls. Time-lapse images collected at 2-hour intervals over 6 hours show that both GluR1Ct and GluR2Ct decrease branch lifetimes. Ultrastructural analysis indicates that synapses formed onto neurons expressing the GluRCt are less mature than synapses onto control neurons.

These data suggest that the failure to form complex arbors is due to reduced stabilization of new synapses and dendritic branches. Although visual stimulation increases dendritic arbor growth rates in control tectal neurons, a weak postsynaptic response to visual experience in GluRCt-expressing cells leads to retraction of branches (Fig. 5). These results indicate that AMPA-R-mediated transmission underlies experience-dependent dendritic arbor growth by stabilizing branches and support a competition-based model for dendrite growth.

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GENETICS OF MEMORY IN *DROSOPHILA*

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

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

The *Drosophila* model system also offers an economy of scale to discover and develop hypotheses in a relatively cost-effective and rapid manner. Given the remarkable evolutionary conservation of genetic, cellular, and behavioral mechanisms, the findings from *Drosophila* can ultimately be informative to work in mammalian model systems.

Discovery of Genes Involved in Memory

J. Dubnau [in collaboration with T. Tully and N. Sinha, Cold Spring Harbor Laboratory]

In collaboration with the Tully lab, we have used a combination of behaviorally specific training protocols and expression profiling with DNA chips to identify transcriptional responses during memory consolidation (Dubnau et al., *Curr. Biol. 13:* 286 [2003]). We have used this approach to compare gene expression profiles after spaced training, which induces both short-term and protein-synthesis-dependent long-term memory, and massed training, which only induces short-lived memory. We have identified a large number of candidate memory genes (CMGs) differentially expressed at three different retention intervals after spaced versus massed training. Using real-time polymerase chain reaction (PCR) follow-up assays, we have confirmed differential expression for 60 of these transcripts.

This effort has identified CMGs that serve as entry points for molecular genetic investigation of gene function in memory. These CMGs have become fodder for in vivo genetic manipulations to forge mechanistic connections between individual gene pathways and memory formation at the behavioral level. With that aim in mind, we are focusing on local translational control, one of several pathways suggested from the array experiments. Our genetic studies already support a role in memory for several components of this pathway. These include *staufen* and *oskar*, which are known components of a cellular mRNA localization machinery in oocytes, and *pumilio*, which is a translational repressor protein. A large number of the known components of the mRNA localization machinery, as well as of the apparatus for regulating cytoplasmic polyadenylation-stimulated translational control, also are differentially expressed. Genetic reagents to manipulate these pathways are extant in *Drosophila*.

In addition to this obvious point of convergence between the microarray and behavioral screen, many additional hypotheses are suggested by the above two gene discovery efforts. Several projects in the lab now focus on testing hypotheses derived from this data set.

Identification of Synaptic Targets of *Drosophila Pumilio*

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

Drosophila melanogaster Pumilio (Pum) protein is one of the founding members of the PUF RNA-binding protein family. Its function in the posterior body patterning of *Drosophila* embryos is relatively well studied. The 3′-untranslated region (3′UTR) of maternal *hunchback* (*hb*) mRNA contains two copies of sequence elements named Nanos response elements (NREs), which are essential for normal abdominal segmentation. It was later shown that Pum binds these

elements, recruits Nanos (Nos) and Brain Tumor (Brat), and represses the translation of maternal *hb* mRNA. More recently, we identified Pum as having a key role in long-term memory (Dubnau et al., *Curr. Biol. 13:* 286 [2003]), and Pum was also recently shown to regulate excitability at the neuromuscular junction via translational regulation of sodium channels. Despite these genetic observations, few neuronal targets of Pum have been identified.

To screen for potentially relevant neuronal (and in particular synaptic) targets of Pum, we (in collaboration with G. Chen and M. Zhang) have used a combination of informatics and experimental approaches. Our first step to identifying new Pum targets was to characterize and model the Pum-binding sites. We then used our models to predict the presence of NREs in the 3′UTRs of mRNAs coding for synaptic proteins. Several of these were validated by in vitro binding assays (Q.S. Zhang and A. Krainer). Finally, we used an established in vivo functional assay to validate *pumilio*-dependent regulation of several of the predicted targets.

To generate in vivo support for the target prediction method, we chose to use the Pum response assay originally described for the *hb* gene. This assay relies upon the requirement that maternally supplied *hb* mRNA is repressed by Pum/Nos in posterior regions of the early embryo. We started with the canonical genomic *hb*-rescuing transgene in which the endogenous NRE motifs have been deleted. In the absence of functional NRE elements, this construct causes a dominant sterility in transgenic females due to ectopic *hb* translation in the posterior half of the embryos produced. Such embryos are unable to form abdominal segments. Insertion into this canonical construct of a functional NRE motif restores Pum-mediated repression in the posterior, allowing production of viable progeny. Using this strategy, we tested the functional capacity of the predicted NRE motifs from *Ace* and *dlg1*. We chose these two putative targets because they showed robust in vitro binding and also because both transcripts are induced by spaced training (not shown).

We generated a series of *hb*-transgene constructs (Fig. 1) in which the two endogenous *hb* NREs had been deleted entirely (hbΔ), had been replaced with a single *hb* NRE, NRE2 (hb2), had both *hb* NRE elements reinserted (hb1,2), had been replaced with putative NRE elements from *Ace* or *dlg1* genes (Ace or dlg1), or had been replaced with an antisense version of the predicted *dlg1* NRE (dlg-anti). We found that the predicted NRE from *dlg1* is sufficient to partially restore abdominal patterning when compared with

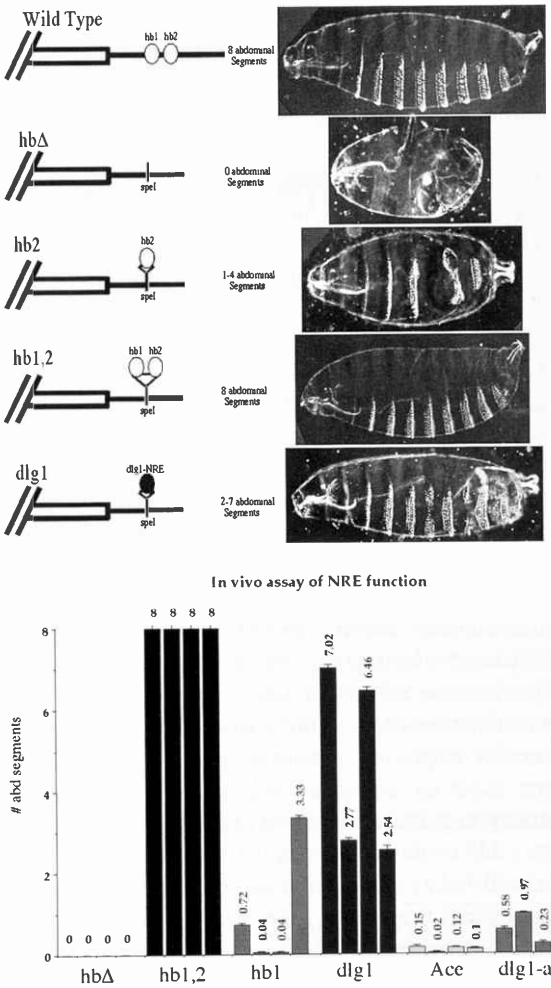

FIGURE 1 Predicted Pum-binding site in *dlg* is able to restore normal Pum-dependent regulation to the *hb* gene in the embryo.

hb1,2 (Fig. 1), which provided full rescue as expected. It is worth noting that the rescue observed with the single *dlg1* NRE is superior to that observed with a single copy of the *hb* NRE. A single *hb* NRE (hb2) yields partial rescue. In contrast, control transgenic lines in which no functional NRE was provided or in which the *dlg1* NRE was inserted in opposite orientation (dlg-anti) generate progeny nearly devoid of abdominal segments.

Together, these findings indicate that *dlg* may be a synaptic target of Pum. Dlg is the sole *Drosophila* member of a family of membrane-associated guanylate kinases (MAGUKs) that in mammals have been shown to have a key role in assembling the postsynaptic density in glutamatergic synapses. In *Drosophila*, Dlg expression is both pre- and postsynaptic at type I boutons at the neuromuscular junction (NMJ), and

mutants exhibit postsynaptic structural defects as well as increased transmitter release. Dlg is thought to have a key role in clustering GluRIIB receptors at the NMJ as well as Shaker K^+ channels throughout the central nervous system (CNS).

Like Dlg, Pum also appears to have both pre- and postsynaptic effects at the NMJ and is colocalized with Dlg at type I boutons. In addition to morphological effects on synapse structure, Pum appears to regulate excitability via an effect on expression of *para* Na^+ channels. The regulation of *para* may be direct, or it may depend on Pum's proposed role in regulating translation of eIF-4E. Pum expression itself is induced by behavioral training that results in long-term memory (Dubnau et al., *Curr. Biol. 13:* 286 [2003]). Thus, one hypothesis that seems reasonable is that activity-dependent increases in Pum expression have a homeostatic role by reducing excitability via repression of *para*.

Our findings indicate an additional role of Pum is directly regulating *dlg1* expression, suggesting that Pum antagonizes the effects of Dlg on neuronal structure and/or function. We cannot distinguish whether this interaction is pre- or postsynaptic (or both). Current efforts focus on testing the role in memory of Pum-dependent regulation of several of the predicted targets.

Functional Anatomy of Memory Consolidation

A. Blum, M. Cressy, H. Qin, J. Dubnau [in collaboration with A.S. Chiang, Taiwan]

In both vertebrate and invertebrate animals, anesthetic agents cause retrograde amnesia for recently experienced events. In contrast, older memories are resistant to the same treatments. In *Drosophila*, anesthesia-resistant memory (ARM) and long-term memory (LTM) are genetically distinct forms of long-lived memory that exist in parallel for at least a day after training. Genetic and pharmacological experiments indicate the presence of at least five mechanistically distinct temporal phases of memory (short-term, middle-term, ARM, and LTM). Although a neural structure called the mushroom bodies (MBs) has a demonstrated role in early memory and in memory retrieval at several time points, very little else is known about the anatomical circuitry underlying each of the above memory phases.

To map the neural circuits required for memory formation, storage, and retrieval, we take advantage of a large panel of Gal4 "driver" lines that express in small, reproducible sets of neurons. These can be used to drive expression of florescent reporters to generate high-resolution images of the neuronal connectivity, or they can be used to drive expression of any other gene of interest to investigate functional requirements for behavior of each circuit component. We are using two main strategies to dissect the functional role of these identified neuronal circuits. First, we use the Gal4 lines to map the temporal and spatial requirements for genes involved with memory formation. Second, we are using a temperature-sensitive dynamin transgene, which disrupts synaptic transmission reversibly and on a time scale of minutes. With the former approach, we are able to determine the anatomical circuits in which each genetic/cellular pathway functions. With the latter approach, we are able to reversibly silence reproducibly specific groups of neurons in vivo to directly probe the functional role of each identified neuron type.

We already have demonstrated a role for synaptic transmission in MB neurons during memory retrieval but, surprisingly, not during acquisition of early memory (Dubnau et al., *Nature 411:* 476 [2001]). These data suggest that the synaptic plasticity underlying olfactory associative learning initially resides in MB dendrites and/or upstream of the MB and that the resulting alterations in synaptic strength modulate MB output during memory retrieval. But virtually nothing is known about the neural circuitry involved in the subsequent consolidation of short-term, middle-term, anesthesia-resistant, or CREB-dependent LTM phases. And nothing is known about the downstream circuitry of memory retrieval.

To select Gal4 lines for functional anatomy investigations, we have used two approaches. First, Gal4 insertion mutations in genes involved in memory permit a first-pass evaluation of the green fluorescent protein (GFP) reporter expression pattern of biologically relevant genes. These are then used in combination with the dynamin mutant to reversibly silence synaptic transmission. A second approach that we are using to select relevant Gal4 driver lines is based solely on their expression pattern, rather than on their insertion in a "memory gene." In collaboration with A.S. Chiang (Taiwan), we have access to a growing panel of Gal4 enhancer lines that yield remarkably specific expression in neurons that send projections into different substructures of the MB. These identified MB "extrinsic" neurons constitute a significant fraction of the inputs, outputs, and modulation of MB. With the dynamin method, we can functionally manipulate these neuronal populations.

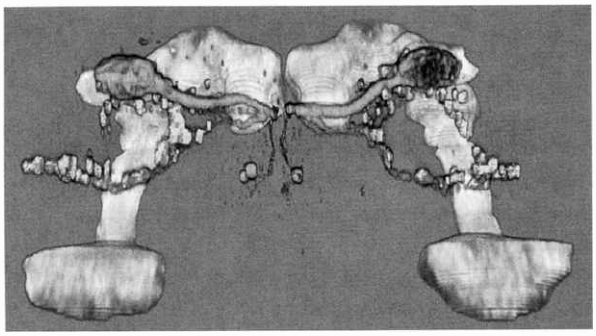

FIGURE 2 VAM neurons are output neurons of MB alpha lobes.

The ventral anterior medial (VAM) neuron driver, for example, yields expression in four neurons whose dendrites project to the axon outputs of the MB alpha lobe (Fig. 2). Axon outputs of the VAM neurons terminate in the posterior superior protocerabrum (PSP). With the dynamin approach, we have established a role for these neurons in an intermediate-term memory (Fig. 3). We used transient disruption of transmission in VAM neurons at different time points after training to examine the temporal requirements for these MB output neurons. When VAM neurons are blocked during the first 30 minutes after training, memory (measured 3 hours after training) is partially disrupted. In contrast, transient inhibition of VAM neuron function at later time points appears to have little or no effect. These VAM neurons appear to be dispensable for learning (not shown), suggesting that these MB output neurons are specifically required to maintain a memory trace or to consolidate memory. Ongoing efforts are focused on filling in the rest of the circuitry for these "early" memory phases, as well as for LTM and for memory retrieval.

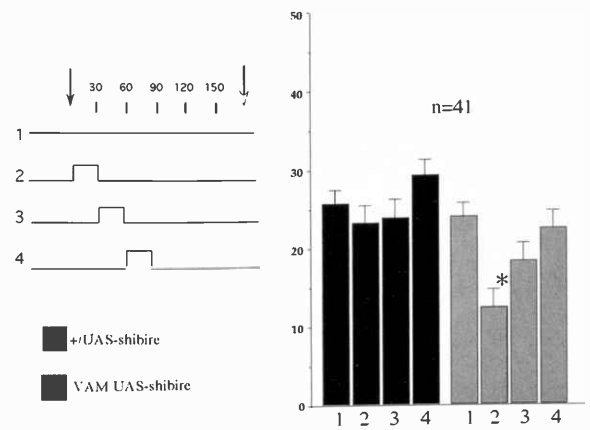

FIGURE 3 Transient inhibition of dynamin-mediated neurotransmission in VAM neurons reveals a temporal window where VAM mediates memory storage. Inhibition times are 0–30 min (2), 30–60 min (3), or 60–90 min (4) after training.

Selective Breeding of *rutabaga* Suppression

M. Cressy, E. Kockenmeister, J. Dubnau [in collaboration with P. Mitra, Cold Spring Harbor Laboratory]

Genetic investigation of memory has revealed that underlying mechanisms are highly conserved across phyla. The cAMP cascade, for example, has been implicated in memory in both invertebrate and vertebrate animals, including humans. The informative power of genetics derives in part from identification of genes that influence phenotype but also from analysis of gene interaction. Saturation mutagenesis for embryonic patterning, for instance, identified most of the relevant genes. Equally important, however, were second-site suppressing and enhancing screens. This approach allowed cell-signaling pathways and mechanistic insights to be distilled from what otherwise would be unconnected gene lists.

In the case of complex behavioral phenotypes such as memory, forward mutagenesis has identified a number of relevant genes (Margulies et al., *Curr. Biol. 15:* R700 [2005]). Here too, the most informative instances are where mechanistic interactions among genes are understood. cAMP signaling is the most notable example. Assembly of genes into functional networks, such as the cAMP cascade, is the most challenging aspect of genetics. Although suppressor/enhancer screens have yielded some success, this approach is often not feasible for complex quantitative traits such as memory. Moreover, modifier screens are only designed to detect interactions between pairs of genes, but they generally do not identify more complex gene networks. We are using experimental evolution with identified memory mutants (Dubnau et al., *Curr. Biol. 13:* 286 [2003]) in *Drosophila* as a strategy to screen for networks of gene interaction capable of suppressing or enhancing the role of cAMP signaling.

Our approach relies on artificial selection over the course of multiple generations to "evolve" combinations of known and molecularly tagged gene variants that interact to suppress the learning defect of *rutabaga* mutants. We are able to follow the "evolution" of allele combinations by using a high-throughput genotyping strategy. The initial stage of this project involved

a series of Mendelian crosses to establish founding populations that are homozygous mutant for *rutabaga* and heterogeneous for a large collection of autosomal recessive alleles of memory-related genes (Dubnau et al., *Curr. Biol. 13:* 286 [2003]). We now are conducting the selective breeding strategy in parallel with high-throughput genotyping of each population.

Poly-ADP-Ribosylation in Memory

J. Dubnau, C. Jurgensen [in collaboration with Ladurner Lab, EMBL]

In all species that have been studied, long-term memory involves a cascade of CREB-dependent DNA transcription, a process that is fundamentally regulated at the level of chromatin structure.

One of the most dynamic ways to manipulate chromatin is through chemical changes on the histone proteins themselves. Among these, poly-ADP-ribosylation is one of the first characterized and least understood posttranslational changes of proteins. Recent in vitro and in vivo evidence suggests that this modification alters the transcriptional activity of genes. Crucially, this modification has a known role in long-term memory in *Aplysia*, as well as in neurodegeneration in *Drosophila* and mammals.

The overall aim of this project is to understand the function of the poly-ADP-ribose pathway in memory. We are capitalizing on existing mutant strains and transgenic lines for the single fly *PARP* and *PARG* genes. To complement existing mutant *Drosophila* strains, we will use a structure-guided biophysical approach to design novel protein variants with altered enzymatic activity and with changed poly-ADP-ribose-binding function. We will then exploit *Drosophila* molecular genetic approaches to determine how these rationally designed as well as existing mutant PAR pathway components regulate the formation of memory.

STEM CELLS, SIGNAL TRANSDUCTION, AND DIFFERENTIATION

G. Enikolopov J. Encinas J.-H. Park
J. Hemish N. Peunova
T. Michurina K. Ravi

New neurons are continuously produced in the adult brain. The birth of new neurons is a dynamic process and it changes in response to a wide range of stimuli. The role of this constant supply of new neurons is still unclear; however, there is increasing evidence that adult neurogenesis may be important for learning, memory, and mood.

We study signals that regulate distinct steps in the cascade converting stem cells into neurons. We also study whether similar signals regulate stem cells in other tissues. We are particularly interested in how neurogenesis may be linked to mood regulation and may underlie the action of antidepressant therapies. We have developed a new approach for identifying and quantifying cellular targets of neurogenic stimuli and have used it to determine cell populations targeted by antidepressants in the adult brain. We are now using this approach to examine whether different types of treatments that regulate mood have a similar effect on the neuronal differentiation cascade and to identify signaling molecules that translate these treatments into an increased number of new neurons. Much of our effort is related to a versatile signaling molecule, nitric oxide, which is involved in controlling cell division and differentiation. Furthermore, we continue to generate new animal models to study stem cells and neurogenesis.

NEURAL STEM AND PROGENITOR CELLS IN THE ADULT BRAIN

New neurons of the adult brain are generated from neural stem and progenitor cells and become fully functional several weeks after they are born; during this time, cells undergo symmetric and asymmetric divisions, exit the cell cycle, express a range of markers, change their morphology, establish connections with other cells, and become integrated into the existing neuronal circuitry.

The transition from stem cells to fully differentiation neurons, the neuronal differentiation cascade, occurs through defined steps, and different classes of neuronal precursors can be distinguished by their morphology, expressed markers, and mitotic activity. We developed a transgenic approach that allows identification, in vivo visualization, isolation, and accurate enumeration of various classes of stem and progenitor cells in the adult brain. For that, we generated a series of reporter mouse lines in which neural stem and progenitor cells express various fluorescent proteins (GFP, CFPnuc, H2B-GFP, DsRedTimer, mCherry) under the control of the regulatory elements of the nestin gene. In particular, nuclear localization of the fluorescent signal in nestin-CFPnuc and nestin-H2B-GFP mice greatly simplifies the distribution pattern of neural stem and progenitor cells and allows accurate quantitation of changes induced by neurogenic agents in distinct classes of neuronal precursors. Using the reporter lines, we were able to dissect the neuronal differentiation cascade into several discrete steps (Fig. 1) and to evaluate the changes induced by various neurogenic and antineurogenic stimuli.

NEUROGENIC TARGETS OF ANTIDEPRESSANTS

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

Using our transgenic reporter lines and the scheme of the neuronal differentiation cascade in the adult brain, we decided to determine the cell population targeted by fluoxetine. We found that the drug does not affect division of stem-like cells, but it increases sym-

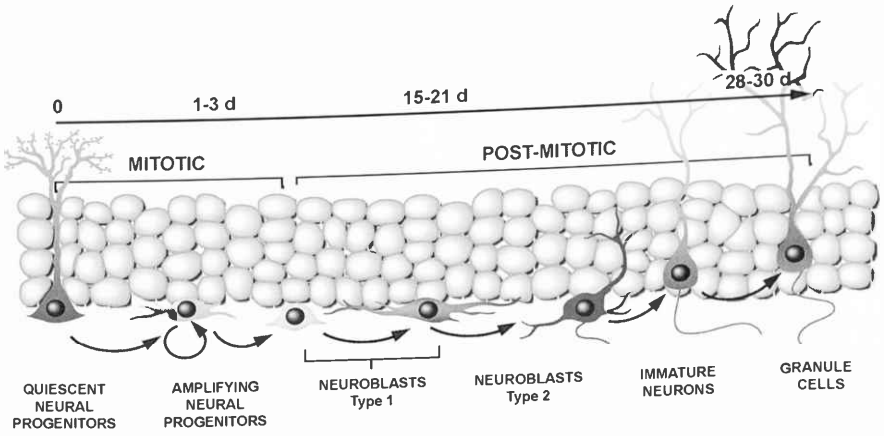

FIGURE 1 Neuronal differentiation cascade in the hippocampus.

metric divisions of an early progenitor cell class. We further demonstrated that this is the sole class of neuronal progenitors targeted by fluoxetine in the adult brain and that the subsequent fluoxetine-induced increase in new neurons arises as a result of the expansion of this class of cells.

Importantly, these studies suggested a general strategy to explore the changes induced by other antidepressant treatments. We are now using the same approach to determine the neurogenic targets of antidepressant drugs of different families and of the other types of antidepressant treatments, such as electroconvulsive shock and deep brain stimulation, trying to determine whether they converge on the same neurogenic targets. We also study whether these therapies may target other types of progenitor cells in the developing brain. Furthermore, we study whether neurogenesis and the action of antidepressants in the adult nervous system may differ under normal conditions and when affected by a disease (e.g., Parkinson's). Finally, we are using the reporter lines and the information that we gained about distinct steps of neuronal maturation to identify the signals that control the quiescence and self-renewal of stem cells and that direct the differentiation cascade in the adult brain.

STEM CELLS IN NONNEURAL TISSUES

Tissue maintenance requires a constant supply of new cells. These new cells replace cells lost to stress and damage or destroyed as part of the normal cell death program; moreover, in response to physiological and pathological stimuli, they may convey new properties to the tissue. The replacement of old cells by new ones is made possible either by self renewal of differentiated cells or through the activity of adult tissue-specific stem cells. Adult stem cells are usually morphologically unspecialized, can undergo long-term self-renewal, and are located in specialized niches that restrict their division and support their undifferentiated status.

We found that in our nestin-GFP and nestin-CFPnuc reporter mouse lines, expression of the reporter transgene marks stem and progenitor cells in several nonneural tissues. We have demonstrated that such GFP/CFPnuc-expressing cells can be found in the bulge region of the hair follicle, where they represent a population of cells with stem properties and a capacity to generate neuronal cells in vivo and in vitro; in testis, where they persist as precursors to the steroidogenic Leydig cells; and in the skeletal muscle, where they correspond to the satellite cells, the quiescent myogenic stem cells. They are also seen in the liver, where they reside as oval cells that can serve as stem cells after exposure to carcinogens or when the usual mode of liver regeneration through division of hepatocytes is suppressed. Furthermore, they are found in the anterior pituitary, where they serve as adult stem cells, able to generate all six endocrine lineages of the adult gland.

These findings suggest a close relationship between the expression of nestin and stem-like properties of cells in the adult tissues. Furthermore, they provide a means to isolate stem cells from various adult tissues and examine the requirements for such cells for tissue maintenance, response to injury, repair, or transplantation.

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Tatyana Michurina

CONSTRUCTION AND PLASTICITY OF THE GABAERGIC CIRCUITS IN NEOCORTEX AND CEREBELLUM

Z.J. Huang G. diCristo, Y. Fu, M. He, S. Kulhman, C.A. Paul, H. Taniguchi, P. Wu, X. Wu, H. Yang

A major challenge in modern neuroscience is to understand how neuronal functions emerge from the underlying neural networks and their cellular constituents. In many areas of the vertebrate brain, GABAergic interneurons are crucial in establishing the functional balance, complexity, and computational architecture of neural networks. For example, GABAergic inhibition regulates synaptic integration, probability, and timing of action potential generation in glutamatergic principal neurons. Furthermore, interneurons generate and maintain network oscillations, which provide the temporal structures for orchestrating the activities of neuronal populations. Therefore, the specific spiking patterns in dynamic ensembles of principal neurons, the neural code underlying their functional output, are largely determined by the spatial and temporal distribution of inhibition in the network.

The rich variety and fine details of inhibition are achieved by stunningly diverse types of GABAergic interneurons, which display distinct morphology, physiological properties, connectivity patterns, and biochemical constituents. Decades of studies suggest that the biophysical properties of different classes of interneurons are optimized for generating a rich array of distinct firing patterns, which constitute the "physiological vocabulary" of interneurons to control electrical activity in neurons and networks. Furthermore, each class of interneurons displays highly characteristic axon arbors and innervation patterns, which distribute their temporal firing patterns to discrete spatial locations, cell types, and subcellular compartments within the network. In addition, like the glutamatergic connections, GABAergic synapses can also be modified by usage, leading to reconfiguration of the inhibitory circuits by prior experience.

Understanding the design, operation, and plasticity of GABAergic circuits is therefore key to deciphering the precise organization and functional architecture of neural networks. My laboratory combines genetic, imaging, and physiological approaches to study the construction, plasticity, and function of GABAergic circuits, using cerebral and cerebellar cortex as complementary experimental systems.

SUBCELLULAR ORGANIZATION OF GABAERGIC SYNAPSES: L1CAMs AND ANKYRIN MEMBRANE SKELETONS

A striking feature of GABAergic innervation is the targeting of different classes of inhibitory synapses to subcellular compartments of principal neurons (spines, dendrites, soma, and axon initial segment [AIS]). Subcellular organization of inhibitory synapses is superimposed upon the intrinsic biophysical compartmental architecture of principal neurons and is essential in regulating input integration, spike probability, timing, and back propagation, but the underlying mechanism is poorly understood. Combining mouse genetics and high-resolution imaging, we have established in vivo and in vitro systems to visualize and manipulate defined classes of GABAergic interneurons.

Our study is beginning to outline the mechanisms underlying subcellular synapses organization. First, we demonstrated that subcellular targeting of GABAergic synapses is largely guided by genetically determined mechanisms and does not involve experience-dependent neural activity. Second, we discovered that the subcellular distribution of neurofascin, a member of the L1 family immunoglobulin cell adhesion molecules (L1CAMs), recruited by the ankyrinG membrane cytoskeleton, is a key molecular mechanism, which directs GABAergic innervation to AIS of Purkinje neurons. Third, we discovered that GABAergic innervation of the Purkinje dendrite is guided by an intermediate scaffold of Bergmann glial fibers. Interestingly, this glial scaffold appears to direct both the geometric and subcellular organization of GABAergic axons and involves another member of L1CAMs, CHL1 (close homolog of L1) as a major molecular signal (F. Ango et al., *submitted*).

Our general hypothesis is that members of L1CAMs recruited to subcellular domains by different ankyrins contribute to subcellular organization of GABAergic synapses. These studies raise many questions: What are the mechanisms for GABAergic synapse targeting in other brain circuit such as neocortex? What are the receptors in different classes of GABAergic neurons for L1CAMs?

ACTIVITY-DEPENDENT MATURATION AND PLASTICITY OF INHIBITORY SYNAPSES AND INNERVATION PATTERNS: A NOVEL FUNCTION OF GABA

The development of inhibitory circuits is shaped by neural activity, but the underlying mechanisms are unclear. We have established organotypic culture and in vivo systems to visualize and genetically manipulate defined classes of GABA interneurons in neocortex. We demonstrate that the maturation of perisomatic innervation by basket interneurons in the visual cortex is regulated by neural activity and visual experience.

Most recently, we made a fundamental discovery that GABA acts beyond its classic role as an inhibitory transmitter to sculpt the maturation, and likely plasticity, of inhibitory synapses and innervation patterns (Fig. 1) (Chattopadhyaya et al. 2007). We found that different aspects of GABAergic development are differentially sensitive to GABA synthesis by its rate-limiting synthetic enzyme GAD67. Although many earlier steps, such as cell migration and differentiation, proceed normally with reduced GAD67 expression, the maturation of perisomatic synapses from the basket interneuron in the adolescent neocortex is significantly deficient even with the loss of one *GAD67* allele (~30% reduction of neocortical GABA content).

These results suggest a surprisingly stringent requirement for GAD67 and GABA signaling in shaping the fine architecture of inhibitory circuits. Because GAD67 level is strongly coupled to neuronal inputs, activity-dependent GABA synthesis and signaling may provide a cell-wide as well as synaptic mechanism to sculpt GABAergic connectivity patterns. Our findings suggest an unexpected parallel between the role of glutamate in regulating the morphogenesis of excitatory synapses and that of GABA for inhibitory synapses; yet, they also imply fundamental differences between these two systems: Activity-dependent cell-wide regulation of "GABA resource" implies a novel logic for the plasticity of synaptic innervation patterns. Our discovery begs a revision in the current concept of GABA function in vertebrate brains and also raises a whole series of questions for future studies.

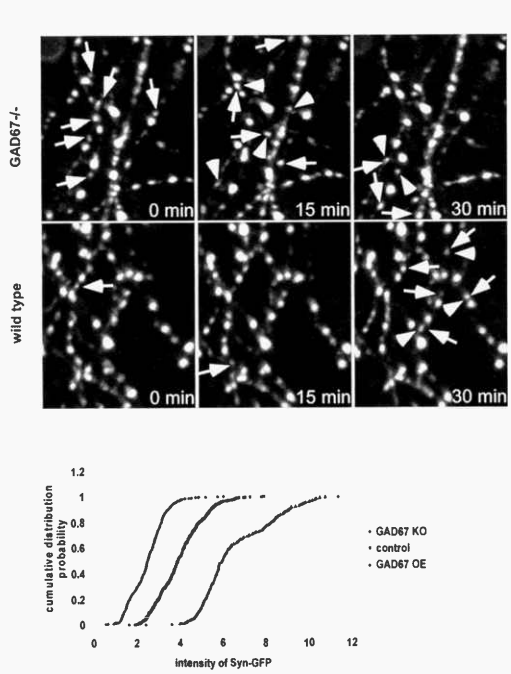

FIGURE 1 GAD67 promotes the maturation of inhibitory presynaptic terminals. (*Top*) Basket interneuron axons are labeled with red fluorescent protein (tdtomato) and synaptophysin–green fluorescent protein (GFP). The dynamics and stability of inhibitory terminals are imaged using two-photon microscopy in wild-type, GAD67$^{-/-}$, and GAD67 overexpressing basket cells. (*Bottom*) GAD67 promotes the accumulation of synaptophysin-GFP in presynaptic boutons.

MATURATION OF GABAERGIC TRANSMISSION AND CRITICAL PERIOD PLASTICITY IN VISUAL CORTEX

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

Maturation of GABAergic inhibition promotes the onset of the critical period for OD plasticity, but the underlying cellular mechanism remains elusive. Combining simple modeling and brain slice physiology, we found that a sufficient level of synaptic inhibition was critical to ensure that temporal correlation of inputs, rather than their initial synaptic strength, controlled postsynaptic spike timing. These results suggest that maturation of inhibition in the visual cortex ensures that the temporally coherent, open eye inputs control postsynaptic spike times of binocular neurons, a prerequisite for Hebbian mechanisms to induce OD plasticity (S. Kuhlman et al., *submitted*).

VISUALIZATION AND MANIPULATION OF GABAERGIC NEURONS AND CIRCUITS IN VIVO

For decades, the heterogeneity and complexity of the GABAergic network has hampered progress in understanding their development and function. Genetically engineered mice provide an ideal strategy to study the GABAergic system, but useful tools thus far are very limited. What is particularly needed are lines of mice in which genetic manipulations can be performed in specific classes of GABAergic neurons in restricted brain regions during a defined developmental window.

We have received major funding from the NIH Neuroscience Blueprint Project to achieve this goal using the Cre-loxP–based binary genetic system (UO1-MH078844-01). We are systematically generating more than 20 "driver lines" expressing Cre or inducible Cre recombinase in different classes of GABAergic neurons and their progenitors. In addition, we are constructing a new generation of Cre-activated "reporter" mice at the Rosa26 locus to achieve high-level GFP (or other gene of interest) expression by incorporating a variety of amplification strategies.

In collaboration of Sacha Nelson at Brandeis University (subcontractor), we will characterize these driver and reporter lines and establish a Web-based platform for disseminating the mice, reagents, and expression data to other investigators. These GABAergic driver and reporter lines will significantly accelerate progress in understanding all aspects of the development and function of the GABAergic system.

GENE EXPRESSION PROFILE AND PROGRAMS IN DIFFERENT CLASSES OF GABAERGIC INTERNEURONS

The stunning heterogeneity and complexity of GABAergic interneurons in their intrinsic and synaptic physiology, morphology, and connectivity are likely conferred by differential gene expression. A systematic characterization of gene expression profiles among GABAergic cell types may provide the most comprehensive and quantitative description of their molecular make-up and yield fundamental insight in their classification, biophysical repertoire, and the genetic program directing their development. We have generated an array of bacterial artificial chromosome (BAC) transgenic mice expressing GFP in different classes of GABAergic neurons.

In collaboration with my lab, Sacha Nelson at Brandeis University has developed a method to manually purify GABA neurons for microarray analysis (Sugino et al. 2006). We are applying this method to the development of neural circuit in cerebellar cortex. The cerebellum is appealing since various transgenic mice label every major class of GABAergic neurons and glia cells throughout development. These studies may reveal novel biophysical properties, signaling pathways, and transcription programs among interneurons and also yield better tools (such as genes that define novel cell types) for more refined genetic manipulations.

Such a non-hypothesis-driven approach is essential to elucidate the genetic design of the GABAergic system, its function, and capacity for plasticity. Using genetic strategies including MADM (mosaic analysis with double markers), we recently succeeded to reliably label individual basket and stellate interneurons to their entirety throughout their development in vivo. The lattice-like circuit architecture in the cerebellar cortex, centered on Purkinje neurons, allows high-resolution imaging and quantitative morphometry of basket and stellate interneurons with their synaptic targets. Together, the combined genetic, imaging, and genomic approaches promise to reveal new insights into the genetic logic of the development of GABAergic circuits in the cerebellum.

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BIOPHYSICAL BASIS FOR NEURAL COMPUTATION

A. Koulakov D. Tsigankov

We continued to focus on the theoretical model for development of connectivity in the visual system. Our model combines the effects of binding and activation of chemical labels, such as Eph receptors and their ligands, ephrins, and Hebbian plasticity. This year, we also included the effects of growth factors, such as BDNF, in our model. We studied both the final connectivity configurations and the dynamics of axons and dendrites when this connectivity is formed. Our model allows us to study the role of various factors on neural development and to make experimentally testable predictions. Thus, we showed that in some cases, Hebbian mechanisms can contribute to the formation of neural connectivity with a single-neuron precision.

General methods advanced by us this year allow us to represent formation of a more abstract neural network as a result of interplay between genetic factors represented by molecular labels and environmental information given by the neuronal electric activity. Our theoretical methods will allow us to address a broad range of questions pertaining to the dynamics and configurations of biologically realistic neural networks in the future.

A Unifying Model for Activity-Dependent and Activity-independent Mechanisms of Topographic Map Development

D. Tsigankov, A. Koulakov

In the developing brain, axons are capable of finding and recognizing their targets based on specific chemical cues, which are emitted into intercellular space or localized on the cellular membranes. Further refinement of neural connectivity is dependent on correlated neural activity. In some cases, neuronal connectivity is known to be assembled with a single-cell precision. To understand the relationship between the effects of chemical labels and activity-dependent refinement, we investigated a quantitative model that is capable of including both of these factors.

Our model generalizes Sperry's chemoaffinity principle. We postulate that the system of axons follows a gradient and optimizes the binding affinity of axons to their targets. The affinity includes both contributions from binding and activation of chemical labels and activity-dependent contributions. Our model takes as inputs the distribution of chemical cues expressed by axons and dendrites, the affinity matrix between these cues, and the correlation function of electric activity between connecting cells. As a result of this model, we obtained the complete connectivity matrix between cells.

To test our model we investigated the formation of topographic connectivity between the retina and superior colliculus or optic tectum that depends on binding and activation of the Eph family of receptor tyrosine kinases by their ligands, ephrins. Using our model, we obtained the following results: (1) Combined effects of chemoaffinity and correlated activity can help organize connectivity with a single-neuron precision. (2) In

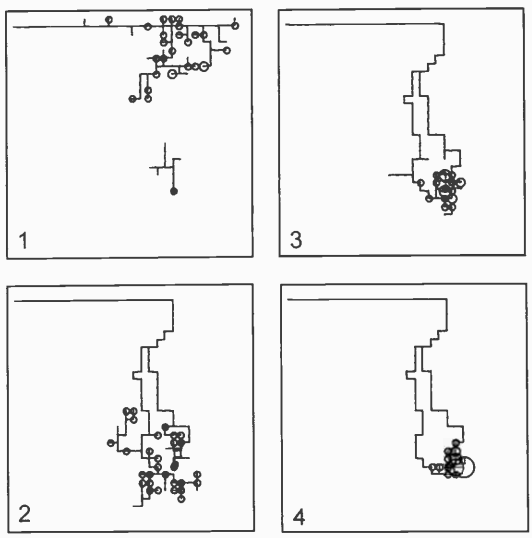

FIGURE 1 Coordinated dynamics of axon, dendrite, and synapses of this axon (*black circles*) in our model. The arbors are shown for four numbered time points. This dendrite and axon end up having 80% of their synaptic connections with each other, which implies a single-neuron innervation. The single-neuron innervation is possible because the arbors of axon and dendrite are highly correlated in geometric space. The soma location determines the level of ephrin expression. The simulation included 900 axons and 900 dendrites (only one pair is shown).

Isl2/EphA3 knockin mice described recently, the requirements imposed by molecular cues may contradict the activity-dependent Hebbian factors. In these animals, the winning factor depends on the relative strength of these contributions. (3) The axonal and dendritic branch dynamics observed in the optic tectum of *Xenopus* tadpoles is reproduced in detail by our model. (4) Geometric connectivity inferred from the overlap of dendritic and axonal arbors does not necessarily reflect functional connectivity. Our model therefore suggests a general method to combine disparate contributions affecting neural circuitry.

Speed Accuracy Trade-off in Olfaction

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

We report the first direct observation of speed accuracy tradeoff (SAT) in olfaction. We developed a behavioral paradigm in which both the time of odor exposure and the difficulty of the odor discrimination task were controlled by the experimenter. The accuracy of odor discrimination performance increases with the duration of odor exposure, and the rate of this increase is slower for harder tasks. We also present a unifying picture of two previous, seemingly disparate, experiments studying the timing of rodent odor discrimination (Uchida and Mainen, *Nat. Neurosci. 6:* 1224 [2003]; Abraham et al. *Neuron 44:* 865 [2004]). The presence of SAT in olfaction provides evidence for temporal integration in olfaction and constrains the applicability of different models of olfactory information processing.

Combinatorial On/Off Model for Olfactory Coding

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

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

Saha Formula in Olfaction

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

Olfactory perception is based on interactions between odor molecules and olfactory receptor proteins. The molecular specificity of binding and activation of the receptors by odorants forms the basis for discrimination of smells of different qualities and quantities. In this study, we considered the reaction of binding between odor molecules and receptor protein and derived the general relationship for the degree of receptor binding. We show that this relationship has a form of the Saha equation. We argue that the olfactory Weber law may be a consequence of the Saha equation for general assumptions about specificities of odorant-olfactory receptor-binding affinities.

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THE NEURAL ORGANIZATION OF OLFACTORY BEHAVIOR

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

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

We recently found that the orbitofrontal cortex (OFC) integrates spatial and reward information to construct maps of behavioral goals. Ongoing recordings focus on temporal coding in the olfactory cortex and the dynamics of representations during learning. From a broader neuroeconomic perspective, we are also studying how the brain deals with the costs of decisions (effort, delay, uncertainty).

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

Olfactory Target-Background Segmentation

N. Xu

Figure-ground segmentation is a fundamental issue faced by most sensory systems. Rats, which rely heavily on olfaction for getting around the world, may frequently have to identify important odors in the presence of various background odors. In some circumstances, the concentrations of the background odors are likely to be much higher than the significant odors. How do rats segment the target odors from the background, instead of perceiving the target-background mixture as an inseparable object? What are the neural mechanisms that can solve such a problem?

A bottom-up mechanism for odor segmentation has been proposed based on odor-specific adaptation of the neural response in the anterior olfactory cortex. This mechanism requires the background odor to be present prior to the target odor, so that the cortical neurons adapt to the background while keeping the responsiveness to the target odors. However, if encountering the target and background odors at the same time, could rats still solve the problem? If so, what could be the neural mechanism? Could the memory of the target odor have any role in this processing? Could active top-town (feedback) processing participate in target odor identification?

To explore these questions, we designed a behavioral paradigm in which rats learn to identify one of two chosen target odorants delivered simultaneously with one of many different background odorants. A custom-made 64-channel olfactometer is used to deliver various odors with precise timing. Currently, we have trained and tested rats with one pair of target odors versus more than 60 background odors. The behavioral data show a clear trend of improvement of performance. We are now testing rats' performance with randomly rewarded novel background odors interleaved with learned background odors. Data acquired so far suggest that rats can segment target odors from simultaneously encountered background odors, even when backgrounds have a much higher concentration.

Our future work will focus on the neural mechanisms underlying behavioral performance. We will use chronic multielectrode recordings in the olfactory cortex while rats perform the segmentation task. We wish to test whether olfactory cortex neurons contain background-invariant representations of the target odors. Also of particular interest is to test the hypothesis that the olfactory cortex generates a predictive representation of the target odors that might serve as a "search image" of the target odor.

Motivating Rats to Trade Speed for Accuracy

H.A. Zariwala, N. Uchida, A. Kepecs

Time can have an important role in the process of deciding between competing options. It is commonly observed that human subjects can sacrifice speed for accuracy in many types of decisions, and a large class of formal decision models are based on the integration of information over time. However, the time scale of integration may vary depending on the nature of the task and the neural systems underlying it.

In a reaction time two-alternative choice odor mixture discrimination task, we found that rats performed the most difficult discriminations with a very small increase in reaction time (~10%) compared to the easiest discriminations (Uchida and Mainen, *Nat. Neurosci. 6:* 1224 [2003]). As a follow up to these observations, one goal was to motivate rats to use longer odor-sampling times in order to increase accuracy.

In a first set of experiments, we manipulated task parameters to discourage speed and/or encourage accuracy. We introduced a variety of manipulations including (1) air puff for incorrect choices to increase the cost of errors, (2) minimum delays to reward delivery and between trials to eliminate incentives for rapid responding, and (3) carefully controlled restriction of water intake to increase the reward value of a successful discrimination. Although each of these manipulations slowed the reaction times of the rats, none produced the expected increase in accuracy, suggesting intrinsic limits on temporal integration in mixture discrimination.

In a second set of experiments we used a forced sampling duration task, training rats to withhold responding until an auditory "go" signal. This allowed us to enforce a minimum odor-sampling duration more strictly. Using this paradigm, odor-sampling durations increased from about 250 msec in the reaction time task to more than 1000 msec. Again, despite large increases in time for integration of information, the accuracy on the delay task was not greater than in the reaction time version of the task across all difficulties.

These results show that rapid olfactory reaction times are not simply a question of motivation but rather reveal fundamental underlying neural constraints. Important factors that may favor rapid processing of olfactory information are decreasing information rates due to sensory adaptation and chunking of information by the sniff cycle. Coding of elementary olfactory information into discrete snapshots may facilitate higher-order olfactory computations such as associating olfactory cues with spatial landmarks or navigating toward odor sources.

Active Sensation and Olfactory Behavior: Rapid and Precise Control of Sniffing

A. Kepecs, N. Uchida

Sensory organs are not passive receivers, rather they are active instruments controlled by the brain. A familiar example of active sensation is saccadic eye movement control known to be critical to vision. Olfactory perception also relies on an active sampling process—sniffing—to rapidly deliver odorants from the environment to the olfactory receptors. The respiration cycle strongly patterns the flow of information into the olfactory systems, but the behavioral significance of particular sniffing patterns is not well understood.

We used a nasal thermocouple (small temperature-sensing probe) that allowed us to monitor respiration in freely behaving animals. This method monitored the frequency and timing of nasal respiration in rats performing an odor mixture discrimination task. We found that respiration frequencies varied widely from 2 to12 Hz, but odor discrimination was dependent on 6–9-Hz sniffing: Rats almost always entered and maintained this frequency band during odor sampling, and their accuracy on difficult discrimination dropped when they did not. Moreover, the switch from baseline respiration to sniffing occurred not in response to odor delivery, but in anticipation of odor sampling and was executed rapidly, almost always within a single cycle. Interestingly, rats also switched from respiration to rapid sniffing in anticipation of reward delivery but in a distinct frequency band, 9–12 Hz. These results demonstrate the speed and precision of control over respiration and its significance for acquisition of olfactory information.

Chemotaxis by Rats: The Where Problem in Olfaction

M. Smear

Animals use spatiotemporal patterns of odor concentration to locate what they smell, a form of navigation known as chemotaxis. Chemotaxis is essential for animals to find mates and food or to avoid predators and toxins. To understand how the brain uses spatiotemporal

patterns of odor concentration to locate odor sources, we are studying chemotaxis in rats. We know that rats excel at odor-guided navigation, but we do not know what behavioral strategies and neural circuits they use.

In species in which chemotaxis has been studied in more detail, a common feature is that the behavior seems to take two states: one for when olfactory signals indicate that the animal is taking the correct trajectory and one for when the animal's trajectory is incorrect. Thus, microorganisms such as *Escherichia coli* and *Caenorhabditis elegans* navigate in straight runs in an increasing concentration gradient and turn more frequently in a decreasing concentration gradient. Moths navigate in odor plumes dispersed by wind. Their chemotaxis behavior also seems to have two states: They fly upwind as they encounter filaments of an odor plume, and in the absence of filaments, they fly perpendicular to the course of the wind.

To test whether rats use a similar two-state chemotaxis strategy, we built an olfactorium in which rats would have to experience both correct and incorrect trajectories as they navigate to odor sources. In preliminary studies, we have shown that rats can correctly navigate to odor sources >90% of the time, but not without first initiating and correcting an incorrect trajectory on 50% of correct trials. We will next study how rats behave when they experience correct and incorrect trajectories to an odor source and how they behave when we make the chemotaxis problem more difficult. In this way, we hope to understand what features of the spatiotemporal pattern of odor concentration can serve as the rat's cue for chemotaxis. In the future, we will combine this task with neural recordings as we seek to uncover the brain's olfactory "where" pathway.

Activity of Orbitofrontal Neurons during Initial Task Learning

G. Felsen

Numerous behavioral studies have demonstrated that animals are capable of learning complex sequences of actions in order to obtain rewards. Models based on reinforcement learning theory provide predictions about the processes that underlie task learning: For example, that there exist representations of the task states and values that can be used by the animal to determine the optimal action corresponding to each state. However, how the nervous system mediates such learning remains unclear, especially since relatively few studies have examined neuronal activity in animals that are learning, as opposed to those already well-trained in complex tasks.

To address these issues, we simultaneously recorded from multiple neurons in rats as they progressed through a series of tasks, culminating in a two-alternative choice odor discrimination. In the first task, the rats learned to "nose poke" into one of two goal ports (on the left and right sides of the environment) in order to receive a water reward. Next, the rat was required to first nose poke into a centrally located port before obtaining the reward at the goal port. Finally, the rat learned to sample an odor at the center port, the identity of which would determine at which of the two goal ports it would be rewarded.

We focused our recordings on the orbitofrontal cortex (OFC), an area known to represent reward value and that has more recently been shown to be spatially selective in rats well-trained on the two-alternative choice task. Such spatial activity can be thought of as a "state map," in which the location of the rat (which in our tasks corresponds to a state) is represented by the firing rates of OFC neurons.

We addressed two questions: First, does spatial selectivity develop immediately after exposure to the behavioral task or is extensive training on the task required? Second, do the dynamics of OFC responses reflect task learning? We found that from the earliest behavioral sessions, populations of neurons were selective for all relevant spatial locations (left and right goal ports and center odor port) and for movement toward these locations. This selectivity could provide real-time information about the current task state, which would be useful for exploring and evaluating actions during early training stages. In addition, a subset of neurons showed different response timings in later trials than in earlier trials within the same session. Specifically, we found that the peak response of movement-selective cells occurred earlier during later trials. This peak shift was not due to a change in movement speed but was correlated with improvement in behavioral performance over the course of the session. These results suggest that OFC responses may have a role in guiding task learning.

Activity of Orbitofrontal Neurons during Acquisition of Novel Odor Discriminations

C.E. Feierstein

Adaptive, flexible behavior relies on the ability to learn from the consequences of ones actions and to use

that knowledge to guide our decisions. This form of decision-making is known as "goal-directed" behavior. We are interested in understanding the neural basis of decision-making and goal-directed behavior, and the OFC is a brain area thought to be central to these functions. To sustain such behavior, the brain should represent two aspects that are essential to this process: the desired outcomes (or goals) and the actions leading to those outcomes. We previously recorded the activity of OFC neurons in rats performing a two-alternative choice discrimination task and found that OFC neurons represented the behaviorally relevant spatial locations, the "goals" in the context of the task (Feierstein et al. 2006). Moreover, OFC neurons encoded the outcomes of each trial (rewarded or not rewarded, i.e., the success or failure of the rat's choice). These results indicate that the OFC neurons encode a signal that reflects the performance of the rat; such a signal could be used to update behavior when outcomes are not favorable or could be used to learn the consequences of actions by trial and error.

To test the possibility that OFC neurons encode error signals and to explore the role of OFC during learning, we trained rats in a novel-odor discrimination paradigm. In this task, rats started each session with a well-learned two-odor discrimination (A/B). After approximately 100 trials, a new odor pair (C/D) was introduced and the rats had to learn the contingencies associated with that novel pair while continuing to discriminate the original A/B pair. In each recording session, a different novel pair was introduced. Rats learned new odor associations fairly rapidly. Usually, a performance accuracy of 80% for the novel pair was achieved in 1 to 60 trials, while maintaining intact performance for the original pair (>90%).

We next recorded the activity of OFC neurons during the acquisition of novel odors. Preliminary data suggest that OFC neurons fire selectively to novel odors, whereas our previous recordings using a well-learned pair showed that there is relatively little odor selectivity in OFC. The selectivity for novel odors developed extremely rapidly (one or a few trials) after these stimuli were introduced. Although many neurons responded to novel odors during the odor-sampling period, as might be expected, the introduction of novel stimuli was also associated with a more widespread change in response properties, including both increases and decreases in firing outside the odor presentation period (e.g., during movement or reward).

These data suggest that OFC circuits are engaged by novel olfactory stimuli. By integrating representations of novel stimuli with representations of actions leading to reward, OFC may participate in the learning of new stimulus response outcome contingencies. Further analyses will address how outcome representations evolve with learning.

Neural Representation and Behavioral Impact of Uncertainty in Decision-making

A. Kepecs, N. Uchida, H.A. Zariwala

Uncertainty has long been studied in economics, psychology, and behavioral ecology as a decision variable critical for understanding behavior. More recently, several computational schemes have been proposed for how populations of neurons might be able to represent not only a value of interest, but also the uncertainty associated with it. However, despite the recognition of uncertainty as a pivotal variable for decision-making and neural coding, little is known about how and where it is represented in the brain.

We therefore explored the neural representation of uncertainty and its behavioral impact in an odor-mixture categorization task. Rats were trained to report the category (dominant component) of binary mixtures of two odors and were rewarded at either the left or the right choice port for correct decisions. Discrimination accuracy for pure odors was close to 100%, but for the most difficult mixtures, performance dropped to almost 60%. Since reward was always provided for correct choices, the outcome was uncertain only because rats sometimes were not able to discern and/or remember the correct category of the odor mixture. We hypothesized that the OFC—a region of the prefrontal cortex known to be activated during choice behaviors involving uncertainty in humans—represents the degree of uncertainty or confidence associated with choices.

What would a representation of uncertainty look like? First, uncertainty will vary with the difficulty of stimulus. Second, for a fixed stimulus, on average, a good estimate of uncertainty will predict the accuracy of a decision (correct/error). We formalized and checked these predictions using two different models of decision-making. Both models agreed in their qualitative predictions: For correct choices, uncertainty increases with stimulus difficulty, whereas for incorrect choices, on average uncertainty is always larger but decreases with stimulus difficulty (Fig. 1A). We recorded the activity of OFC neurons during this decision task and focused our analysis on the reward anticipation period after the choice was made but before reward feedback was provided. We found that about 30% neu-

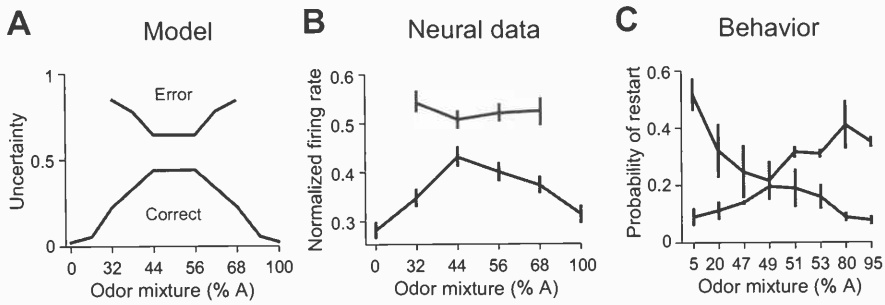

FIGURE 1 Comparison of uncertainty model (*A*), neural recordings from OFC (*B*), and behavioral data (*C*). See text for description.

rons predicted trial outcome (reward/no reward), and their firing rates varied consistent with the model (Fig. 1B). This suggests that orbitofrontal neurons provide an estimate of uncertainty during decision-making.

Next, we tested whether the rats could use uncertainty to guide their behavior. We modified the decision task by delaying reward and allowing rats to abort and restart trials during the delay. This behavior could be compared to a human's when looking for a particular exit on an unfamiliar road based on a fuzzy road sign: The decisions of whether and when to turn around depends on the uncertainty about whether the exit was already passed. Similarly, rats preferentially aborted uncertain trials: Those with stimuli closer to the category boundary and when they made erroneous decisions (Fig. 1C). Thus, rats, like humans, are able to estimate the uncertainty associated with their decisions and use it to guide their behavior. Our results emphasize that uncertainty is a fundamental quantity for decision-making—one that is easily and most likely automatically computed during the decision process to support adaptive behavior—similar to other previously studied decision variables such as choice probability and reward value.

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

M. Murakami

Consider the situation in which you wait for a bus but it is delayed. You have no idea when it will arrive. There is another option, taking a cab, which costs much more but is available immediately. How long do you wait for the bus? When do you give up waiting for the bus and decide to take a cab? In such circumstances, your brain must compute the costs and benefits of the choices and use that information for choosing an action—waiting or giving up.

The medial prefrontal cortex (mPFC) is reciprocally connected to the OFC, which is suggested to be involved in value representation of stimuli. Previous studies show that inactivation of the mPFC impairs the ability for an animal to wait. A recent electrophysiological study also suggested the involvement of the mPFC in waiting. The mPFC neurons send output to the striatum, which is thought to be involved in action generation. Thus, the neuronal circuit centering on the mPFC is well-situated to use value information to choose between waiting and giving up. In this study, we address the question of how this circuit decides to wait or not to wait.

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

We first plan to record neuronal activity from the mPFC and other areas connected with the mPFC and then examine whether neuronal activities from these areas can predict the choice of waiting time and other aspects of the behavior. Preliminary results showed that the time a rat is willing to wait for a given set of reward amounts and delays varies randomly from trial to trial. On some trials, the rat will respond immediately after

the first tone, but on others, it will withhold responding until after the second tone, and on others, it will wait well past the first tone, but give up before the second. Brain areas responsible for the choice of either to wait or to give up waiting should be able to predict how long a rat will wait and when it will give up waiting.

Diversity and Precision of Neural Activity in Serotonergic Brain Stem Nuclei

S. Ranade

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

In this project, we recorded from raphe nuclei in rats performing a two-alternative choice odor discrimination task. Rats were trained to associate individual odors with water reward at one of two choice ports. Correct responses were rewarded probabilistically after a variable delay. This paradigm allowed us to study sensory, motor, and reward-related responses with high temporal precision by aligning to nose poke events.

After training, rats were chronically implanted with a six-tetrode recording drive. Tetrodes were targeted to the raphe using a guide cannula. Recordings were obtained from 54 neurons in seven rats over an average of four to eight sessions per rat. Recording locations were verified histologically following termination of experiment. Raphe neurons showed diverse firing properties with respect to waveform characteristics, firing rate, and sleep state modulation. By conventional criteria, 10% of neurons were wide-spiking putative serotonin neurons.

Neuronal responses were analyzed with respect to four epochs: odor sampling, movement, reward anticipation, and reward consumption. Firing rates of >70% neurons were modulated during at least one behavioral epoch, with a large fraction tuned to multiple events. Many neurons responded to behavioral events within 100 msec, whereas some were even more precisely time-locked (order 20 msec), showing a very strong (>40 sp/sec) phasic response, apparently to the click produced by water valve opening.

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

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

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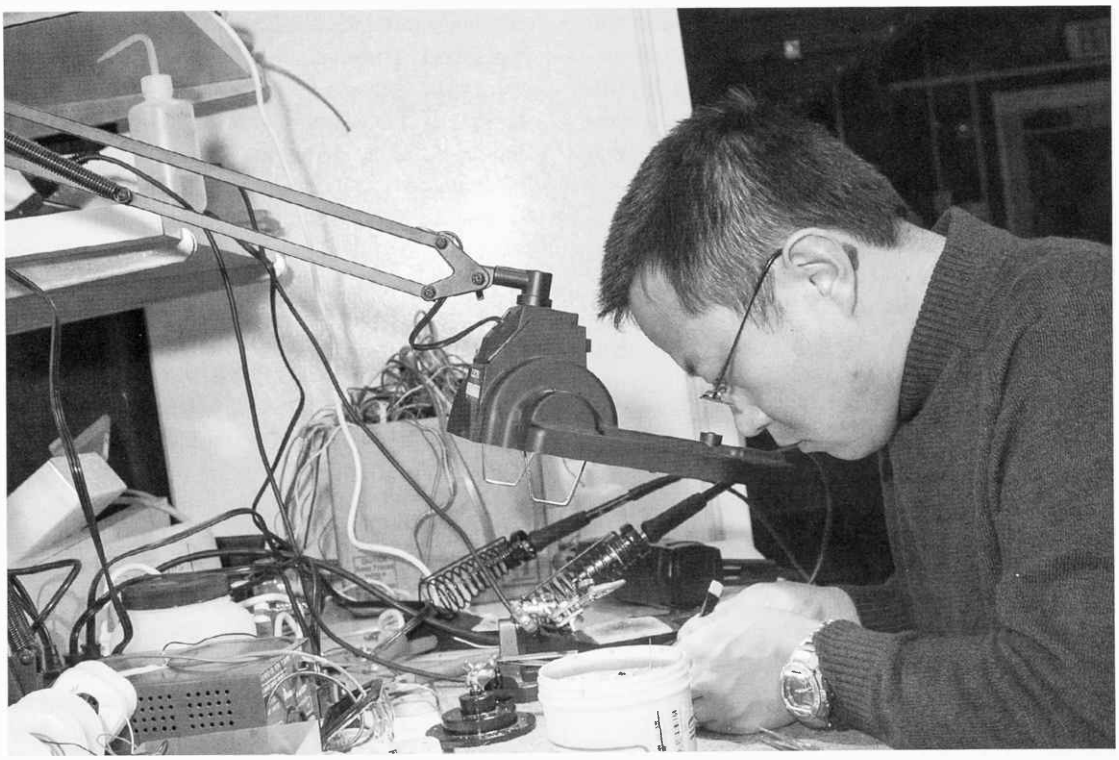

Ning Long Xu

TRANSMISSION AND PLASTICITY IN MAMMALIAN CENTRAL SYNAPSES

R. Malinow W. Benjamin, J. Boehm, N. Dawkins-Pisani, I. Ehrlich, H. Hsieh, H. Hu, H. Kessels, C. Kopec, B. Li, T. Takahashi, W. Wei

My laboratory is directed toward understanding synaptic function, synaptic plasticity, and synaptic dysfunction. Through such an understanding, we hope to elucidate how learning and memory are achieved and how diseases corrupt them. This year, we continued to examine the regulation of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and *N*-methyl-D-asparate (NMDA) receptors at synapses that may underlie plasticity and the malfunction that could lead to disease. Some of our studies are summarized below.

AMPA-R Removal Underlies Aβ-induced Synaptic Depression and Dendritic Spine Loss

H. Hsieh, R. Malinow [in collaboration with S. Sisodia, University of Chicago]

Beta amyloid (Aβ), a peptide generated by neurons, is widely believed to underlie the pathophysiology of Alzheimer's disease when overproduced. Recent studies indicate that this peptide can drive endocytosis of AMPA- and NMDA-type glutamate receptors. We now show that Aβ uses signaling pathways of long-term depression (LTD) to drive endocytosis of synaptic AMPA receptors (AMPA-R). Synaptic removal of AMPA-R is key, because it is necessary and sufficient to drive loss of dendritic spines and synaptic NMDA responses. Our results indicate that increased levels of Aβ tap into endogenous physiological processes to depress synaptic structure and function.

PSD-95 Is Required for Activity-driven Synaptic Development

I. Ehrlich, R. Malinow

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

Signaling by *Neuregulin/ErbB4*, Genes Implicated in Schizophrenia, Is Critical to Proper Maturation of Excitatory Synapses

B. Li, R. Malinow [in collaboration with L. Mei, College of Georgia]

Neuregulin 1 (*NRG1*) and its receptor *ErbB4* activate signaling cascades that are essential for the development and function of many organ systems including the neural system. However, the function and mechanism of *NRG1* and its receptors in the central glutamatergic synapse are poorly understood. Here, we show that *NRG1* signals via *erbB4* to control spine growth, synaptic maturation, and synaptic plasticity at the Schaffer collateral-CA1 synapse. Our data suggest that the defect in glutamatergic synapse development caused by a perturbation in the *NRG1/erbB4* signaling pathway may contribute to the etiology of schizophrenia.

Postsynaptic Receptor Trafficking Underlying a Form of Associative Learning

S. Rumpel, A. Zador, R. Malinow [in collaboration with J. LeDoux, New York University]

To elucidate molecular, cellular, and circuit changes that occur in the brain during learning, we investigated the role of a glutamate receptor subtype in fear

conditioning. In this form of learning, rodents associate two stimuli, such as a tone and a shock. In this study, we found that fear conditioning drives AMPA-type glutamate receptors into the postsynapse of a large fraction of neurons in the lateral amygdala, a brain structure essential to this learning process. Furthermore, memory was reduced if AMPA-R synaptic incorporation was blocked in as few as 10–20% of lateral amygdala neurons. Thus, the encoding of memories in the lateral amygdala is mediated by AMPA-R trafficking, is widely distributed, and displays little redundancy. We have continued these studies by examining dendritic spines in vivo before and after fear conditioning.

Spine Enlargement Precedes AMPA-R Exocytosis during LTP

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

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

Differential Subcellular Overexpression of AMPA-R Subunits in CA1 Neurons

H. Kessels, M. Klein, R. Malinow

The role of AMPA-type glutamate receptors in synaptic plasticity can be studied by the introduction of recombinant AMPA subunits in glutamatergic neurons. Here, we show that whereas transient expression of AMPA subunits leads to considerable overproduction in neuronal somata of organotypic hippocampal slices, at dendrites, they remain close to endogenous levels. These results provide evidence for a tightly controlled transport mechanism of AMPA-R complexes traveling from soma to dendritic compartments. We have now evidence that the AMPA-R-associated protein, stargazin, is a rate-limiting protein in the transport of AMPA-Rs from cell bodies to dendrites. This transport system is selective for certain AMPA-R subunits.

Direct Phosphorylation by PKC at a Novel Site on AMPA-R Subunit Glur1 Controls Synaptic Incorporation during LTP

J. Boehm, R. Malinow [in collaboration with R. Huganir, Johns Hopkins University]

Incorporation of GluR1-containing AMPA-Rs into synapses has an important role in several forms of neural plasticity. A number of signaling pathways have been identified, but the direct modifications of GluR1 that control its synaptic incorporation have not yet been found. Previous studies indicate that activation of protein kinase C (PKC) is required to generate LTP, a leading model of synaptic plasticity. However, the targets of PKC responsible for LTP have not been determined. We find that GluR1 Ser-818, which is highly conserved evolutionarily, is phosphorylated by PKC in vitro and by LTP in hippocampal slices. Acute phosphorylation by PKC at GluR1 Ser-818 as well as molecular mimicking phosphorylation at this site drives GluR1 into synapses. Preventing GluR1 Ser-818 phosphorylation blocks LTP and PKC-driven synaptic incorporation of GluR1. Thus, GluR1 Ser-818 is rapidly phosphorylated by PKC during LTP induction and is required for its incorporation into synapses.

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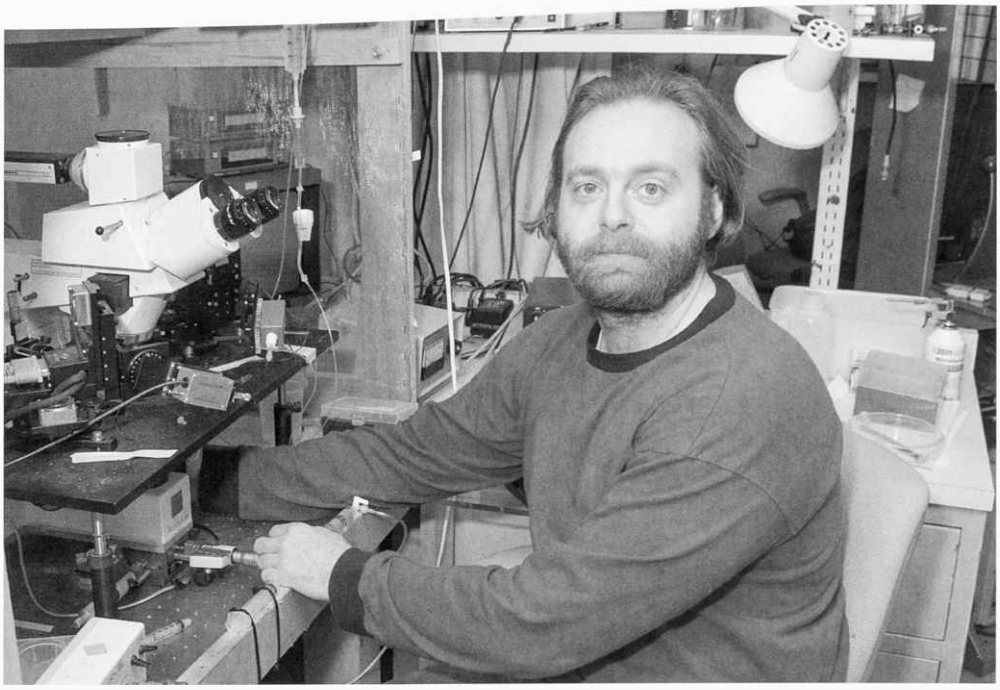

Alfonso Apicella

INTEGRATIVE SYSTEMS NEUROBIOLOGY

P. Mitra C. Allen H. Bokil J. Serkhane H. Wang
P. Andrews H. Maniar D. Valente

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

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

Continuing in our lab in 2006 are Hiren Maniar, (scientific informatics analyst), Hemant Bokil (postdoctoral fellow), and Peter Andrews (scientific informatics manager). Joining us in 2006 are Haibin Wang, Dan Valente, and Jihene Serkhane (postdoctoral fellows) and Cara Allen (scientific informatics manager). We also have close collaborative ties with multiple research groups at CSHL and other institutions, which currently include a collaboration with Nicholas D. Schiff and Keith Purpura at the Weill Medical School of Cornell University, where Dr. Mitra is an adjunct associate professor; a consortium of zebra finch researchers at four different universities, including a long-standing collaboration with Ofer Tchernichovski at City College of New York (CCNY); an integrative analysis of memory formation in *Drosophila*, as part of the Dart Neuro-genomics Alliance at CSHL; and the Brain Architecture Project, begun in 2006 with coinvestigators at Harvard/Massachusetts General Hospital, California Institute of Technology, and the University of Southern California.

Chronux: Open Source Software for Neural Signal Processing

H. Bokil, P. Andrews

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

We made improvements to the Chronux software package in 2006, largely to increase its usage in the field. We created several new tutorials that discuss signal-processing theory and provide step-by-step instructions for how to handle common data analysis scenarios. We have also added several new GUIs for specific analysis tasks, including spike sorting and audio segmentation. Since 2001, we have used Chronux in the annual MBL Neuroinformatics summer course, and this year, we presented a one-day tutorial at the Dynamical Neurosciences Workshop preceding the 2006 Society for Neuroscience Annual Meeting in Atlanta, Georgia. Use of Chronux in the neuroscience community is increasing, and the package has been downloaded more than 2000 times (1300 in 2006), with more than 240 users registering for support and updates (170 in 2006).

LBEX: A Technique for EEG/MEG Source Localization

H. Maniar

Electroencephalography (EEG) and magnetoencephalography (MEG) provide noninvasive images of electrical and magnetic fields resulting from internal neural activity. Localizing the spatial origins of this neural activity from EEG/MEG images is a challenging problem requiring both an understanding of human brain function and sophisticated mathematical and signal-processing expertise. Our objective is to provide the research community with a tool kit for reliable and

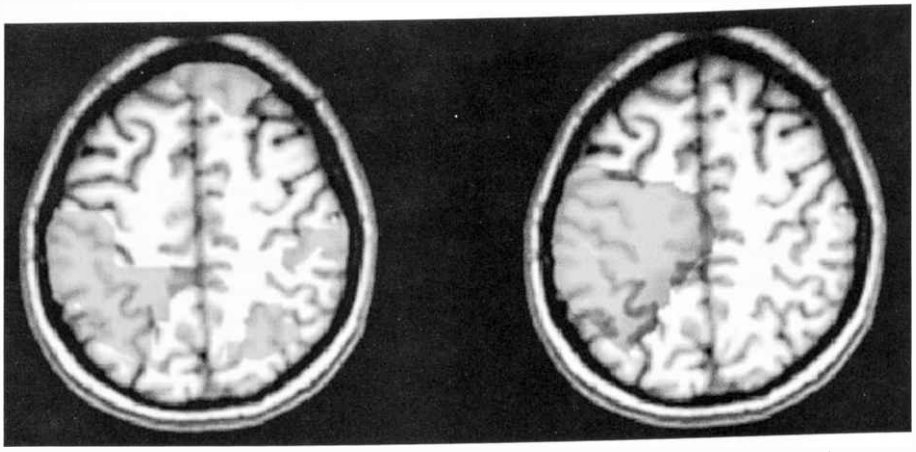

FIGURE 1 Localization of brain activity in a working memory task, where EEG and MEG were simultaneously measured. Localization based on EEG (*left*) and MEG (*right*) show overlapping regions of activation. Since these are independent measurements, this increases our confidence in the source localization technique.

accurate source localization of neural activity, and during the past few years, we have developed a promising new localization technique: local basis expansions for linear inverse problems (LBEX). With theoretical aspects of LBEX now well-characterized, we initiated a collaborative study with Dr. Richard Coppola at the National Institute of Mental Health to apply the technique to real EEG-MEG data. Figure 1 compares unimodal localization of neural activity in the 8–12-Hz frequency range (alpha band) using EEG (left) and MEG (right) recordings. Since information captured by EEG and MEG is complementary, we expect that bimodal localization using simultaneous EEG and MEG recordings will be more robust and accurate than unimodal localization using EEG or MEG images separately.

Integrative Analysis of Memory Formation in *Drosophila*

D. Valente

This is the second project year of the Mitra lab's participation in the Dart NeuroGenomics Alliance at CSHL. Our group represents the alliance's principal theoretical modeling component and has a broad goal of integrative data analysis and modeling of memory formation in the fruit fly *Drosophila melanogaster*, spanning genetic, cellular, neural, and behavioral levels.

We are developing several experimental setups to probe memory formation at the neural network and behavioral levels. The first is a flight simulator (Fig. 2) for tethered flight conditioning assays. A fly is tethered

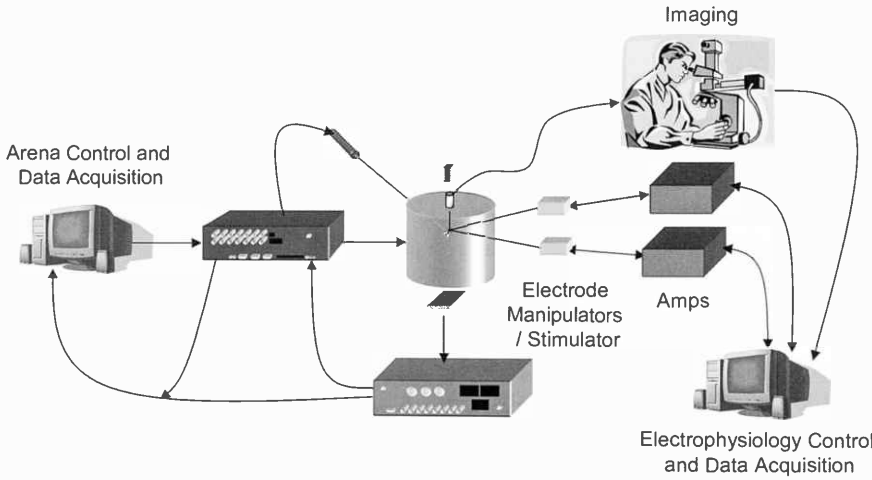

FIGURE 2 Flight simulator for *Drosophila melanogaster*. A fly is tethered in the apparatus and is presented with a visual panorama that rotates in response to the flight behavior.

in the center of a cylindrical arena, where visual stimuli are presented. The simulator can be run in an open-loop mode, in which the visual stimuli are independent of the fly's behavior, or in a closed-loop mode, in which the fly's wing-beat pattern is used as feedback to control the visual panorama.

Another important step in creating an integrative model of memory is the phenotypic characterization of memory mutants and wild-type flies. The majority of memory mutants have no obvious phenotype besides memory deficiency, but pleiotropic effects on other behaviors cannot be ruled out, and their characterization may further elucidate the function of particular genes in memory processes. As one approach to this characterization, we will quantify locomotor behavior with an automated system allowing for high-throughput experiments. For this purpose, we are developing an open-field arena for observing exploratory walking behavior, along with trajectory tracking software. Our tracking software is in use by our group and Tim Tully's group here at CSHL and by our collaborator in this work, Dr. Ilan Golani of Tel Aviv University.

Song Learning in the Zebra Finch

H. Wang

Our research on zebra finch song learning is part of a consortium of researchers at four different institutions. This collaborative approach toward understanding the neural mechanisms of vocal learning seeks to provide a quantitative description of the relationship between physiological variables and vocal performance in the zebra finch over the course of song development. The goals of the Mitra lab component of this collaboration are to provide an informatics infrastructure, to build an integrative theoretical framework, and to develop signal-processing algorithms for data integration and hypothesis discovery and testing.

We have set up an external portal for the consortium at zebrafinch.org and are currently in the process of building an internal portal that will feature collaborative tools such as a bulletin board, a file archive, and a database for experimental data. Since the data sets generated by the consortium are very large, we have had to devote significant effort to the hardware and software aspects of terabyte-scale databases, but expect this to have significant payoffs in terms of the ability to perform analysis of the song system at an unprecedented scale. Semantic Web methods, which we have chosen to employ in our work, may facilitate the integration of neurobiological data across modalities and scales, an aspect of data analysis currently under intense investigation and therefore of potentially far-reaching significance for neuroscience.

Quantitative Analysis of Speech Development in Human Infants

J. Serkhane

Brain-imaging techniques are revolutionizing research in human psychology and neuroscience, but their application to the infant human brain will remain limited due to ethical and technical reasons. An alternative and complementary approach can be found in quantitative human ethology, with noninvasive high-resolution recording and fine analysis of natural behavior.

The purpose of this project is to study the development of speech in human infants through quantification of vocalizations in ecological conditions. Previous studies of early language acquisition have largely consisted of linguists transcribing infant sounds by hand or of relatively short acoustic recordings (a few hours per week or month), although infant vocalizations can change significantly from day to day. Instead, we will use more continuous, dense recordings of vocalizations (audio and video) and will also develop associated analysis and data management tools. Before collecting data from a larger target population, we are conducting preliminary analyses of smaller data sets to guide the development of analysis and classification software. One data set, from Dr. April Benasich (Rutgers University), consists of analog audiovideo recordings of infants at 6, 12, 24, and 36 months of age. A second data set, consisting of a several-month-long audio recording, has been acquired from the Max-Planck Institute in Nijemegen in The Netherlands.

Engineering Principles in Biological Systems

P. Mitra

We have begun a series of workshops and conferences intended to foster an emerging approach to theoretical biology with a formal emphasis on design or engineering principles. Through the process of evolution, living systems retain accidentally found solutions to problems they must solve in order to survive. Although such solutions or designs are not engineered but instead evolve incrementally, the premise of this theo-

retical approach is that they may nevertheless be studied in their existing forms in the framework of theories developed alongside human-engineered systems. Our goal is to determine whether major existing engineering theories (controls, communication, computation) can be applied to biological systems and, if not, what modifications are in order.

The first workshop, entitled "Design Principles in Biological Systems," was held at CSHL's Banbury Center in May 2006. Each session included a tutorial overview of an engineering theory, followed by participant presentations about corresponding examples from biological systems, chosen to span organizational levels, from populations to molecular pathways. The small workshop format provided an opportunity for biological researchers to learn about engineering theories relevant to their work and for engineering theorists and computer scientists to learn about biological questions they might help answer. A larger meeting, "Engineering Principles in Biological Systems," held at CSHL in December 2006, allowed for presentation of a broader range of relevant research. A second Banbury workshop is planned for 2007 and another CSHL meeting for 2008.

The Brain Architecture Project

P. Mitra, C. Allen

Although the human genome has been sequenced, the architecture of the human brain, in terms of its connectivity patterns and functional subsystems, remains incompletely characterized, despite the classical origins of human neuroanatomy research. The Brain Architecture Project is a new collaborative effort based in the Mitra lab with a long-term goal to produce a comprehensive draft of the connectivity matrix of the human brain, along with analytical and visualization tools. A main focus will be collation and integration of existing but fragmented information about human neuroanatomy into a comprehensive database. Data from a large-scale functional brain-imaging study will be analyzed with respect to project goals, and we will also consider the architecture of rodent and non-human primate brains to exploit homologies across species. The results of the project will be made readily accessible to researchers for scientific and clinical applications.

The Brain Architecture Project is supported by the W.M. Keck Foundation and draws on the expertise of coinvestigators in neuroanatomy (Larry Swanson, University of Southern California), human brain imaging (Hans Breiter, Massachusetts General Hospital), and analysis of connectivity graphs (John Doyle, California Institute of Technology). The project begins 2007 with a newly recruited full staff, several additional collaborators, and a board of prominent external advisors.

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GENE COPY-NUMBER VARIATION AND ITS ROLE IN HUMAN DISEASE

J. Sebat W. Choi, R. Kim, M. Kusenda, P. Lin, J. Lloyd, D. Malhotra, S. McCarthy, K. Pavon, P. Roccanova, S. Yoon

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

Determining the Genetic Basis of Autism

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

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

The mutations that we detected occurred at many loci throughout the genome, and few were detected more than once. These results are consistent with the findings of linkage studies and cytogenetics and support the hypothesis that there are many genes in the genome that potentially contribute to autistic spectrum disorder (ASD), with each gene contributing to only a small fraction of cases. Our findings implicate specific genes in autism including genes involved in sterol metabolism, hormonal signaling, and synaptic transmission; however, further studies are needed to determine the contribution of each gene to the disorder in the broader population of patients.

To this end, we have begun candidate gene studies, including sequencing and fine-scale CNV analysis in a large sample of patients and controls. Preliminary results suggest that more complete ascertainment of variation within candidate regions may reveal the greater extent of an individual gene's contribution to ASD. For instance, our recent work done in collaboration with Dan Geschwind (University of California, Los Angeles) has found a convergence of evidence from analysis of SNPs and CNVs implicating the gene *CNTNAP2* in ASD.

A Genomic Analysis of Schizophrenia and Bipolar Disorder

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

A high spontaneous rate of structural mutation is a property of the human genome and exists independently of the phenotypes that emerge from it. Thus, our findings in ASD may serve as a model for other disorders. We hypothesize that rare highly penetrant mutations, including a significant proportion that are CNVs, may also have a role in the etiology of schizophrenia (Scz) and bipolar disorder (BD). We have therefore recently initiated genetic studies of Scz and BD, with a focus on families with sporadic and young-onset cases. In addition, a small collection of families have been obtained that each have more than three individuals diagnosed with Scz. To date, we have scanned the genomes of 350 Scz patients and more than 500 controls.

Spontaneous copy-number mutations have been identified in our studies, suggesting that the approach described above will be effective in these

disorders as well. However, because twin studies of both Scz and BD suggest that the penetrance of the mutations involved are lower on average compared to risk factors for ASD, we may expect that the proportion of variants that are familial (as opposed to spontaneous) will be higher. Therefore, our studies will likely require a larger sample size, and we will also rely to a greater extent on detecting the association of rare variants by observing recurrent non-identical mutations at individual loci in the genome.

A Case Control Study of Parkinson's Disease

J. Sebat, D. Malhotra, S. Yoon

Parkinson's disease (PD) is the most common neurodegenerative movement disorder characterized by age-dependent increases in bradykinesia, muscular rigidity, gait abnormalities, and a rest tremor. PD can arise from multiple etiologies, including genetic mutations that for the most part are uncommon. Evidence has been lacking for the existence of common genetic variants with large effect, but it does exist for multiple genes including common alleles with small effect and rare highly penetrant alleles. Such complex etiology is inherently difficult to study using traditional genetic approaches. Analysis of genome copy-number polymorphisms holds great promise in identifying structural variants predisposing to PD pathology.

We used an 85K probe representational oligonucleotide microarray analysis (ROMA) to screen 300 late-onset sporadic PD cases and an equal number of matched controls from Caucasian and Chinese cohorts. Findings include the detection of deletions in both familial and sporadic cases involving two genes, *ZIC1* and *ZIC4*, which are related to cerebellar development. Also detected were deletions of *Parkin*, a gene previously implicated in recessive early-onset PD. These findings suggest that heterozygous deletions of *Parkin* also may have a role in late-onset PD. More comprehensive studies of candidate genes identified in this study will help us to determine the contribution of these genes to the disease.

Analysis of Large-scale Copy-number Variation in the HapMap

J. Sebat, S. Yoon, J. Lloyd, D. Malhotra

[in collaboration with K. Ye and B. Lakshmi, Cold Spring Harbor Laboratory]

Much remains to be learned about the extent of "normal" structural variation in the human genome and its implications for genetic studies of populations. We have initiated a CNV-discovery effort that will focus on DNA samples from the International HapMap Project. The key advantage of using the HapMap samples for this study is the availability of high-density SNP genotypes on the same individuals. CNV data can be combined with SNP data to determine, for example, whether a polymorphic duplication or deletion is associated with a unique SNP haplotype (indicating that the variant results from an ancestral event that was inherited by descent) or with many different haplotypes (suggesting recurrent structural mutation at that site). In addition, direct analysis of copy-number variation is helpful for resolving aberrant patterns in the HapMap data that result from CNVs, such as apparent non-Mendelian patterns of inheritance. Here, we analyzed copy-number variation in 30 CEPH trios using ROMA.

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EXPERIENCE-DEPENDENT PLASTICITY OF SYNAPSES AND SYNAPTIC CIRCUITS

K. Svoboda I. Bureau, V. DePaola, C. Harvey, A. Holtmaat, A. Karpova, T. O'Connor, G. Tervo, L. Wilbrecht, C. Zhang

The functional properties of the brain change in response to salient sensory experiences. The nature of these changes, at the level of synapses, neurons, and their networks, also known as the engram, is unknown. We are dissecting the mechanisms of experience-dependent plasticity in the developing and adult neocortex.

Neocortical tissue is dauntingly complex: 1 mm^3 contains nearly a million neurons, each of which connects to thousands of other neurons. To probe neurons and synapses within the intact network, we build and use sensitive tools. Two-photon laser-scanning microscopy (2PLSM) allows us to image single synapses in intact tissues and to track changes in intracellular calcium and signal transduction events. Excitation of neuronal elements by focal uncaging of neurotransmitters allows us to probe the connectivity of neural networks with high efficiency. We combine these optical methods with electrophysiological measurements of synaptic currents and potentials and molecular manipulations of neurons.

We use both in vivo measurements to address system level questions and in vitro methods to get at detailed mechanisms. As a model system, we use the rodent barrel cortex, where whiskers are represented in a topographic manner, with information from each whisker represented by a small cortical region (barrel). Whisker maps are shaped by experience during development and reshaped in the adult. The cellular mechanisms underlying sensory map plasticity are likely to share mechanisms with those underlying learning and memory in other brain regions and other species.

[Ca^{2+}]-dependent Signaling in Single Dendritic Spines

C. Harvey

Postsynaptic Ca^{2+} activates diverse Ca^{2+}-dependent signal transduction mechanisms. We have designed high-sensitivity fluorescence resonance energy transfer (FRET) sensors for Ras, mitogen-activated protein kinase (MAPK), and protein kinase A (PKA) activation and cAMP levels. Fluorescence lifetime imaging microscopy (FLIM) allows us to image the Ca^{2+}-dependent signal transduction cascades activated by synaptic transmission in dendrites and even individual spines. We can thus study the biochemical dynamics of Ca^{2+}-dependent Ras signaling in neuronal microcompartments. For example, we find that Ras acts as a Ca^{2+}-dependent switch in neurons. Activation of single synapses leads to local activation of Ras, but activated Ras escapes from spines and invades the dendrite and neighboring spines. The spread of Ras activation likely underlies subtle coupling between the properties of neighboring synapses.

Experience-dependent Plasticity in the Adult Cortex In Vivo

V. DePaola, A. Holtmaat, L. Wilbrecht [in collaboration with G. Knott, Lausanne]

Sensory representations in the adult brain are stable, yet we are able to learn. To understand the underpinnings of stability and plasticity, we image structural dynamics in vivo. We find that the large-scale arborization of axons and dendrites is stable but that neurons display a rich repertoire of micrometer-level structural plasticity of dendritic spines, axonal terminals, and axonal branch tips. Experience-dependent changes in spines and boutons are cell-type specific. By combining in vivo imaging with retrospective serial section electron microscopy we have found that new spines grow to make synapes and that they preferentially grow toward existing boutons in the neuropil. We find that mutant mice that are defective in experience-dependent plasticity and learning also show profound defects in structural plasticity.

Circuit Mechanisms of Cortical Plasticity and Dysfunction

I. Bureau, A. Karpova

The wiring diagram is fundamental to understanding cortical function and plasticity. However, little quantitative information about functional circuits is available. What are the sources of input to a neuronal subtype in a particular layer and column and what are their relative strengths? Which connections change with novel sensory experience?

We developed laser-scanning photostimulation (LSPS) into a quantitative and rapid tool for circuit analysis in brain slices. Using LSPS, we performed an unbiased search for circuit phenotypes in animal models of fragile-X mental retardation. We found specific defects in the function and plasticity of excitatory L4→L2/3 synapses.

Molecular Methods to Reversibly Inactivate Synapses In Vivo

G. Tervo, A. Karpova

Inducible and reversible silencing of selected neurons in vivo is critical to understanding the structure and dynamics of brain circuits. We have developed molecules for inactivation of synaptic transmission (MISTs) that can be genetically targeted to allow the reversible inactivation of neurotransmitter release. MISTs consist of modified presynaptic proteins that interfere with the synaptic vesicle cycle when cross-linked by small-molecule "dimerizers." MISTs based on the vesicle proteins VAMP2/Synaptobrevin and Synaptophysin induced rapid (~10 min) and reversible block of synaptic transmission in cultured neurons and brain slices. In transgenic mice expressing MISTs in Purkinje neurons, administration of dimerizer reduced learning and performance of the rotarod behavior. MISTs allow for specific, inducible, and reversible lesions in neuronal circuits and may provide treatment of disorders associated with neuronal hyperactivity. We are currently investigating the mechanisms underlying MIST-dependent silencing. We are also constructing knock-in mice expressing MISTs in specific neuronal populations.

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NEUROGENETICS OF MEMORY IN *DROSOPHILA*

T. Tully J. Barditch, K. Bell, F. Bolduc, Y. Chen, H. Cox, Y. Hua, J. Kui, W. Lin, M. Regulski, N. Sinha, H. Wang, Y. Wang, S. Xia

Studies on Pavlovian learning in many animals have revealed remarkably similar behavioral properties of associative learning. Surprisingly, Pavlovian learning in fruit flies also displays these behavioral properties, suggesting a common underlying mechanism. The neural architecture of the *Drosophila* brain bears no resemblance to that of mammalian brains, of course, which suggests that the behavioral "homology of function" must result from conserved cellular/molecular mechanisms.

Consistent with this view, several genes and genetic pathways have been identified to function in both vertebrate and invertebrate associative learning, including *N*-methyl-D-aspartate (NMDA) receptors (NMDA-Rs), cAMP signaling, cAMP response element binding (CREB)-dependent gene transcription, and *staufen*- and fragile-X mental retardation protein (FMRP)-mediated local regulation of protein translation. This systematic rationale approach now is beginning to connect the biochemistry of memory formation with the molecular bases of heritable forms of mental retardation.

MUTANTS AND MICROARRAYS

We continue work derived from earlier experiments on regulated gene expression induced during memory formation. To date, we have confirmed 53 of 170 candidate memory genes (CMGs) using quantitative polymerase chain reaction (Q-PCR). Although perfectly adequate to identify a few genes for further in vivo analyses, this approach is too slow to provide a genome-wide confirmation of CMGs. Thus, this year, we designed and hybridized (with independent RNA extracts) a custom microarray from Nimblegene, with isothermal oligo probes corresponding to each of our CMGs from the original Affymetrix experiments. We currently are using "concordance" between the two platforms to (1) determine the optimal analytic method for these data and (2) provide simultaneous confirmation of all CMGs from our original experiments.

We also are beginning to "connect the dots" among the memory mutants we have identified either from a past behavioral screen at CSHL (supported by the Hartford Foundation) or from a more recent behavioral screen at Tsinghua University in Beijing (done in collaboration with Dr. Yi Zhong here at CSHL). A few years ago, X. Ge and coworkers at Tsinghua University showed that overexpression of *Notch* could enhance long-term memory (LTM) formation, providing some of the first evidence that this canonical developmental gene is involved in adult behavioral plasticity. In this past year, Q. Meng at Tsinghua identified the X-linked *chi* mutant from a behavioral screen for memory defects 1 day after spaced training. In collaboration with Meng, Y. Zhong, and others, we have shown that *chi* disrupts *PTP10D*, a protein tyrosine phosphatase, the mouse homolog of which is *PTPN5* (see Publications). Preliminary genetic experiments in flies have confirmed claims in the literature that *PTP10D* interacts with *gp150*, which itself interacts with *Notch*. In collaboration with M. Matsuno and M. Saitoe at Tokyo Metropolitan Institute for Neuroscience, we have more recently shown that the memory-enhancing effects of *Notch* overexpression can be blocked in the *ruslan* mutant, which was identified from our CSHL behavioral screen and which disrupts the cell adhesion molecule Klingon (Fig. 1).

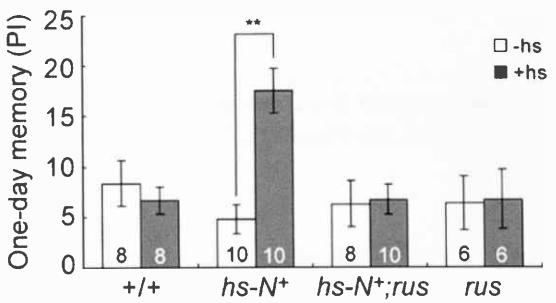

FIGURE 1 One-day memory after one training session is enhanced in transgenic fliesw (*hs-N*$^+$) after heat-shock-induced overexpression of *Notch* compared to wild-type flies (+/+) or *ruslan* (*rus*) mutants. When *Notch* is overexpressed in a *rus* mutant background (*hs-N*$^+$;*rus*), however, the enhancement of 1-day memory is blocked. This observatoin suggests that *klingon* function downstream from *Notch* during LTM formation (in collaboration with M. Matsuno and M. Saitoe, Tokyo Metropolitan Institute for Neuorscience).

CIRCUITS AND PHASES

We continue to perform spatiotemporal experiments, which reveal where in the adult brain LTM formation occurs. This year, we have shown that modulations of the NMDA-R subunit genes, *NR1* and *NR2*, restricted to adult mushroom bodies do not affect 1-day memory after spaced training. In regions outside the mushroom bodies, however, these same modulations can either disrupt or enhance LTM. Thus, we are beginning to believe that NMDA-R-dependent memory formation requires anatomical structures in the adult fly brain outside of, or in addition to, the mushroom bodies.

We have established a reliable new method to cell-sort green fluorescent protein (GFP)-expressing neurons from dissociated adult brains. This approach, in principle, will allow us to extract proteins and RNA from specific types of neurons involved with memory formation. The amount of material collected is too small for most subsequent biochemical assays, however. To address this issue, we are collaborating with Dr. Lee Henry here at CSHL to develop a novel solid-phase method of RNA amplification. Preliminary results to date suggest that this method can reliably amplify relatively small starting amounts of RNA.

GENETIC MODELS OF DISEASE

We have succeeded to demonstrate an acute (biochemical) requirement for FMR1 during LTM formation in flies. Pharmacological study of *fmr1* mutants also has begun to establish the molecular mechanism by which FMRP functions during behavioral (and presumably synaptic) plasticity.

We also have begun studies on a fly model of Parkinson's disease (transgenic overexpression of human α-synuclein). We want to challenge these flies with various stressors and then to look genome-wide for early changes in gene expression in response to these conditions. We are particularly interested in gene expression changes that may occur in both Parkinson's disease and memory formation—ultimately as a means to identify contributing factors to the cognitive decline detected in early-stage Parkinson's patients.

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NEURAL SUBSTRATE OF SELECTIVE AUDITORY ATTENTION IN THE CORTEX

A. Zador H. Asari, D. DeWeese, T. Hromadka, S. Jaramillo, S. Lima-Mainen, G. Otazu, H. Oviedo, L.-H. Tai, Y. Yang

We use a combination of theoretical and experimental approaches to study how the brain computes. The brain is able to solve hard computations that remain far beyond the reach of the fastest computers. Our goal is to understand this computation at the synaptic, cellular, behavioral, and algorithmic levels.

One example of such a hard computation is the "cocktail party problem." When we tune in to one voice at a cocktail party, and tune out the others—a task that remains beyond the capacity of modern computers—we are engaging in a form of selective auditory attention. Our ability to attend selectively is not limited to the auditory domain: Analogous tasks demand selective attention in the visual and even somatosensory domains. In monkeys, visual attention selectively enhances neural activity even in the earliest stages of visual cortical processing. This enhancement is surprising because the areas associated with these first stages of visual processing have traditionally been thought of as representing the sensory world faithfully, in a way that depends only on the properties of the sensory input itself. The discovery of attentional modulation overturns the notion that the peripheral sensory cortex is a passive "TV screen" available for viewing by a "homunculus" buried deep within the cortex.

The specific projects in the laboratory fall into two main categories. First, we are interested in how neurons represent auditory stimuli, and how these representations are computed from the cochlear inputs half a dozen synapses away. To address these questions, we are using electrophysiological and imaging approaches in anesthetized rats, as well as computational approaches, to characterize the properties of natural sounds. Second, we are interested in how these representations are modified dynamically—within seconds to hours or longer—in awake behaving rats by attention and other forms of learning.

Sparse Representations for the Cocktail Party Problem

H. Asari [in collaboration with B. Pearlmutter, Cold Spring Harbor Laboratory]

To extract the behaviorally relevant information imbedded in natural acoustic environments, animals must be able to separate the auditory streams that originate from distinct acoustic sources (the cocktail party problem). The auditory cortex has several orders of magnitude more neurons than does the cochlea, so that many different patterns of cortical activity may faithfully represent any given pattern of cochlear activity. We have been exploring the hypothesis that the cortex exploits this excess "representational bandwidth" by selecting the sparsest representation within an overcomplete set of features. We have constructed a model showing how sparseness can be used to separate sources perceived monaurally. The model makes testable predictions about the dynamic nature of representations in the auditory cortex. Our results support the idea that sparse representations may underlie efficient computations in the auditory cortex (Asari et al. 2006).

Context Dependence and Response Predictability in Rat Auditory Cortex

H. Asari

Sensory signal processing in the brain depends on stimulus history and contexts. Classical linear encoding models with rather shorter time scales (typically, hundreds of milliseconds), however, have failed to fully capture neural dynamics in the auditory cortex. Here, we used whole-cell recordings in vivo to assess the relevant time scales and how neural responses depend on the stimulus context. We found that the

changes in lower-order sound properties (e.g., intensity) had larger and longer effects than the changes in higher-order properties (e.g., amplitude modulation); and the context dependence sometimes lasted as long as 4 seconds in some neurons. We also showed that the predictive power for the best nonlinear model, but not for the linear models even with static nonlinearities, asymptotically approached the predictable response power on the time scale of seconds. These results suggest that complex modulations on longer time scales should be considered as well for fully understanding the auditory cortical activities and functions.

Sparse Synchronous Inputs Drive Neurons in the Auditory Cortex In Vivo

M. DeWeese, T. Hromadka

Cortical neurons receive most of their synaptic drive from other cortical neurons. This fact imposes a strong constraint on models of cortical activity: The dynamics of one neuron's spiking output must be consistent with the ensemble activity of the population of neurons that synapse onto it. The usual solution to this self-consistency constraint posits that both excitatory and inhibitory neurons fire asynchronously, varying their rates only slowly. According to this model, subthreshold fluctuations in membrane potential should be well-described by a random walk. However, based on our experiments using the in vivo whole-cell patch-clamp technique, we have developed an alternate model in which inputs are organized into synchronous volleys. According to this model, subthreshold fluctuations should be small most of the time, punctuated by large excursions corresponding to the arrival of volleys. Our results show that activity is organized into synchronous volleys superimposed on a relatively quiet background. This observation has implications for coding and computation. In particular, the correlations we have found among the synaptic population are precisely what is required for sparsely encoded signals to successfully propagate from one stage of cortical processing to the next (Deweese and Zador 2006).

Identifying Neurons with ChR2 during In Vivo Recording

S. Lima-Mainen, T. Hromadka

Neural circuits consist of a heterogeneous mixture of neurons with different neuroanatomical projections and patterns of molecular expression. Recordings of neural activity in behaving animals reveal tremendous functional heterogeneity as well: nearby neurons often respond very differently to the same stimulus or action. However, little is known about how this structural circuit-level heterogeneity contributes to function, in part because of the technical difficulty of identifying neurons during in vivo recordings in behaving animals.

To overcome this difficulty, we have developed a technique that allows us to "tag" subpopulations of neurons for identification during in vivo electrophysiological recordings. The tag is a light-gated ion channel—the algal protein channelrhodopsin-2 (ChR2)—whose expression can be genetically restricted to a subpopulation of neurons. In the subpopulation of neurons expressing ChR2, a brief flash of blue light triggers a single action potential with millisecond precision.

We are using this approach to test the hypothesis that neuroanatomical connectivity represents one important structural correlate of the functional diversity in the rodent cortex. To do this, we restrict ChR2 expression to subsets of neurons in the rat auditory cortex (ACx). ACx pyramidal neurons project to multiple brain regions, including the amygdala, the posterior parietal cortex, or the contralateral ACx, and presumably carry different information about auditory stimuli to these centers. To target ACx neurons specifically based on their projection pattern, we inserted the ChR2-coding region into a herpes simplex virus (HSV). HSV travels in a retrograde fashion through the axons of infected neurons. ChR2-tagged neurons, i.e., neurons projecting to the infected area, can be identified by their low latency and reliable spiking response to a brief light flash. Thus, for example, we have used this approach to identify the subpopulation of layer-5 ACx neurons that project to the contralateral cortex.

This approach is general, in that any population to which expression of ChR2 can be genetically restricted can be tagged. Promising future applications include tagging of different subpopulations of neurons based on promoters (e.g., for subclasses of inhibitory interneurons) and tagging of neurons in different cortical layers.

Separation of Sound Sources by Awake Behaving Animals

G. Otazu, L.-H. Tai

Sounds in the natural world rarely occur in isolation, but rather as part of a mixture. To survive, the auditory system must be able to attend selectively to one sound

source and ignore others, and it does so more effectively than any artificial system yet devised. To understand how this is performed, we are using multielectrode recording (tetrode) technology to monitor the activity of many neurons simultaneously in awake, behaving rodents performing an auditory discrimination task.

Mapping of Auditory Cortex Circuitry Using Laser-scanning Photostimulation

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

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

Using Cortical Timing Information to Guide Behavior

Y. Yang, G. Otazu, M. DeWeese

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

We have therefore adopted a direct approach to probe the precision with which cortical timing information can be used to guide behavior in the rat. To bypass subcortical auditory pathways, we stimulate the primary auditory cortex directly, using transient biphasic current trains delivered via chronically implanted intracortical microelectrodes. The behavioral paradigm we use is a two-alternative choice task in which stimulus 1 consists of the simultaneous stimulation of two intracortical sites (A and B), and stimulus 2 consists of sequential stimulation of the two sites separated by a brief interval dt (A – dT – B). After the subjects are trained to perform to criterion (1–2 weeks) with the initial long interstimulus interval (dT > 50 msec) for stimulus 2, we probe the subjects' psychophysical threshold by reducing dT, until the subjects can no longer distinguish between the two stimuli. Our initial experiments indicate that the cortex can make use of information on a time scale as short as 10 msec and possibly much less.

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NEURAL BASIS OF LEARNING AND MEMORY IN *DROSOPHILA*

Y. Zhong H.-C. Chiang, I. Hakker, I.S. Ho, K. Lijima, A. Mamiya, M. Pagani, Y. Wang

We are investigating the neural basis of learning and memory by using *Drosophila* to combine functional analyses with genetic manipulation. Currently, we are pursuing two major projects. First, we are establishing *Drosophila* models for studying genes involved in human neurodisorders that impair learning and memory. In particular, we are interested in genes known to contribute to neurofibromatosis 1 (*Nf1*), Alzheimer's disease, and Noonan syndrome. For *Nf1*, we have shown that different fragments of *NF1* are involved in the regulation of different signal transduction pathways and have distinct roles in mediating formation of short-term memory (STM) versus long-term memory (LTM). For Alzheimer's disease, we have shown that although aggregation proneness is critical for the toxicity of Aβ42, increasing or decreasing the aggregation propensity of Aβ42 does not simply change the level of toxicity, but it can also result in qualitative shifts in the pathologies they induce in vivo. For Noonan syndrome, our preliminary data suggest that gain-of-function mutations associated with the disease may cause learning and memory defects. In the second major project, we are investigating learning-associated changes in the activity of neurons. For this project, we have found that a specific group of neurons in the mushroom body, a brain structure critical for insect learning and memory, responds greater to an odor after that odor has been associated with aversive stimulus. The specific projects are described below.

Two Functional Domains of NF1 Independently Control Learning and Memory Formation

I.S. Ho, I. Hakkar, M. Pagani

Neurofibromatosis type 1 (NFI) is an autosomal dominant genetic disorder characterized by benign tumors of the peripheral nervous system called neurofibromas, café au lait spots, and extreme freckling. In addition, at least 40% of afflicted children have learning difficulties. The NF1 protein contains a highly conserved GTPase-activating protein (GAP) domain that inhibits Ras activity, and the carboxy-terminal region regulates G-protein-dependent activation of adenylyl cyclase (AC). Behavioral analysis has indicated that learning and memory are also disrupted in *Drosophila* and mouse NF1 models; however, the learning defect in flies is attributed to altered activation of the cAMP pathway, whereas the mouse learning deficit results from increased Ras activity. Because of the enormous difference in the timescale involved in training paradigms for mice (water maze) and flies (odor-foot shock association), we suspected that different components of memory were being affected. We found for the first time that not only STM, but also LTM was defective in Drosophila *Nf1* mutants. Analysis of clinically relevant human NF1 mutations and deletions expressed in Drosophila *Nf1* mutants allowed us to dissect the molecular basis for the role of NF1 in mediating both STM and LTM. We found that the GAP-related domain with its GAP activity and binding with Ras was necessary and sufficient for LTM, whereas the carboxy-terminal domain of NF1 that is required for G-protein-dependent activation of AC was critical for STM. Thus, this study shows that two functional domains of the same protein participate independently in formation of two memory components.

Mutant Aβ42 with Different Aggregation Propensities Induce Distinct Pathological Phenotypes in *Drosophila*

K. Iijima, H.-C. Chiang, I. Hakker, Y. Zhong

The amyloid-β-42 (Aβ42) protein has been suggested to have a central role in the pathogenesis of Alzheimer's disease (AD). The neurotoxicities of Aβ42 have been well established in various experimental models and often correlated with its aggregation-prone nature, leading to the hypothesis that reducing aggregation proneness of Aβ42 decreases its toxicity. To revisit this hypothesis in vivo, we examined the effects of expression of mutant Aβ42 with

altered aggregation propensities in the *Drosophila* brain. We found that some aspects of Aβ42 toxicity do not positively correlate with aggregation proneness. Rather, Aβ42 with different aggregation properties can induce distinct pathological phenotypes. Aβ42 with the Arctic mutation (Aβ42Arc), which causes early-onset familial AD, is more aggregation-prone, whereas Aβ42 with an artificial mutation (Aβ42art) is known to suppress aggregation. Consistent with in vitro observations, Aβ42Arc expressed in the *Drosophila* brain formed more oligomers and Aβ deposits than wild-type Aβ42, whereas Aβ42art flies showed less formation of both oligomers and deposits. Age-dependent locomotor dysfunction and premature death correlated well with aggregation proneness, with Aβ42Arc showing the strongest phenotypes and Aβ42art the weakest. However, Aβ42art induced a more severe memory defect than did Aβ42. Strikingly, these flies developed different pathologies. Aβ42Arc caused extensive neuron loss, whereas Aβ42art flies showed the strongest neuropil degeneration. This may be partly explained by the different distribution patterns of these Aβ peptides. Aβ42Arc accumulates primarily in the cell body as large deposits, and Aβ42art preferentially accumulates in neurites, and Aβ42 showed both properties. Thus, although aggregation proneness is critical for the toxicity of Aβ42, our results demonstrate that increasing or decreasing the aggregation propensity of Aβ42 does not simply change the level of toxicity but can also result in qualitative shifts in the pathologies they induce in vivo.

Experience-dependent Behavioral Alteration in a *Drosophila* Model of Noonan Syndrome

M. Pagani

Noonan syndrome is a pleiomorphic dominant genetic disorder showing developmental defects, high incidence of leukemia, mental retardation, and behavioral disorders. Recently, it has been identified that missense mutations in the *PTPN11* gene, which encodes the protein tyrosine phosphatase nonreceptor 11, SHP-2, are associated with Noonan syndrome, as well as with several types of leukemia. It is thought that gain-of-function mutations are associated with both Noonan syndrome and leukemia. The SHP-2 protein is highly conserved among human, vertebrates, and invertebrates and has an essential role in growth factor and integrin signaling. However, the genetic and molecular mechanisms that mediate behavioral alterations in Noonan syndrome have not been explored.

To examine the role of SHP-2 in behavior and the mechanisms underlying its effect on behavior, we started studying transgenic *Drosophila* flies carrying mutations associated with Noonan syndrome, leukemia, or both disorders in the *Drosophila* orthologous gene, *csw*.

We have found that gain-of-function mutations associated with Noonan syndrome, leukemia, or both disorders may cause learning and memory defects. Preliminary studies showed that learning defects are not dependent on developmental alterations of the nervous system. In addition, although normal learning, STM and LTM were dependent on *csw* function, the mechanisms involved for each stage seem to be different. For example, learning was affected by an increase in the dosage of the *csw* gene but not by missense mutations, whereas LTM seems to be sensitive to both mutation and the dosage of csw.

Further genetics, behavioral, and physiological studies will help us to identify the molecular mechanisms involved in experience-dependent behavioral alteration mediated by SHP-2 in Noonan syndrome as well as in normal individuals.

Imaging of Memory Traces in the *Drosophila* Mushroom Body

Y. Wang, A. Mamiya

The *Drosophila* mushroom body (MB) is a higher-order brain center that is crucial for olfactory associative learning and memory formation. Depending on their birth orders, the MB neurons can be divided into three subtypes, the γ, α′/β′, and α/β neurons, which give rise to separate axonal branches. Axons of the γ neurons form the horizontal γ lobe. Axons of α′/β′ neurons bifurcate to form vertical α′ lobes and horizontal β′ lobes, whereas axons of α′/β neurons bifurcate to form vertical α lobes and horizontal β lobes. As an initial step in understanding the neural mechanisms underlying learning and memory formation, we are investigating how an odor-evoked response of different types of MB neurons changes after the odor has been associated with electric shock. To do this, we express GCaMP, a genetically encoded Ca^{2+} sensor, in MB neurons and monitor the neuronal-activity-induced increase in the Ca^{2+} at different regions of MB using a two-photon microscope.

We took three different in vivo and in vitro approaches. In the first approach, we used an isolated fly brain and stimulated the antennal nerve (AN) to mimic olfactory input and the ventral nerve cord (VNC) to mimic electric shock. Pairing of AN and VNC stimulation was used to mimic odor-aversion conditioning, and the response to AN stimulation before and after pairing was compared. In the second approach, flies were trained in a traditional T-maze where they were presented with an odor with electric shock, followed by a different odor without electric shock. In this approach, responses to shocked odor and nonshocked odor were compared. In the third approach, flies were fixed and conditioned under the microscope by presenting an odor together with electric shock to its body. The response to an odor before and after odor–electric shock pairing was compared.

In all three approaches, we observed enhanced odor-evoked Ca^{2+} influx after conditioning in the axonal branches of the α'/β' MB neurons, but not in the other types of MB neurons nor the dendritic region. The enhancement lasted for more than an hour and was abolished by disrupting the G protein signaling that impairs olfactory learning. Furthermore, immediate memory was impaired by disrupting G protein signaling in the α'/β' neurons or blocking their synaptic output. Therefore, the observed enhanced Ca^{2+} influx after learning in the α'/β' neurons may represent a memory trace in the MB.

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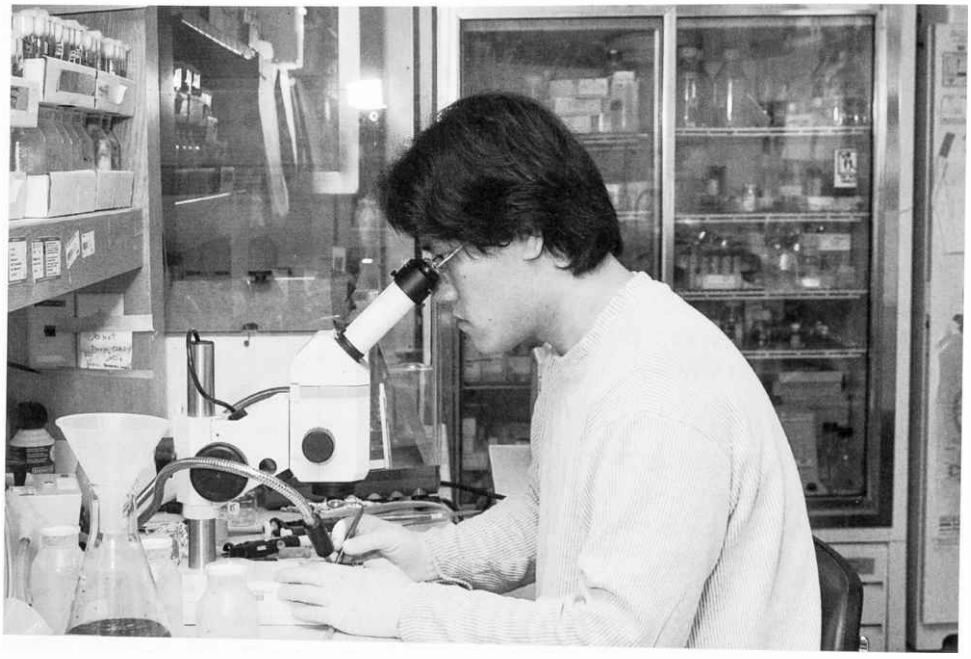

Hsueh-Cheng Chiang

PLANT GENETICS

From orchids to dandelions, the dazzling diversity of plants' shapes, colors, and forms are an endless source of wonder. But all of these traits, including those of tremendous agricultural significance, have been shaped by evolution or by human selection. Uncovering the molecular processes that govern how plants grow and develop is thus of fundamental biological interest and importance.

David Jackson's lab aims to identify the genes and signals that regulate plant signaling and morphogenesis. In the last year, they have isolated a gene that controls how plant cells communicate through small channels, called plasmodesmata. This gene is expressed in the growing regions of the plant and may control the allocation of nutrients and developmental signals during plant growth. Jackson's lab also continues to use positional cloning to identify maize genes with defined roles in development. In the past year, candidate genes that control branching, stem cell proliferation, and inflorescence organ identity have been identified. One of these, *RAMOSA3*, appears to function through modulation of a hypothetical sugar signal, highlighting a novel mechanism by which plants regulate their growth. Finally, Jackson and colleagues have also begun to compile a collection of fluorescent-protein-tagged maize lines that will be used for studies in cell and developmental biology. This is the first collection of its kind, and it promises to enhance maize genetics research through characterization of developmental gene pathways and identification of maize promoters.

The specialized shapes of leaves have a vital role throughout plant biology. Moreover, within each leaf, the functions of the upper and lower surfaces are very different. Marja Timmermans's work is focused in part on understanding how unspecialized stem cells create this dorsoventral (upper/lower) axis of leaves. Her group has identified two genes—*Leafbladeless1* (*Lbl1*) and *Rolled leaf1* (*Rld1*)—that are essential for controlling this process. Timmermans discovered that these genes are in turn controlled by the opposing action of two particular microRNAs. One significant aspect of these findings is that like classical protein "morphogen gradients," microRNAs can act as diffusible developmental signals that move through tissues and thereby control stem cell specialization. Timmerman's findings suggest that small RNAs may be mobile, and this offers the intriguing possibility that they can function as signals in development. Moreover, these results reveal a novel patterning mechanism, whether in plants or animals, in which opposing fates along a developmental axis are established by two distinct small RNAs.

Unlike most developing animal embryos, plant embryos begin to differentiate from the very first cell division. An unequal division of the fertilized egg cell creates daughters with radically different fates—embryonic and extraembryonic. Wolfgang Lukowitz studies the molecular steps that regulate this fate decision and its impact on the subsequent tissue specializations. His group has identified a number of proteins that are likely forming a signaling pathway controlling this earliest step in plant embryonic development. One of these proteins appears to act through the sperm cell and to link the onset of signaling to fertilization.

Gene expression can depend on many factors, not only on a gene's DNA sequence, but on its position. A major aspect of work in Robert Martienssen's lab involves shedding light on the mystery of gene expression by studying the process of "position effect variegation." First described in the 1930s, position effect variegation refers to plant color diversity caused by the inactivation of a gene in some cells due to its position near a condensed clump of chromosomal material called heterochromatin. Martienssen and colleagues are investigating the role of RNA interference in heterochromatin, epigenetics and plant development. This work holds the promise of yielding fundamental insights into plant biology and has implications for an understanding of human disease as well.

PLANT SIGNALING AND DEVELOPMENT

D. Jackson J. Anker, Y. Benitez, P. Bommert, D. Bouyer, O. Dmytrenko, A. Gallavotti, K. Lau, B.H. Lee, J. Linder, A. Maizel, A. Mohanty, N. Satoh-Nagasawa, J. Wang, C. Whipple, Y. Yan, P. Yin

Research in our lab focuses on the molecular mechanisms of cell-to-cell communication and development in plants. We use genetics to identify developmental regulators, genomic tools to isolate the corresponding genes, and various biochemical and cell biology techniques to investigate the underlying mechanisms. In the last year, we have made very significant advances in several areas, notably in the ability to isolate maize mutant genes by positional cloning and in the identification of a gene that regulates cell-to-cell transport, as discussed below.

Maize is the premier model system for the cereal crops and probably the best genetic plant model. In the past, isolation of genes identified by mutations has been slow; however, with the emerging rice and maize genome sequences, we have been among the first to report the use of positional cloning in maize. This last year we reported the isolation of *RAMOSA3*, a gene involved in inflorescence branching. Remarkably, we found that *RA3* encodes a trehalose phosphate phosphatase, giving the first inkling that certain sugars may act as very specific developmental signals (Satoh-Nagasawa et al. 2006). We have also isolated genes regulating vegetative branching, inflorescence organ identity, and stem cell proliferation and are in the process of characterizing their gene products.

Several years ago, we identified a novel mechanism of cell-cell communication in plants that involves the specific transport of regulatory proteins between cells. In the last year, we have begun to identify the plant genes that regulate this process. We have also started to compile a collection of fluorescent-protein-tagged maize lines that will be used for studies in cell and developmental biology. This is the first collection of its kind, and it promises to enhance maize genetics research through characterization of developmental gene pathways and identification of maize promoters.

Characterization of the Function of the *RAMOSA3* Gene in Maize

N. Satoh-Nagasawa, A. Mohanty, A. Gallavotti, Y. Yang, J. Linder [in collaboration with H. Sakai and N. Nagasawa, Dupont; S. Malcomber, California State University, Long Beach; S. Swaminathan, Brookhaven National Laboratories, New York]

To elucidate the genetic and molecular mechanisms of inflorescence branching, a major yield trait in crops, we have been working with the *ramosa3* (*ra3*) mutant of maize. By observation of the mutant phenotype and double-mutant analyses, we found that *RA3* functions upstream of another *RA* gene (*RA1*) to establish the correct identity and determinacy of axillary meristems in the maize inflorescence. The *RA3* gene was cloned, and we found that it encodes a trehalose-6-phosphate phosphatase (TPP). TPPs are considered to be metabolic enzymes that catalyze the conversion of trehalose-6-phosphate (T6P) to trehalose, a disaccharide. Interestingly, *RA3* is expressed outside of the cells whose fate it regulates, at the base of axillary meristems, and this novel expression pattern is conserved throughout the grasses.

From these studies, we developed two hypotheses for the molecular mechanism of *RA3* function. First, T6P or trehalose may act as a mobile signal during axillary meristem development. Second, the RA3 protein itself may directly control the expression of other genes, like *RA1*. To test these hypotheses, we investigated RA3 protein localization, and preliminary results show that a RA3 yellow fluorescent protein (YFP) fusion is localized in the cytosol and nucleus. This leads us to speculate that RA3 protein could act as a regulator of gene expression in the nucleus, a role distinct from its proposed metabolic activity. To determine whether this is the case, we made catalytically inactive RA3 mutants, after three-dimensional struc-

ture modeling of the TPP domain. The mutant RA3 proteins expressed in bacteria did not have enzymatic activity. We are currently testing whether this mutant protein can complement the *ra3* phenotype. We are also attempting to measure the concentration of trehalose and T6P in young inflorescences of wild type and ra3 mutants and have started to purify RA3 antisera. To further investigate the mechanism of RA3 action, we used microarray experiments to search for RA3-regulated genes, which may also provide direction for further genetic analysis.

While we are examining *RA3* function in maize, we are also interested in the function of *RA3*-like genes in other plants and genetic interaction of *RA3* with other genes in maize. We analyzed the expression of *RA3* and its closest homolog, *sister of RA3* (*SRA*) and are characterizing mutants in other grasses and in *Arabidopsis* in order to define the function of those genes. With these experiments, we aim to more fully understand the function of *RA3* and fit it into an emerging network of regulators of inflorescence development in maize.

Regulation of Phyllotaxy in Maize

B.H. Lee, Y. Yan [in collaboration with J. Traas, Lyon, France; H. Sakakibara, RIKEN, Yokohama, Japan; C.-X. Xie, Chinese Academy of Agricultural Sciences, Beijing, China]

One of the most fascinating aspects of plant morphology is the regular geometrical arrangement of leaves and flowers, called phyllotaxy. The shoot apical meristem (SAM) determines these phyllotactic patterns, which vary depending on species and developmental stage. For example, maize develops leaves in alternating patterns, and *Arabidopsis* rosette leaves display a spiral arrangement. Unlike normal maize, *abph1* (*aberrant phyllotaxy1*) mutants show decussate leaf phyllotaxy, where two leaves develop at the same node, paring at 180°. *ABPH1* encodes a cytokinin-inducible type-A response regulator and is believed to function in cytokinin hormone signaling. Since auxin and polar auxin transport have also been implicated in phyllotaxy, we focused our studies on the interactions of cytokinin and auxin signaling in phyllotaxy.

We observed reduced *ABPH1* expression in polar auxin transport-inhibited SAMs, suggesting that the *ABPH1* expression is dependent on auxin. Using antibodies for the *Arabidopsis* PINFORMED1 (PIN1) protein, which functions as a polar auxin efflux carrier, we found that the maize SAM has a PIN1 protein expression domain similar to that in *Arabidopsis*. Quantitative reverse transcriptase–polymerase chain reaction (RT-PCR) for a maize PIN1 homolog showed reduced expression level in the *abph1* SAM. These results are consistent with our observations of delayed expression of the maize PIN1 protein at the incipient leaf initiation sites in *abph1*, as found by three-dimensional reconstructions of sections probed with a PIN1 antisera. Consistent with these observations, auxin levels were lower in *abph1* mutants than in the wild type.

Together, these results suggest a positive feedback loop involving ABPH1, auxin, PIN1, and polar auxin transport. In the past, we proposed that ABPH1 acts as a negative regulator in cytokinin signaling. Consequently, we now propose that ABPH1 operates at a junction point that modulates a negative feedback in cytokinin signaling and a positive feedback in auxin signaling in the maize SAM. To further characterize this proposed network, we used microarrays to compare gene expression in normal and *abph1* embryos. We selected 22 putative *ABPH1*-regulated genes and have independently confirmed 11 of these as having altered expression in *abph1*. Interestingly, several of these encode predicted hormonal or developmental regulators, and their functions will be examined using a reverse genetics approach.

We also generated transgenic maize containing a YFP-tagged ABPH1 construct (ABPH1-YFP) under the control of the endogenous *ABPH1* promoter, and it showed the native expression pattern. We also found that the transgene complements the *abph1* mutant phenotype, suggesting the YFP fusion is functional. Thus, these lines will be very useful for in vivo studies of *ABPH1*.

A second maize abnormal phyllotaxy mutant has been identified. This one is dominant and we have called it *Abph2*. We mapped this gene at the top of chromosome 7 in a region that shows synteny to the top of rice chromosome 7. There are about 200 rice genes in the syntenic region defined by two maize markers that flank *ABPH2*. We are in the progress of refining this mapping information, with a goal of identifying the *ABPH2* gene.

Role of the Phytohormone Auxin in Maize Branching

A. Gallavotti, Y. Yan [in collaboration with M.J. Zanis, Purdue University; R.J. Schmidt, University of California, San Diego]

One of the most striking features of plants is their ability to constantly adapt their development in response

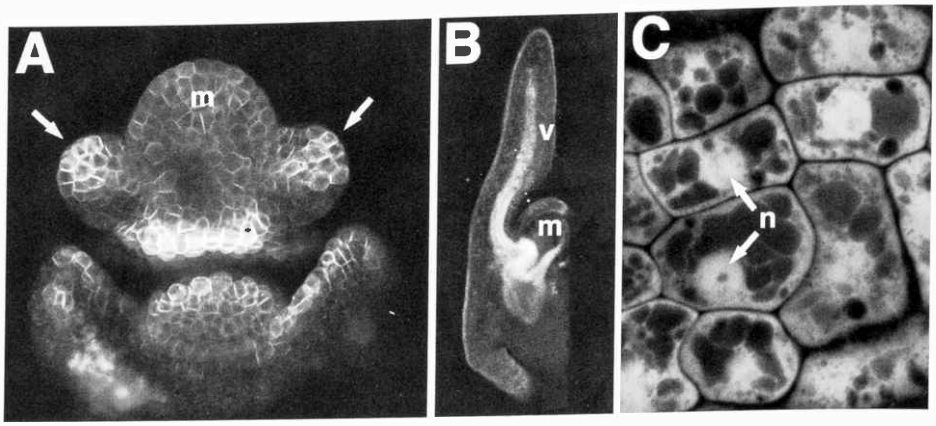

FIGURE 1 Fluorescent protein markers for hormonal regulators in maize. (*A*) Maize developing flower meristems (*m*) showing spikes of ZmPIN1a-YFP expression at sites of organ initiation (*arrow*), indicative of auxin maxima. (*B*) ZmPIN1a-YFP expression in the maize embryo. YFP expression is detected in the shoot apical meristem (m), epidermal layer of the scutellum, and vascular bundles (v). (*C*) HP1-RFP expression in callus. This protein is localized to the nuclei (n) and also present throughout the cytoplasm. All images captured using the confocal microscope.

to environmental changes. This plasticity is made possible thanks to few groups of cells, called meristems, that are responsible for postembryonic development of organs such as leaves, branches, and flowers. In the past few years, it has become clear that the formation of new meristem and organ primordia is triggered by a universal mechanism involving the creation of dynamic gradients of the plant hormone auxin. These gradients are the result of the subcellular localization and activities of auxin efflux and influx carriers. We are studying the role of auxin transport throughout maize development. Maize has unique features that make it an attractive system to study the formation of new meristems and new organs. Most noticeably, maize inflorescences are formed by a progression of different meristem types, each characterized by distinct identity and fate.

The maize *PINFORMED1* auxin transporter gene, *ZmPIN1a*, is expressed throughout vegetative and reproductive development and acts as an auxin efflux carrier in a heterologous system. To monitor the dynamics of auxin transport during the formation of branches and organs, we made transgenic lines expressing a fusion of *ZmPIN1a* with YFP. We are also generating maize transgenic lines carrying a synthetic auxin responsive promoter, "DR5rev," fused to red fluorescent protein (RFP), to monitor auxin maxima during development. With the goal of describing a unified model for branching in maize, from the formation of vegetative axillary meristems to the formation of floral organs, we are characterizing the localization of ZmPIN1a (Fig. 1) and the direction of auxin flow using these marker lines. Taking advantage of the existing large collections of maize mutants, we are also crossing several branching mutants with our fluorescent protein reporters to understand how they interact with the auxin pathway. For several of these mutants, the underlying genes have been recently isolated. This analysis will help to gain a comprehensive view of the molecular mechanisms behind meristem formation and activity in maize.

Using Trichomes as a Tool to Study Intercellular Protein Transport

D. Bouyer, J. Wang

Cell-to-cell communication is a prerequisite to ensure the coordinated development of multicellular organisms. The development of leaf hairs, so-called trichomes, in *Arabidopsis* involves cell-to-cell communication in order to establish a regular pattern on the leaf epidermis. The *TRANSPARENT TESTA GLABRA 1* (*TTG1*) gene is necessary for seed pigmentation and trichome initiation and acts in a non-cell-autonomous matter. Moreover, this protein is able to move between cells. Interestingly, this protein mobility is a characteristic that is absent in some *TTG1* homologs. In contrast to *TTG1*, the petunia homolog *AN11* is not able to rescue the *Arabidopsis ttg1* mutant when expressed from the subepidermis, a tissue that does not make trichomes, but can complement the mutant when expressed using the ubiquitous CaMV35S promoter. This attribute of mobility can be localized to a small

domain within the TTG1 protein. In summary, whereas the role in anthocyanin pigment regulation is highly conserved between AN11 and TTG1, the *Arabidopsis* homolog is additionally involved in several patterning processes, which require intercellular communication.

In plants, protein transport between cells is enabled by small channels, plasmodesmata, that connect cells and allow the exchange of signals. Although these structures have been known for quite some time, their composition and regulation are poorly understood. To gain more insight into the function of plasmodesmata, we are performing a genetic screen based on the ability of TTG1 to be transported from the subepidermis to the epidermis. In this screen, mutants that are defective in protein transport should lack trichomes. In a second screen, the inability of AN11 to move from the subepidermis to the epidermal layer will be used as a tool to screen for mutants that have "dilated" plasmodesmata, leading to the restoration of trichomes. Taken together, the analysis of TTG1 mobility may serve as an excellent way to investigate the mechanism and evolution of mobile protein signals in plants.

We also performed a screen using the KNOTTED1 homeodomain protein, this was the first plant transcription factor found to move cell-to-cell. A fusion of the homeodomain of KN1 with the GLABROUS1 (GL1) protein was able to complement trichome formation when expressed in the non-trichome producing subepidermal cells of a *gl1* mutant line. We showed that this rescue was dependent on the ability of the KN1-GL1 fusion protein to move into the epidermal cells. Four mutants have been identified in this screen, and we have mapped each to a small region in preparation for positional cloning of the corresponding genes. Analysis of these genes should be informative as to the mechanism of intercellular transport of KN1 and other mobile plant signals.

Molecular and Genetic Characterization of Plant Intercellular Channels

Y. Benitez, O. Dmytrenko

We are interested in mechanisms regulating transport through plant intercellular channels, plasmodesmata. We previously reported the isolation of mutants by screening of approximately 1000 M2 EMS (ethylmethane sulfonate) lines in a pSUC2-GFP (green fluorescent protein) background. These mutants were affected in GFP unloading from the phloem vasculature and in cell-to-cell transport to the meristematic cells of the root tip. We focused our efforts on two mutants, called "*gfp aberrant trafficking*" or *gat* for short. These mutants are recessive and show a seedling-lethal phenotype.

We have performed experiments to further characterize these mutants and identify the genes responsible for the mutant phenotype. We analyzed the targeted trafficking of developmental proteins, such as SHORTROOT (SHR) and SHOOTMERISTEMLESS (STM), in the mutant meristems. SHR::GFP, expressed under the SHR promoter, normally traffics from the stele to the endodermis, and we found that the transport is unaffected in gat mutants, suggesting they may affect general PD permeability rather than transport of specific targeted proteins.

We have isolated *gat1* by positional cloning. By allelism tests with insertional mutants in genes in the candidate region, we found that the CSHL gene-trap transposon line GT12462 failed to complement *gat1*, suggesting that *GAT1* is encoded by the gene tagged by the gene trap. Furthermore, we found that a construct expressing this gene can complement the *gat1* mutant phenotype; therefore, *GAT1* has been cloned. Interestingly, we were not able to find any mutation in the coding or promoter sequence in *gat1* homozygous mutants, suggesting that this might be an epiallele. Preliminary experiments to check the methylation status of this gene in *gat1* mutants indicate that the promoter is methylated, consistent with the idea that it is indeed an epiallele. Polymerase chain reaction (PCR) analysis of genomic DNA digested with methylation-sensitive enzymes and analysis of double mutants with genes in DNA methylation pathways are being performed. We hope to show that a reduction of methylation in the *gat1* EMS allele is able to rescue the *gat1* phenotype.

The GT12462 gene-trap line carries a β-glucuronidase (GUS) reporter gene, and by using GUS staining, we have detected expression in shoot and root meristems. We also found strong expression in the leaf and root vasculature and leaf primordia. In embryos, staining appeared at the transition from heart to torpedo stage and was also localized in root and shoot apical meristems. This staining is greatly reduced in homozygous mutants, suggesting that the transposon insertion in the intron of this gene affects its expression. To further explore the expression and subcellular localization of *GAT1*, we made a YFP-tagged construct of the gene under the control of its own promoter. Expression of

this construct together with the results of in situ localization experiments will be critical in understanding the role of this gene in plant development and cell-to-cell transport. At the same time, we are planning experiments to study the effect of misexpression of the gene. We have made several constructs carrying the wild-type gene under the control of tissue-specific promoters for vascular, meristematic, and mature tissues. These experiments will help dissect the potential developmental and physiological functions of *GAT1* and will elucidate its importance in meristem development and in intercellular trafficking.

Hormonal Signaling in Maize

A. Mohanty, Y. Yang [in collaboration with A. Chan, The Institute of Genomic Research; A. Sylvester, University of Wyoming]

Plant hormones have critical roles in growth and development. Among them, cytokinins are especially interesting as they regulate a variety of agronomic processes including crop yield and nodulation. Cytokinins are perceived by histidine kinase receptors (HKs), which upon binding of cytokinins initiate a phosphorelay signal transduction pathway. The phosphorylation is transferred to B-type response regulators (RRs) via histidine phosphotransfer (HP) proteins, which translocate from the cytoplasm to nucleus. In turn, the B-type response regulators act as transcription factors to activate A-type response regulators.

We have taken a localization-based approach to decipher cytokinin signaling pathways in maize. We have generated fusions of fluorescent proteins to the HPs, HKs, and RRs, as well as to cytokinin oxidase (CKO) genes that degrade cytokinins, and have obtained transgenic maize plants harboring these constructs. Some of the questions that we are interested in addressing include Is the subcellular localization of these proteins regulated in tissue-specific manner? Does the nucleocytoplasmic shuttling of HPs observed in transient assays in *Arabidopsis* hold true for these maize genes? Preliminary analysis of transgenic maize calli expressing HP1-RFP shows localization in the cytosol as well as nucleus (see Fig. 1). Another area of interest is the interplay between cell cycle control and cytokinin signaling in the meristem. Cyclin D (CYCD) has been proposed to be a sensor for cytokinins, and we have made a maize CYCD-YFP fusion construct. We are currently generating transgenic plants harboring this gene. It will be interesting to determine the expression pattern and regulation of this gene in the meristem, and analysis of these lines should greatly aid our understanding of cytokinin biology.

FIGURE 2 Pink maize kernels expressing a fusion of a seed storage protein with the red fluorescent protein.

Since the tools for subcellular localization in maize are very limited, we have also generated a series of marker lines for nuclei, microtubules, tonoplast membrane, plastids, peroxisomes, protein storage bodies, and other structures. We are evaluating expression patterns of these markers in multiple organs and cell types so as to develop an atlas of expression patterns in maize. In addition, our use of native promoters will allow us to generate useful promoters for tissue- and developmental-stage-specific regulated expression. The expression of fluorescent proteins in maize appears to be robust and stable. In one particular example, to develop an endosperm and protein-storage-body-specific marker, we used RFP to tag an endosperm-specific gene, *FLOURY2* (*FL2*), encoding a zein class storage protein. Confocal image analysis of transgenic maize showed the endosperm-specific expression of *FL2-RFP* driven by its native promoter and localization in protein bodies, consistent with previous studies on this gene. Furthermore, the *FL2-RFP* kernels have a distinct pink color to the naked eye, due to accumulation of high levels of the FL2-RFP fusion protein (Fig. 2). This feature could be exploited for use as a reporter for selection of transgenic seeds.

Regulation of Inflorescence Meristem Size in Maize

P. Bommert, K. Lau, J. Anker, J. Wang [in collaboration with W. Bruce, Pioneer Hi-Bred Intl.]

The development of the maize tassel and ear is dependent on the activity of the inflorescence meristems. Our interest is in the analysis of mechanisms that control the size of the inflorescence meristem using genetic and biochemical approaches. Our previous analysis of the *fasciated ear2* (*fea2*) and *thick tassel dwarf1* (*td1*) mutants in maize suggested that the *CLAVATA* (*CLV*) signaling pathway, which regulates meristem size in *Arabidopsis*, is conserved in maize, since *FEA2* encodes an LRR receptor protein orthologous to *CLV2*, and *TD1*, an LRR receptor-like kinase, orthologous to *CLV1*.

Currently, we are continuing to isolate the FEA2-containing receptor complex by using GFP and cTAPi (a modified, plant-specific version of the yeast TAP-tag) as epitope tags. Initial gel-filtration experiments found that the FEA2 protein is a member of a 450-kD complex, and subsequent analysis of both epitope-tagged FEA2 proteins revealed that they are also incorporated in high-molecular-weight complexes of a similar size. We were also able to show that both tagged complexes are located at the plasma membrane, indicating that adding of the epitope tags does not interfere with the correct intracellular localization. Our eventual aim is to perform a mass spectrometric analysis of the purified complexes. Identified proteins and their encoding genes will then be analyzed by expression profiles, mutant phenotypes, and genetic interactions with *fea2* and *td1*.

We are also following a genetic approach to isolate other fasciated mutants, such as *fasciated ear3* (*fea3*) and *compact plant2* (*ct2*). Both mutants develop abnormally enlarged inflorescence meristems, indicating that they also regulate meristem size. Using a map-based cloning strategy, we have identified the *ct2* gene. A combination of molecular markers used on a population of 450 *ct2* mutants was sufficient to narrow down the chromosomal location of *ct2* to an interval of 0.5 cM on chromosome 1. As this region is highly syntenic to rice, we were able to follow a candidate gene approach, which allowed us to identify *ct2* as a predicted kinase. Preliminary in situ hybridization experiments show that ct2 is expressed within the inflorescence meristem, which, together with the phenotypic analysis, suggests that *CT2* might be a newly identified factor in the *CLAVATA* signaling pathway. Map-based cloning of the *fea3* gene is also in progress.

Tassel sheath Mutants and the Regulation of Bract Suppression in Grasses

C. Whipple

Shoot development can be interpreted as a reiteration of three basic units: the leaf, stem, and an axillary meristem that will itself grow out and continue this pattern. Together, these units constitute a "phytomer," that is repeated throughout plant growth and development. It is by modification of this phytomer that changes in morphology occur. Distinct phytomer morphologies characterize transitions in the plant life phase (e.g., vegetative to reproductive), as well as interspecies differences. Although there is a lot known about the development of individual phytomer components, little attention has been given to how phytomer growth is modified throughout either the life cycle of a single plant or through evolution. One interesting phytomer modification that occurs in many plant species is the suppression of leaf development after the transition to reproductive development. For example, in most grass species, inflorescence leaves (called bracts) fail to grow, although a rudimentary bract primordium does form. Interestingly, in a few grass taxa, as well as in close relatives of the grass family, bracts are not suppressed and grow out normally. It thus appears that a mechanism for bract suppression evolved in the common ancestor of all grasses and was subsequently lost in a few species. Understanding the mechanism by which bract suppression occurs will shed light on an interesting modification important both in the development of a single species and in evolution.

To understand the regulation of this process in grasses, we have begun an investigation of maize mutants that fail to suppress bract development. These mutants are known as *tassel sheath* (*tsh*). The mutants often have well-developed bracts in the inflorescence. We have collected several mutants with a *tassel sheath* phenotype and are beginning genetic characterization. Initial complementation and mapping studies suggest that at least three distinct loci (*tsh1*, *tsh2*, and *tsh3*) exist. A *tsh1 tsh2* double mutant has a synergistic phenotype indicating that they interact in a common pathway of bract suppression (Fig. 3). We have used a map-based cloning approach to isolate the *tsh1* gene, which

FIGURE 3 A *tsh1 tsh2* double mutant has a synergistic phenotype, indicating that they interact in a common pathway of bract suppression.

encodes a predicted transcriptional regulator containing a putative GATA zinc finger domain. Currently, we are performing an expression analysis of *TSH1* as well as other genes known to be important for leaf initiation and growth in order to understand the mechanism by which this gene regulates bract suppression. Ultimately, we hope to investigate *TSH* genes from other grass species and outgroups to see if this pathway is involved in the evolution bract suppression in the grass family.

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Clinton Whipple

CELL-FATE DECISIONS IN THE EARLY PLANT EMBRYO

W. Lukowitz T. Awoyomi M. Bayer R. Devenyi M. Galli T. Nawy J. Nemiroff S. Peters J. Williams

In most plants, the early stages of embryogenesis follow a predictable sequence of cell divisions and cell-shape changes reflecting the coordinated fate decisions that lay down the reference points of the body plan. Our goal is to identify and understand the genetic network regulating this process. We are working with the small weed *Arabidopsis*, an inexpensive experimental model with very good comparability to economically important species.

Paternal Control of Embryonic Patterning

W. Lukowitz, M. Bayer, M. Galli

Plant development begins with an asymmetric division of the zygote. This division entails a fundamental fate decision that sets the stage for all subsequent patterning events: The small apical daughter cell will produce the proembryo, whereas the large basal daughter cell will mainly form the extraembryonic suspensor. The mitogen-activated protein kinase kinase (MAPKK) kinase gene *YDA* promotes suspensor fate in the basal cells. Loss of *YDA* activity essentially eliminates formation of the extraembryonic suspensor, whereas hyperactive variants of *YDA* suppress formation of the embryo often to the extent that all daughters of the zygote appear suspensor-like. On the basis of these findings, we have proposed that the *YDA* MAP kinase cascade acts a molecular switch that promotes extraembryonic or suspensor fate.

A mechanistic understanding of the *YDA* pathway will require an inventory of its component. Using various approaches, five other genes with a likely function in *YDA*-dependent signaling have been identified. A reverse genetic analysis of *Arabidopsis* MAP kinase genes performed in the lab of S. Zhang (University of Columbia, Missouri) has revealed that *MPK3* and *MPK6* act redundantly downstream from *YDA* in postembryonic development. In collaboration, we have confirmed that the same two MAP kinases also function in the embryo. An ongoing, systematic survey of *Arabidopsis* MAPKK genes in our lab has, so far, implicated *MKK9*: Expression of hyperactive variants in the embryo has an effect similar to expression of hyperactive *YDA* variants. Thus, the *YDA* MAP kinase cascade appears to consist of *YDA*, *MKK9* (and possibly other MKK genes with overlapping function), and *MPK3/6*. This MAP kinase cascade is required throughout the life cycle, regulating, among other processes, the size of the stem-cell population in the shoot apical meristem and the formation of guard cells in the leaf epidermis.

In contrast, two components of the YDA pathway that we originally identified by virtue of their *yda*-like mutant phenotypes in the embryo are not required after germination. The *GRD* gene likely executes suspensor-specific gene transcription in response to the *YDA* MAP kinase cascade. *GRD* encodes a protein of the RWP-RK family, a small group of predicted transcription factors that have only been found in green algae and higher plants. The only members of this family with an assigned function both affect developmental fate decisions: *minus dominance* (*mid*) from the unicellular alga *Chlamydomonas* dominantly determines the mating type of gametes, and *nodule inception* (*nin*) from the legume *Lotus japonicus* promotes the colonization of roots with nitrogen-fixing symbiontic bacteria in response to Nod-factor signaling. The *GRD* gene product contains two possible MAP kinase phosphorylation sites, one of which has a high score with different prediction algorithms and is conserved in the rice homolog of *GRD*. We have constructed variants of *GRD* in which one or both of these sites have been mutated such that they cannot be phosphorylated any more or such that they are mimicking the effects of constitutive phosphorylation. These variants are being tested in mutant plants. If *GRD* is a substrate of the YDA MAP kinase cascade, phosphorylation of *GRD* should be essential for normal function.

Activation of the YDA MAP kinase cascade appears to require the *SSP* gene. *SSP* encodes a member of the receptor-like protein kinase family. In animals, these proteins operate in association with cell surface receptors, such as the interferon-1 receptor com-

plex. We have found that SSP protein is myristoylated at its amino terminus and that this modification effectively anchors the protein in the plasma membrane. Myristoylation of SSP is essential for normal function: Mutation of the modified glycine residue causes mislocalization of the SSP protein to the cytoplasm where it remains inactive. Although SSP has been classified as a protein kinase, key positions of the catalytic domain are not conserved, and SSP variants that harbor transition-state mutations in the ATP-binding pocket fully complement the phenotype of mutant embryos. This would suggest that SSP is a "dead" kinase. On the other hand, a small carboxy-terminal TRP domain of SSP that likely mediates protein-protein interactions is absolutely essential: Even deletions of a few amino acids create completely inactive variants. Taken together, our findings suggest that SSP functions as an adapter protein at the plasma membrane.

What is the role of SSP in the *YDA*-dependent signaling event? Unexpectedly, we found that SSP links activation of the *YDA* MAP kinase cascade to fertilization. Expression of *SSP* mRNA is restricted to pollen, and *ssp* mutations show a rather unusual paternal effect: The phenotype of the zygote and embryo is completely determined by the genotype of the pollen, i.e., wild-type egg cells fertilized with mutant pollen develop as mutants, whereas mutant egg cells fertilized with wild-type pollen develop as wild type. Forced expression of *SSP* in leaves is sufficient to trigger *YDA*-dependent signaling, implying that *SSP* can act as a signal (Fig. 1). This truly unique mechanism immediately raises a number of questions surrounding the signaling event: Is *SSP* mRNA or SSP protein delivered to the zygote? How is *SSP* mechanistically connected to activation of the *YDA* MAP kinase cascade? When after fertilization does the *YDA* pathway because active and for how long does it stay active? Our current research focuses on addressing these issues.

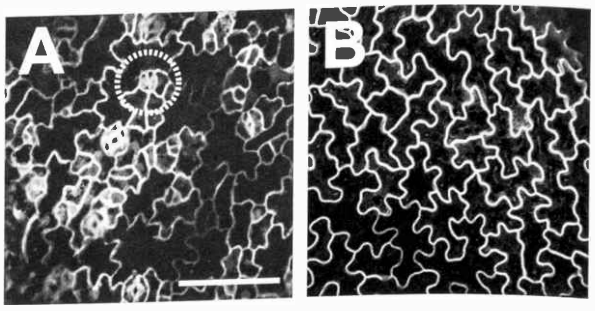

FIGURE 1 Forced expression of SSP protein in leaves eliminates stomata. (*A*) Stomata—the pores on the surface of leaves that allow for gas exchange—are formed by two specialized, kidney-shaped guard cells (an example is circled). Activity of the *YDA* MAP kinase cascade represses the differentiation of guard cells, limiting the numbers of stomata on the leaf. Expression of an inactive variant of SSP that is defective in N-myristoylation has no effect on formation or spacing of stomata. (*B*) In contrast, no stomata are found on leaves expressing an active SSP variant, implying that SSP protein is sufficient to hyperactivate the *YDA* MAP kinase cascade in leaves. Images were taken with a confocal laser-scanning microscope, and both SSP variants were tagged with a fluorescent protein moiety to visualize their distribution in the cell. Bar, 50 μm.

A GATA Factor Organizing Initiation of the Embryonic Root

W. Lukowitz, T. Nawy

The embryonic root of *Arabidopsis* develops at the border between the two cell types established with the asymmetric division of the zygote: the suspensor and proembryo. Root initiation is triggered by an inductive signal from the lower-tier cells of the proembryo to the uppermost suspensor cell. This signaling event is dependent on the plant hormone auxin and results in the formation of a characteristic lens-shaped cell, which functions as the quiescent center of the incipient root meristem and organizes the establishment of the various tissue initials.

Mutations in the GATA-type transcription factor *HAN* eliminate all anatomical hallmarks associated with root formation in the early embryo. Despite this, about 20% of all *han* mutants eventually recover and, after a lag period, form relatively normal, viable seedlings. This feature of *han* mutants is in striking contrast to mutants in the auxin signaling pathway, which typically result in rootless seedlings and affect root formation throughout the life cycle. Thus, we have proposed that *HAN* is not required for root formation in general but specifically for initiating a root in the early embryo.

Surprisingly, an analysis of *han* mutants with a panel of molecular cell fate markers revealed that the expression of root-specific genes is not absent in the mutants but rather shifted toward the center of the proembryo. For example, a *WOX5* reporter gene, normally expressed in the uppermost suspensor cells and, later, the lens-shaped cell is found in a broad stripe across the center of the proembryo (Fig. 2). In summary, our analysis of cell fate markers suggests that *han* mutations change the coordinates of the fate map, shifting it toward the apex.

How can this global effect on the fate map be rationalized? The only known pathway with a global effect

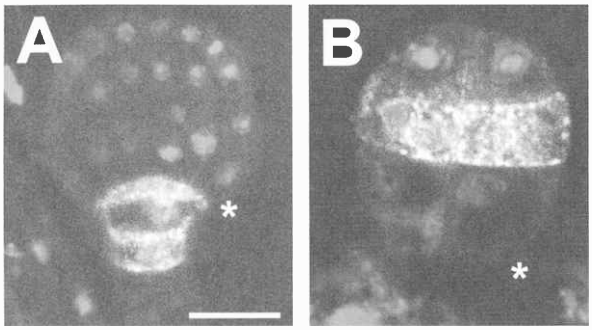

FIGURE 2 Mutations in the GATA-type transcription factor result in an apical shift of the embryonic fate map. (*A*) The homeodomain gene *WOX5* is normally transcribed in the uppermost suspensor cells, which will form the distal part of the root primordium. (*B*) In *han* mutants, *WOX5* is expressed in a broad stripe in the center of the globular proembryo. *WOX5* expression was visualized using a fluorescent reporter gene and imaged with a confocal laser-scanning microscope. Nuclei of the embryo and the surrounding endosperm are seen as weak dots in the background. The star marks the border between the embry and the suspensor. Bar, 20 μm.

on embryonic development is auxin signaling. We have found that mutations in *han* can dominantly suppress the rootless embryo phenotype caused by loss of the auxin response factor gene *MP: mp han/+* embryos often develop a rudimentary root meristem and clearly show expression of auxin-dependent transcripts that are absent in *mp* embryos. These observations indicate that the auxin signaling network is highly sensitive to changes in *HAN* activity. Perhaps the simplest explanation is that *HAN* functions to inhibit select auxin responses. According to this view, *han* embryos should show overactive or inappropriate auxin responses. Indeed, the expression of auxin-responsive markers is expanded in *han* embryos, although not to the extent that they become ubiquitous.

In the early embryo, *HAN* mRNA is expressed in a pattern very similar to that of the *BDL* and *IAA13* genes, two redundant negative regulators of auxin response factors. This observation has led us to investigate whether *HAN* might function by promoting the expression of *BDL/IAA13*, which in turn would negatively regulate auxin response factors. Preliminary results suggest that this is not the case: Expression of *BDL* from a *HAN* promoter has no effect on the phenotype of *han* embryos, and the expression of *BDL* reporter genes appears to be relatively normal in a *han* mutant background. We are now investigating whether *han* mutations affect auxin signaling at the level of hormone transport.

The biochemical function of *HAN* is to regulate gene expression. To identify the set of target genes subject to regulation by *HAN*, we have initiated a global comparison of the transcriptional profiles of wild-type embryos with *han* mutant and *HAN*-overexpressing embryos. This approach will provide an unbiased whole-genome snapshot of transcription that can then be mined to define a mechanistic context for *HAN* function in the embryo.

Role of the *YDA* MAPKK Kinase Gene in Maintaining the Stem Cell Population of the Shoot Apical Meristem

W. Lukowitz, J. Williams [in collaboration with G.V. Reddy, University of California, Riverside]

Plant growth is dependent on the continuous production of new organs, such as leaves, flowers, or lateral shoots. All of these organs are initiated by the apical meristems, a group of cells at the tip of the shoots. The apical meristems contain a small population of stem cells that are the ultimate progenitors of all organs. The shoot meristem of seedlings is located between the petioles of the two embryonic leaves or cotyledons. An analysis with the scanning electron microscope revealed that hyperactive variants of *YDA* frequently cause fusion of the cotyledons and eliminate the shoot meristem. On a molecular level, expression of the CLV3 gene, which marks the stem cells of the meristem, is undetectable in a large portion of embryos harboring hyperactive *YDA* variants.

The small, secreted ligand CLV3 is part of a feedback loop between the stem cells of the shoot meristem and the cells directly below them, called the organizing center. The stem cells secrete CLV3, which is perceived by a cell surface receptor complex including *CLV1* and *CLV2* and represses transcription of the homeodomain gene *WUS*. As a consequence, *WUS* mRNA is absent in the stem cells and only found in the cells of the organizing center (negative branch of the loop). The organizing center, in turn, provides an inductive signal required to maintain the stem cells in an undifferentiated state (positive branch of the loop). This feedback loop is thought to maintain the size of the stem cell population. Since it has been proposed that a MAP kinase cascade mediates transduction of the CLV3 signal from the cell surface to the nucleus, we have investigated whether *YDA* acts in the *CLV* pathway. So far, our results seem to indicate that this is

not the case. For example, expression of hyperactive *YDA* variants in the stem cell population does not have any effect on the phenotype of *clv* mutants.

Alternatively, *YDA* might act on antagonistic transcription factors that regulate the formation of organ primordia. The knotted-type homeodomain gene *STM* is expressed in the center of the meristem to prevent premature differentiation of the stem cells, whereas the myb domain gene *AS1* is expressed at the periphery, in the primordia, to promote differentiation. Misexpression of *AS1* in the center of the meristem represses *STM* and results in a loss of the stem cells. We are now investigating the possibility that hyperactive *YDA* eliminates the meristem by causing ectopic expression of *AS1*. Furthermore, in collaboration with Venu Reddy at the University of California, Riverside, we are developing the tools to forcibly induce *YDA* activity in subsets of cells within the shoot apical meristem. The effect of these manipulations will be followed by imaging cell division and marker gene expression in live meristems and will allow us to directly test the assumption that hyperactive *YDA* variants interfere with primordia formation.

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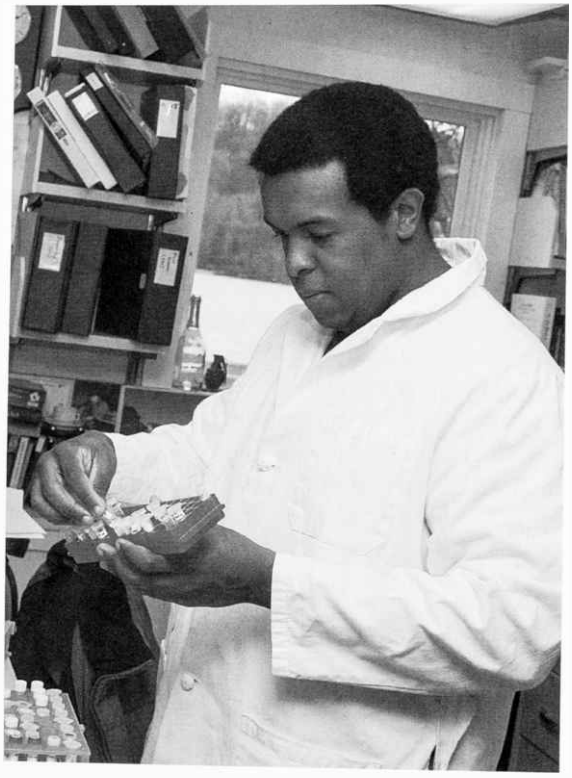

Jason Williams

PLANT DEVELOPMENTAL GENETICS AND FUNCTIONAL GENOMICS

R. Martienssen P. Ferreira, D. Garcia, J. Horn, D. Irvine, A. Kloc, S. Locke, U. Ramu, D. Roh, M. Ronemus, R. Shen, J. Simorowski, R. Schwab, R.K. Slotkin, M. Tanurdzic, M.W. Vaughn, M. Zaratiegui-Biurrun

Research in our laboratory concerns transposon silencing, gene control, and stem cell function in plants, as well as heterochromatic silencing in yeast, which provide useful models for higher organisms. Stem cell function and axis formation in *Arabidopsis* depends on *asymmetric leaves1* and on RNA interference (RNAi) via spatially restricted microRNA (miRNA) and *trans*-acting small interfering RNA (siRNA). In fission yeast, RNAi of centromeric transcripts regulates histone modification by endonucleolytic cleavage, and we have found similar transcripts in *Arabidopsis*, where small RNA, DNA methylation, and chromatin remodeling regulate heterochromatin through transposons and repeats. We continue to develop novel tools that use transposons to probe genomic function.

During the last year, Damien Garcia left for a position in Strasbourg, and Mike Ronemus took a position at Cold Spring Harbor Press. We were joined by postdoc Rebecca Schwab from Germany and graduate student Sarahjane Locke.

Specification of Leaf Polarity in *Arabidopsis* via *trans*-acting siRNA

D. Garcia, R. Schwab [in collaboration with M. Byrne, John Innes Center]

In plants, leaves initiate on the flanks of the shoot apical meristem and subsequently develop distinct proximodistal, dorsoventral (adaxial-abaxial), and mediolateral patterns. The SANT (*myb*) domain gene *PHANTASTICA* (*PHAN*) is required for adaxial fate in the snapdragon *Antirrhinum* and in other plants, but the *Arabidopsis* ortholog *AS1* has milder effects on leaf shape, suggesting the existence of alternate or redundant regulatory functions. We performed a screen for enhancers of *as1* with more elongate and dissected leaves. These enhancers disrupt an RNA-dependent RNA polymerase (*RDR6*), *ARGONAUTE7 (AGO7)/ZIPPY*, *SUPPRESSOR OF GENE SILENCING3* (*SGS3*), and *DICER-LIKE4(DCL4)* which all regulate *trans*-acting siRNA (ta-siRNA). Microarray analysis revealed that the *AUXIN RESPONSE FACTOR* genes *ETTIN* (*ETT*)/*ARF3* and *ARF4* were up-regulated in *ago7*, whereas *FILAMENTOUS FLOWER (FIL)* was up-regulated only in *as1 ago7*double mutants. *RDR6* and *SGS3* likewise repress *ARF3* and *ARF4*, which specify abaxial fate. We found that the ta-siRNA gene *TAS3*, which targets *ARF3* and *ARF4,* is expressed in the adaxial domain (Fig. 1) and that *ett as1 ago7* triple mutants resemble *as1*. Thus, *FIL* is down-regulated redundantly by *AS1* and by *TAS3,* acting through *ETT*, revealing a role for ta-siRNA in leaf polarity. *RDR6* and *DCL4* are also required for systemic silencing, implicating ta-siRNA as a potentially mobile signal. Interestingly, *TAS3* also matches one of six precursor genes for miR165/166, which we have previously shown was abaxially expressed. Misexpression of miR166 in *TAS3* mutants could contribute to the phenotype in some cells, although miR166 target genes were still expressed in *as1 ago7* double mutants.

miRNA-targeted and siRNA-mediated mRNA Degradation Regulated by Argonaute, Dicer, and RdRP

M. Ronemus, M.W. Vaughn

ARGONAUTE1 of *Arabidopsis* mediates the cleavage of miRNA-targeted mRNAs, and it has also been implicated in the posttranscriptional silencing of transgenes and maintenance of chromatin structure. Mutations in *AGO1* severely disrupt plant development, indicating that miRNA function and possibly other aspects of RNAi are essential for maintaining normal patterns of gene expression. Using microarrays, we have found that 1–6% of genes display sig-

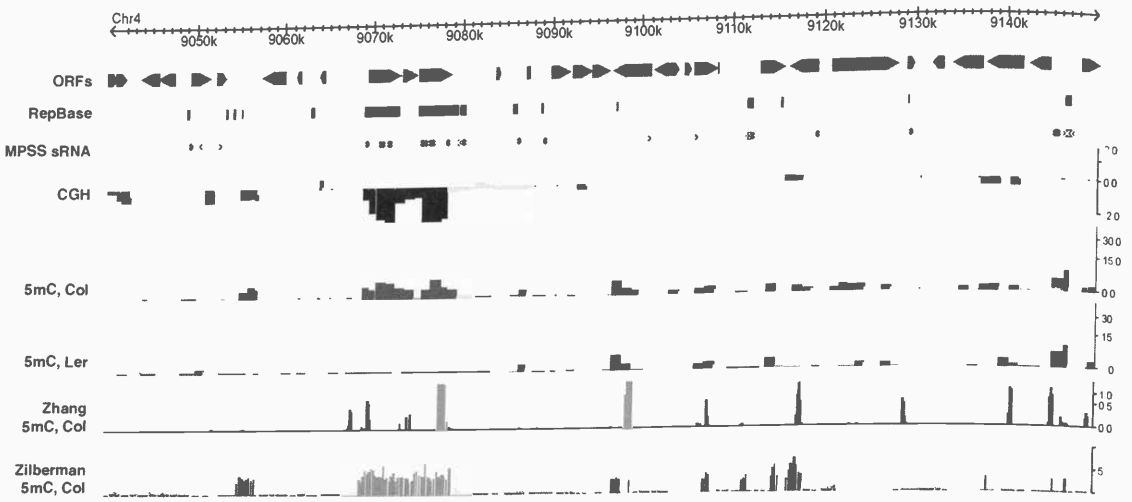

FIGURE 1 Epigenetic variation: DNA methylation profiles of *Arabidopsis* chromosome 4. McrBC digestion-based, and two antibody-based methylation detection profiles are compared along 125 kb of euchromatin 9 Mb from the end of *Arabidopsis* chromosome 4. ORFs (open reading frames) from genes (*light*) and retrotransposons (*dark*) are indicated, along with repeats predicted by RepBase and TandemRepeatFinder. Small RNA matches from massively parallel signature sequencing (MPSS) data are indicated by arrowheads. Tiles that detect significant differences in copy number are highlighted (CGH), whereas tiles that detect significant DNA methylation by means of McrBC-based detection are highlighted for the two strains Columbia and Landsberg *erecta* (5mC, Col and 5mC, Ler). For comparison, the posterior probability of methylation at 35-bp microarray probes as determined by mCIP-based detection on Affymetrix arrays (Zhang et al. 2006) and significant uncorrected log 2 ratios of mCIP antibody-enriched DNA to input DNA for 220-bp Nimblegen probes (Zilberman et al. 2006) are shown for wild-type Columbia. The three detection protocols show significant agreement, especially for more heavily methylated features such as repetitive elements. McrBC-based profiling is more sensitive and detects significant variation between strains.

nificant expression changes in several alleles of *ago1* at multiple time points, with the majority showing higher levels. Several classes of known miRNA targets increased markedly in *ago1*, whereas others showed little or no change. Cleavage of mRNAs within miRNA-homologous sites was reduced but not abolished in an *ago1* null background, indicating that redundant slicer activity exists in *Arabidopsis*; 21–22-nucleotide siRNA, as well as larger (30–65 nucleotides) RNA fragments, corresponding to highly up-regulated miRNA target genes accumulated in wild-type plants, but not in *ago1*, nor in the RNA-dependent RNA polymerase mutants *rdr2* and *rdr6*, nor in the Dicer-like mutants *dcl1* and *dcl3*. Both sense and antisense RNAs corresponding to these miRNA targets accumulated in the *ago1* and *dcl1* backgrounds. These results indicate that a subset of endogenous mRNA targets of RNAi may be regulated through a mechanism of second-strand RNA synthesis and degradation initiated by or in addition to miRNA-mediated cleavage. The 21-nucleotide siRNA corresponding to some targets (*TIR1*-like genes) were cleaved in register with the miRNA target site, resembling ta-siRNA.

The Slicer Function of Argonaute Is Required for Heterochromatic Silencing and Spreading

D. Irvine, M. Zaratiegui, A. Kloc, S. Locke [in collaboration with D. Goto, Hokkaido University, Japan, and L. Joshua-Tor, Cold Spring Harbor Laboratory]

In the fission yeast *Schizosaccharomyces pombe*, the RNAi machinery converts transcripts from pericentromeric repeats into siRNAs, which are required for the assembly of pericentromeric heterochromatin. siRNA guides chromatin silencing via the Argonaute and RNA-dependent RNA polymerase complexes, and this silencing presumably depends on base pairing with either RNA or DNA. We have shown that Argonaute requires the conserved DDH amino acid motif for heterochromatic silencing and histone H3 lysine-9 dimethylation (H3K9me2). Argonaute proteins require this motif for RNase H activity, which cleaves (or slices) target messages complementary to siRNA. H3K9me2 spreads into silent reporter genes when they are embedded within heterochromatic transcripts. Silencing of these reporter genes requires

readthrough transcription by RNA polymerase II (pol II) and processing of these transcripts via Argonaute. Thus, siRNA appears to impact histone modification by cleaving heterochromatic RNA, whereas spreading of the silencing effect depends, ironically, on transcription of this "aberrant RNA" by pol II. Heterochromatic silencing occurs in most eukaryotes and shares some common properties. For example, silencing due to position effect is inherited from one cell to another but is unstable (variegated) in *Drosophila*; similarly, patterns of transposable element control are unstable (cycling) in maize, and centromeric silencing is unstable in *S. pombe*. We are exploring these patterns of variegation and their relationship to histone modification and RNAi.

Maize-targeted Mutagenesis: A Knockout Resource in Maize

P. Ferreira, D. Roh, M. Vaughn [in collaboration with W.R. McCombie and L. Stein, Cold Spring Harbor Laboratory]

Maize-targeted mutagenesis (MTM) population comprises seed and tissue from 44,000 maize plants in which *Mutator* transposons have been mobilized and then stabilized genetically. We estimate this resource has approximately 0.5 to 1 million independent new insertions of *Mutator* elements in the gene-rich portion of the genome, or more than one insertion per kilobase. Tissue was harvested in 18 two-dimensional "grids" of 48 x 48 plants each, and genomic DNA was prepared from row and column pools that permit cross-referencing of any individual sample. These pools have been extensively used to select insertions in target genes using polymerase chain reaction (PCR), as a service to the maize community. Target sites flanking each Mutator insertion can also be amplified en masse from these pools by a variety of PCR methods. We have optimized the use of GWALK (genome walker) PCR to amplify a representation of flanking sequences from row and column pools. Sequencing using massively parallel 454 technology followed by mapping back to the maize genome resulted in identification of a high proportion of the flanking sites, including control insertions previously recovered by conventional means. By first comparing nonintersecting row pools, and then row and column pools, it is possible to distinguish preexisting (parental) insertions from new germinal insertions at row-column intersections. We are currently exploring the use of Solexa sequencing technology to further reduce costs, and we estimate that it should be possible to saturate the maize genome, to the extent possible with *Mutator* insertions, with only a few hundred sequencing reactions.

Polyploidy, Transposable Elements, and Epigenetic Control

M. Tanurdzic, K. Slotkin, J. Simorowski, M. Vaughn [in collaboration with L. Comai, University of Washington; W. Thompson, N. Carolina State University; and R.W. Doerge, Purdue University]

Heterochromatin is composed of transposable elements (TEs) and related repeats. Like TEs, heterochromatin silences genes located nearby and has a major role in epigenetic regulation of the genome. siRNA corresponding to heterochromatic sequences can be detected in plants, animals, and fission yeast, indicating that these sequences are transcribed. In plants, siRNA corresponding to different classes of TEs depends on the DNA methyltransferase MET1, the SWI/SNF ATPase, DDM1, or both, but not on the histone deacetylase SIL1. All three genes are required for silencing transposons in the absence of RNAi, but they depend on siRNA for resilencing in backcrosses. DNA methylation is concentrated in TEs and heterochromatin, but many genes have low levels of methylation in their coding region. Genic methylation is not associated with H3K9me2, is highly polymorphic, does not require DDM1, and is inherited in recombinant inbred lines, but it has little effect on gene expression. Transgene reporters, integrated around the genome by gene-trap mutagenesis, are silenced by position-effect variegation when they are located a few kilobases from the NOR, and depend on SIL1 and MET1 for silencing, but not DDM1. Similar insertions in centromeric satellite repeats are also silenced.

Polyploidy in plants results in a variety of genetic and epigenetic changes in gene expression from generation to generation following the establishment of allopolyploids. The mechanisms that underlie these changes are important in the short term (hybrid vigor) as well as in the long term, as polyploids are stabilized during evolution. We are using microarrays and Solexa sequencing to profile chromatin modifications in synthetic allopolyploids from crosses between tetraploid *A. thaliana* and tetraploid *A. arenosa*. Although changes in heterochromatin are relatively modest, some transposons are activated in the allotetraploids. We are also comparing diploid chromatin modification profiles to the pattern of

replication of chromosomal DNA using BrdU incorporation in synchronized cultured cells.

Epigenetic Natural Variation in *A. thaliana*

M.Vaughn, M. Tanurdzic [in collaboration with W.R. McCombie, Cold Spring Harbor Labratory; Z. Lippman, Hebrew University of Jerusalem, R.W. Doerge, Purdue University, and V. Colot, URGV, France]

Cytosine methylation of repetitive sequences is widespread in plant genomes, occurring in both symmetric (CpG and CpNpG) and asymmetric sequence contexts. We have used the methylation-dependent restriction enzyme McrBC to profile methylated DNA using tiling microarrays of *Arabidopsis* chromosome 4 in two distinct ecotypes, Columbia and Landsberg *erecta*. We have also used comparative genome hybridization (CGH) to profile copy-number polymorphisms (Fig.1). Repeated sequences and TEs, especially LTR (long terminal repeat) retrotransposons, are densely methylated, but one third of genes also have detectable methylation in one or more exons and introns. siRNA are preferentially associated with methylated TEs, but not with methylated genes, indicating that most genic methylation is not guided by siRNA. Whereas TEs are almost always methylated, genic methylation is highly polymorphic, with half of all methylated genes being methylated in only one of the two ecotypes. A survey of loci in 96 *Arabidopsis* accessions revealed a similar degree of methylation polymorphism. Within-gene methylation is heritable but is lost at a high frequency in segregating F_2 families. Promoter methylation is rare, and gene expression is not generally affected by differences in DNA methylation, although highly expressed genes are more likely to have methylated exons.

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PLANT DEVELOPMENTAL GENETICS

M. Timmermans D. Chitwood F. Nogueira
M. Guo T. Phelps-Durr
S. Madi A. Sarkar

Development in higher plants is a continuous process as organs emerge throughout the entire plant life cycle, which for some plants extends over hundreds of years. The growing tip of a plant, referred to as shoot apical meristem (SAM), contains a population of stem cells that divide to maintain the SAM and to generate daughter cells from which lateral organs, such as leaves and flowers, arise. The research in our lab aims to understand the molecular mechanisms that distinguish indeterminate stem cells from their differentiating derivatives. In addition, we are studying the role of stem-cell-derived signals in the patterning of lateral organs. Observations from our lab indicate that microRNAs (miRNAs) are among these meristem-derived signals. Moreover, stem cells produce signals required for the establishment of determinacy. This process involves an epigenetic silencing mechanism, indicating that the switch from stem cell to differentiated cell is not simply encoded in DNA but by proteins associated with DNA.

Adaxial/Abaxial Patterning of Lateral Organs in Maize

F. Nogueira, S. Madi, D. Chitwood

Outgrowth and patterning of lateral organs in plants depend on the specification of adaxial-abaxial (dorsoventral) polarity. This asymmetry is generated in part through the polarized expression of class III homeodomain leucine zipper (HD-ZIPIII) transcription factors that specify adaxial/upper fate. We have reported that in maize, the adaxial-specific expression of the *hd-zipIII* genes is established by a 21-nucleotide microRNA, miR166. miRNAs are endogenous, small noncoding RNAs that mediate the cleavage or translational repression of target transcripts containing complementary sequences, thus controlling gene expression at the post-transcriptional level. We have shown that miR166 accumulates in a gradient on the abaxial side of young leaf primorida and thereby sets up leaf polarity (Fig. 1).

Specification of adaxial fate in maize also requires *leafbladeless1* (*lbl1*), recessive mutations of which lead to formation of radial abaxialized leaves. Double-mutant and expression analyses indicate that *lbl1* is required for the accumulation of *hd-zipIII* transcripts in the developing leaf. Using in situ hybridization analysis, we have shown that loss of *lbl1* leads to misexpression of mature miR166 throughout the initiating and developing leaves. Reverse transcriptase–polymerase chain reaction (RT-PCR) analysis showed that transcript levels for three of the nine maize *mir166* genes are increased in *lbl1* apices compared to wild type. These findings indicate that *lbl1* contributes to leaf polarity by spatially restricting expression of selected *mir166* family members. We have cloned *lbl1* and found that it encodes a homolog of SUPPRESSOR-OF-GENE SILENCING3 (SGS3), a key component of the biogenesis pathway of a second family of small regulatory RNAs termed *trans*-acting small interfering RNAs (ta-siRNAs). Using small RNA blots, we have shown that ta-siRNAs accumulate at detectable levels in wild-type but not in *lbl1* apices, suggesting a functional conservation between LBL1 and SGS3. More importantly our data indicate a role for ta-siRNAs in specification of leaf polarity through repression of abaxial factors such as miR166.

ta-siRNAs are generated in 21-nucleotide phasing as defined by a miRNA cleavage site from noncoding transcripts (referred to as *tas*). Therefore, the ta-siRNAs generated from each *tas* locus can be predicted, enabling the identification of potential target genes using computational approaches. Previous work in *Arabidopsis* had identified a ta-siRNA, tasiR-ARF, that regulates the expression of *AUXIN RESPONSE FACTOR3* (*ARF3*) and *ARF4*. We have identified four *tas* loci harboring tasiR-ARFs in maize, and using in situ hybridization, we have shown that tasi-ARF accumulates on the adaxial side of the incipient and developing leaf primordia, consistent with a possible role in setting up the adaxial side of the leaf (Fig. 1). 5′ RACE (rapid amplification of cDNA ends) analysis showed that maize *arf3a* is a direct target for tasiR-ARF, and expression studies showed that *arf3a* is expressed abaxially in normal developing leaves but is ectopically expressed in the SAM of *lbl1* mutants. As *mir166* precursors are not direct targets for tasiR-ARFs, it is likely that their misexpression in *lbl1*

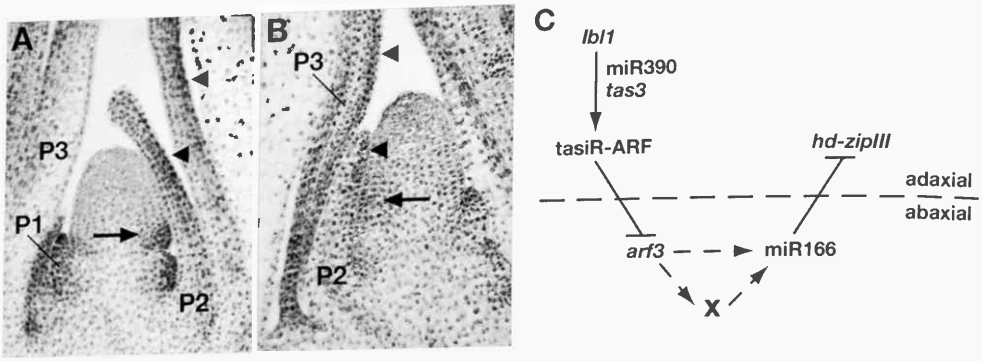

FIGURE 1 The small regulatory RNAs, miR166 and tasiR-ARF, establish opposing domains along the adaxial-abaxial axis of the leaf. (*A*) miR166 is expressed on the abaxial/lower surface of newly initiated (*arrow*) and developing leaf primordia (*arrowheads*). (*B*) tasiR-ARF is expressed on the adaxial/upper surface of leaf primordia. (*C*) A model for adaxial-abaxial patterning via tasiR-ARF and miR166. LBL1, along with miR390, is required for the biogenesis of ta-siRNAs from *tas3* precursor transcripts. tasiR-ARF acts on the adaxial side of the incipient leaf and sets up organ polarity by spatially restricting the expression domain of *arf3*. ARF3, perhaps via additional abaxial determinants, regulates expression of specific *mir166* family members. This leads to the accumulation of miR166 on the abaxial side of the initiating leaf, where it guides the cleavage of *hd-zipIII* transcripts and represses adaxial fate. tasiR-ARF and miR166 thus establish opposing fates along the adaxial-abaxial axis in developing leaves. Direct interactions (*solid lines*) are distinguished from putative interactions (*dashed lines*).

mutants results from the misregulation of ARF3 transcription factors. This possibility is being investigated using genetic approaches in maize and by analyzing *mir166* promoter reporter constructs in *Arabidopsis arf3* and *arf4* mutants.

Our observations indicate that *lbl1/SGS3* is essential for adaxial/abaxial patterning in maize and present the intriguing possibility that dorsoventrality is specified through the opposing action of two small regulatory RNAs, tasi-ARF and miR166; tasiR-ARF defines the adaxial domain by spatially restricting expression of abaxial determinants, whereas miR166 delineates the abaxial domain by restricting expression of the adaxializing *hd-zipIII* genes (Fig. 1). These findings thus reveal a novel patterning mechanism in development. By studying the genetic pathway involving *lbl1* and the maize *hd-zipIII* genes, we hope to further improve our understanding of the role of small regulatory RNAs as developmental signals.

The Spatiotemporal Regulation of miR166 Expression in Developing Leaves

F. Nogueira, D. Chitwood, S. Madi [with contributions from K. Marran and W. Kruesi, Undergraduate Research Program, CSHL]

The dynamic expression pattern and gradient of miR166 accumulation is reminiscent of a movable signal and suggests that expression of the *mir166* genes is under such control or alternatively that miR166 can move between cells. Testing the latter possibility is of key importance, as the knowledge that miRNAs can act as mobile signals will have major implications regarding their role in developmental biology. A number of experiments suggest that miRNAs, unlike siRNAs, do not spread systemically throughout the plant, but we are taking a variety of approaches in maize and *Arabidopsis* to test whether mobility of small RNAs over ranges of just a few cells or within particular developmental contexts does occur.

The maize genome contains at least nine *mir166* loci, suggesting that the dynamic miR166 accumulation pattern may result in part from the differential regulation of individual *mir166* family members. Detection of miRNA precursors by in situ hybridization has been unsuccessful. We therefore employed laser capture microdissection (LCM) in combination with RT-PCR to analyze the expression profiles of *mir166* genes in specific domains of the SAM and young leaves. Although some *mir166* family members displayed overlapping expression profiles, most *mir166* genes exhibit unique tissue and cell-type-specific expression patterns. One of the tissue samples tested included cells from the incipient leaf. Four *mir166* family members are expressed in that domain and are thus important candidates for establishing adaxial/abaxial leaf polarity. Interestingly, the *mir166a* precursor was found to be expressed at the tip of the SAM. This result was surprising, because these cells

do not accumulate mature miR166 and in fact express the miR166-targets *rld1* and *rdl2* at high levels. This suggests that miR166 accumulation may be regulated in part at the posttranscriptional level. LCM/RT-PCR analysis on more precisely defined developmental domains in leaf primordia is being used to establish whether miR166 is mobile.

As mentioned above, maize contains another leaf-polarity-related class of small regulatory RNAs, the ta-siRNAs. *tas* transcripts are direct targets for miR390 cleavage, which is required to process *tas* transcripts into mature ta-siRNAs. LCM separating cells from the epidermal (L1) and sub-epidermal layers (L2) of the SAM shows that *tas* transcripts accumulate in both L1 and L2 layers. In contrast, the precursor of miR390 is detected only in the L1 layer of the SAM. Using in situ hybridization, we have detected the accumulation of mature miR390 in the adaxial domain of the incipient leaf. More importantly, we found that miR390 accumulates in both the L1 and L2 layers of the incipient leaf. Our observations are consistent with a scenario in which miR390 is produced exclusively in the L1 layer and moves into the underlying cell layers. Thus, it is plausible that specific miRNAs could move over extremely short distances of one or only a few cell lengths from specific cell types, such as the epidermal layer, to act as developmental signals during pattern formation. We are currently testing this possibility in *Arabidopsis* as well.

In *Arabidopsis*, we are using GUS-reporter lines to determine the expression patterns of miR166 precursors in developing leaves. Five *MIR166* family members are expressed during early stages of vegetative development, including *MIR166a*. We established that a full-length promoter of *MIR166a* drives expression on the abaxial side of young leaves, reminiscent of the accumulation of mature miR166. Most prominently, its abaxialized expression is restricted to the L1 layer. Last year, we reported our initial results from *MIR166a* promoter deletion analyses. More detailed characterizations of these deletion constructs identified a DNA fragment required for L1-specific expression; however, we were unable to detect any known L1-specific *cis*-elements in the promoter of *MIR166a*. Similar expression studies are under way to determine the precursor expression patterns for other small RNAs with roles in leaf polarity, such as miR390 and tasi-ARF, and, as in maize, precursor expression data will be compared to the expression pattern of the mature small RNA to test for movement.

These precursor reporter lines are also being used to identify genes involved in the spatiotemporal regulation of the polarizing small RNAs. Several lines of evidence indicate that ASYMMETRIC LEAVES1 (AS1) and ASYMMETRIC LEAVES2 (AS2) act together to regulate leaf morphology as well as adaxial/abaxial leaf polarity. We have tested whether the AS1-AS2 pathway affects miR166 expression. Using the *MIR166a::GUS*, we have shown that *MIR166a* is adaxially misexpressed in an *as2* mutant background, and we are currently analyzing whether the AS1-AS2 pathway affects expression of *MIR166a* and other family members directly or indirectly. Similarly, we have generated *arf3* mutants expressing the *MIR166a::GUS* reporter to determine the contribution of the ta-siRNA pathway in regulating miR166 expression in *Arabidopsis* and to begin to analyze the role of the plant hormone auxin in adaxial/abaxial patterning, specifically its contribution to the dynamic expression pattern of miR166.

Establishment of Determinacy during Organ Development

M. Guo, T. Phelps-Durr [with contributions from E. Jusseaux, Denis Diderot University, Paris, France]

Indeterminacy within the SAM is specified in part by the *KNOX* homeobox genes. Down-regulation of *KNOX* expression is a key factor that distinguishes stem cells and their immediate derivatives in the SAM from lateral organ founder cells. Moreover, establishment of determinacy in developing organs requires the continued silencing of the *KNOX* genes. We have previously shown that this process involves the highly conserved MYB domain proteins ROUGH SHEATH2 (RS2) and ASYMMETRIC LEAVES1 (AS1) from maize and *Arabidopsis*, respectively. On the basis of expression and genetic analyses, we proposed that these proteins function as epigenetic regulators, which in response to a stem cell signal keep *KNOX* genes in an "off" state during organogenesis, thus preventing differentiating cells from reverting into indeterminate stem cells. In the past few years, we have gained important new insights into the mechanism of *KNOX* gene silencing by RS2/AS1 that confirm this hypothesis. We have shown that RS2 and AS1 form highly conserved repressor complexes that include the zinc finger transcription factor ASYMMETRIC LEAVES2 (AS2), an RNA-binding protein named RIK, and the histone chaperone HIRA.

Using chromatin immunoprecipitation (ChIP), we identified two sites in the promoter of *BP*, one of the *KNOX* genes, that mediate AS1 complex binding. The significance of these AS1 complex binding sites for

stable *BP* silencing in leaves was demonstrated in vivo using various *BP promoter::GUS* reporter constructs. Deletion of either AS1-binding site resulted in misexpression of BP in wild-type leaves. This year, we have used gel-shift assays to more precisely characterize the *cis* sequences and proteins required for binding of the AS1 complex to *BP*. Consistent with earlier genetic data, we found that AS1 requires AS2 for binding to the *BP* promoter. We also found that AS1-AS2 bind to each of the approximately 200-bp promoter fragments identified by ChIP. Considering that both binding sites are required for stable repression of *BP* in leaves, this result suggests that AS1-AS2 binding creates a loop in the *BP* promoter. These gel-shift assays further identified 6-bp and 8-bp consensus sequences that mediate binding of AS1 and AS2, respectively. We are currently using this information to identify additional genes that are potentially regulated by an AS1-AS2 complex. As mentioned above, we are particularly interested in the contribution of AS1-AS2 to the spatiotemporal regulation of leaf polarity genes.

We have previously reported that AS1-AS2 interact with the chromatin remodeling protein HIRA, which is involved both in heterochromatic gene silencing and in the spatiotemporal regulation of euchromatic genes. We are therefore studying the role of HIRA in *KNOX* repression during leaf development using genetic and biochemical approaches. As a first step, we are testing whether *KNOX* gene silencing is associated with changes in chromatin organization and/or DNA methylation. Preliminary data suggest that the promoter region of *BP* between the two AS1-AS2-binding sites may become methylated in differentiating leaves, but whether this methylation is AS1-AS2- or HIRA-dependent remains to be tested. These data will eventually clarify the assembly of the AS1 complex at the *KNOX* loci as well as the molecular mechanism with which these genes are stably repressed to establish determinacy in differentiating lateral organs.

Global Expression Analysis of Meristem Function and Leaf Initiation

A. Sarkar, S. Madi [in collaboration with M. Scanlon, Cornell University, Ithaca, New York; P. Schnable, Iowa State University, Ames; B. Buckner and D. Janick-Buckner, Truman State University, Kirksvile, Missouri]

Plant meristems comprise distinct histological and functional domains. For instance, the stem cells are located at the most apical tip in the so-called central zone, whereas lateral organ founder cells are located on the flanks of the SAM. Traditional genetic analyses have demonstrated the importance of this meristematic organization for normal plant development and have led to the identification of some genes required for meristem function and lateral organ development. To identify novel and potentially redundant or essential genes that function in discrete domains of the SAM or developing leaf primordia, we are using a technique called laser-capture microdissection (LCM), which allows the isolation of specific cells within a tissue or organ, in combination with microarray analyses to compare global gene expression patterns between different cell populations. Expression profiles between the following cell types have been compared: (1) indeterminate stem cells in the SAM and determinate cells of newly initiated leaf primordia; (2) the epidermal (L1) and subepidermal (L2) layers of the SAM; and (3) SAMs and/or leaf primordia from developmental mutants and wild-type plants.

This year, we have used this approach to compare the gene expression profiles in the SAM and first leaf primordia from wild-type and *lbl1* mutant plants. We identified nearly 300 genes whose expression levels are significantly altered in *lbl1*. The L1 layer of the SAM had been shown to be crucial for adaxial-abaxial patterning of leaves. Interestingly, 22 genes that are differentially expressed between wild-type and *lbl1* are also differentially expressed between the L1 and L2 meristem layers. We are currently in the process of validating these differentially expressed genes by real-time quantitative RT-PCR, followed by in situ mRNA localization, and the function of genes of particular interest will be determined using functional genomics resources available in maize and *Arabidopsis*. We are also taking a bioinformatics approach to identify potential targets of ta-siRNAs among those genes that are up-regulated in *lbl1* apices. Even though this project is still in its infancy, it will soon provide novel insight into gene networks controlled by ta-siRNAs and involved in leaf polarity.

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Mengjuan Guo

BIOINFORMATICS AND GENOMICS

With the full genome sequences of many organisms completed, scientists are increasingly turning their attention to the important task of putting this gene sequence information to work to explore biology, improve agriculture, and advance biomedical research. Much of this will focus on resequencing all or large parts of selected genomes to understand the relationship between sequence variation and phenotypic variation, including disease.

This year, W. Richard McCombie joined a team that has begun sequencing the genome of maize, the most important crop in the United States. McCombie has also continued testing the first of a new generation of DNA-sequencing instruments. As part of this, they have been developing methods to selectively isolate large, targeted genomic regions. The goal of this project is to develop new, more rapid, and cost-effective methods to determine the variation that occurs in these regions among large numbers of individuals. This will be important in understanding the role of sequence variation in disease.

For plant breeders and others interested in the major crop plants—rice, corn, wheat, oats, sorghum, and barley—the availability of a publicly accessible comparative genome database has the potential to speed the development of improved varieties to meet the world's increasing food demands. Lincoln Stein and his colleagues have created just such a database, dubbed Gramene. A major milestone in 2006 was the integration of genome-mapping information on 11 wild rice species to the Gramene database, which could provide information that will allow plant breeders to create new robust varieties of domestic rice. Stein's group is also part of the International Human HapMap Project, an international project to map out regions of common genetic variability in the human genome by genotyping three major world populations across a large number of naturally variable sites. The resulting "haplotype map" will greatly reduce the cost of genetic association studies to find cancer susceptibility genes and other disorders with genetic components. The work is already bearing fruit: During the past year, the HapMap data have been used to identify a gene responsible for age-related macular degeneration (the major cause of acquired blindness in the United States) and to identify several genes that appear to contribute to type-2 diabetes.

Certain proteins have persisted over a billion years or more of evolution, and it is likely that these proteins form critical interactions subject to specific geometric and/or chemical constraints. Conserved sequence patterns reflect these functional constraints and thus contain implicit information regarding underlying structural mechanisms. Andy Neuwald uses comparative methods to study protein mechanisms through statistical analysis of the constraints imposed on protein sequences during evolution. With these methods, Neuwald and his colleagues compared a family of key enzymes called eukaryotic protein kinases (EPKs) with a set of protein kinases called "atypical protein kinases," proteins that do not appear to fit into any known protein kinase family. The result of this analysis indicates that EPK regulatory mechanisms evolved through modification of an ancestral structure. In 2006, the Neuwald lab began extending their statistical models to include detailed three-dimentional structural features and thus to come closer to more directly modeling aspects of protein mechanisms by using structural data in addition to sequence data. Neuwald and colleagues' findings provide clues to the functions of EPKs and EPK-like kinases, many of which are implicated in cancer, diabetes, and other diseases.

The DNA in cells is coated with proteins and chemical modifications that change how and when the DNA code is read. To make sense of one such modification, DNA methylation, Michael Zhang and his colleagues have identified large-scale DNA methylation patterns in the structure of the human brain and developed a new computer algorithm (Human DNA Methylation Finder) that predicts DNA methylation profiles in the human genome. This work has implications for understanding cancer and other diseases.

GENOME SEQUENCE ANALYSIS

W.R. McCombie V. Balija, L. Cohen, F. Katzenberger, M. Kramer, L. Nascimento, B. Miller, S. Muller, T. Rancanelli, L. Spiegel

We have begun a project to sequence the corn genome. The project, headed by the Washington University Genome Sequencing Center, is designed to sequence the "genespace" of the B73 strain of maize by the end of 2008. The maize genome will be the largest plant genome sequenced to date, with a size of about 2.5 billion bases. Maize is an extremely important agricultural commodity in the United States. It has been for many years the largest crop in the United States. The very recent concerns over energy security have significantly heightened its importance within the United States. Major increases in the use of corn to make ethanol are increasing the demand for corn, and this is expected to continue for the foreseeable future.

The strategy we are employing is to focus on regions of the genome that are non-repetitive. This is what we refer to as the "genespace." Entire bacterial artificial chromosomes are shotgun-sequenced and assembled at Washington University. Software developed by Doreen Ware's lab here at CSHL is then used to identify the regions of the assemblies that are non-repetitive. Following this analysis, the data are transferred to groups carrying out the finishing: Washington University, Arizona Genomics Institute, and our lab. These groups then carry out the finishing steps of the sequencing process to produce the final sequence of the nonrepetitive portion of the genome. In 2006, our lab finished clones comprising about 100 million bases of the maize genome. This amount was completed mostly in the second half of the year and we are still ramping up our output.

DEVELOPMENT OF TOOLS AND STRATEGIES TO MAXIMIZE THE UTILIZATION OF NEXT-GENERATION SEQUENCING INSTRUMENTS

S. Powers, G. Hannon

It became clear to us that rapid, possible revolutionary changes are taking place in DNA sequencing technology. Although the new sequencing instruments have great potential, they also have certain limitations. We began aggressively preparing ways to most efficiently use the instruments and to as great an extent as possible avoid their limitations

HYBRIDIZATION-BASED SELECTION OF A DEFINED SUBSET OF THE HUMAN GENOME

Oligo Design for Hybrid Selection Arrays. The process of selecting and isolating specific regions of a genome can be accomplished by very time-consuming and expensive traditional techniques such as polymerase chain reaction (PCR) amplification. Whereas traditional processes may be feasible for small sets of targeted regions, when extremely large numbers of genetic loci are targeted, an alternate approach must be considered. An oligonucleotide-array-based selection scheme was designed to address the issue of ultra-large-scale targeting and selection as well as to offer a rapid technique using significantly smaller amounts of genomic DNA.

To test the recovery of target regions of the genome of interest, we have designed an oligonucleotide array of probe sequences to be synthesized on microarray chips. As a first step in designing the oligonucleotide probes, the pseudomolecules for all chromosomes of the human genome (assembly hg17; May 2004 reference sequence) were downloaded from the genome browser (http://genome.ucsc.edu) at the University of Southern California. Repeat sequences (as noted by RepeatMasker) were masked to lowercase bases before download. The bgl2fragmentor script (written by Scott Powers [CSHL]) was used to create in silico BglII digestion fragments for each chromosome. The bgl2fragmentor script was modified to select BglII fragments between 300 and 800 bp in length. The resulting fragment sequences were then screened, and any fragment that contained repetitive sequence (indicated by sequence in lowercase) was ignored.

To design the oligonucleotide probes for the selection array, the BglII fragments (minus one 50 mer kept out for later selection if necessary) were then chopped into sequential, adjacent (but nonoverlapping) 90 mers. Obviously, these fragments were not

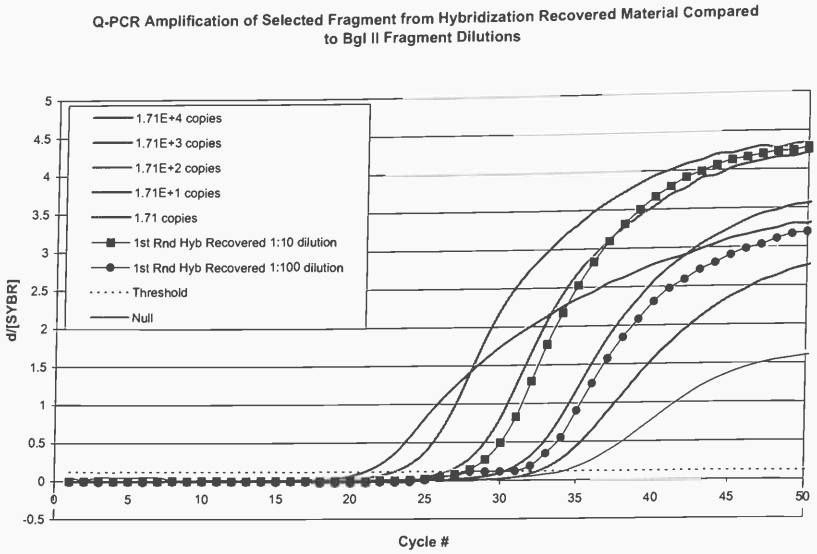

FIGURE 1 Q-PCR analysis of hybridization-selected material. Q-PCRs were performed using serially diluted amounts of BglII-digested human genomic DNA. The hybridization-selected material was also diluted and amplified for a selected fragment alongside the serially diluted BglII fragments.

all equally divisible by 90 bp, so any end sequences smaller than 90 bp were removed. An 18-bp 5′ adapter sequence [TGCTGTTGACAGTGAGCG] and a 16-bp 3′ adapter sequence [TGCCTACTGCCTCGGA] (Cleary et al. *Nat. Methods 3:* 241 [2004]) were then added to each 90 mer from each BglII fragment to create 124-mer probes. These adapters will serve to make the 124-mer probes cleavable and amplifiable. A set of 20,000 124 mers from 4181 randomly selected BglII fragments has been selected to be loaded onto an Agilent array. This will serve as the selection chip. The selection probes were then cleaved from the array using alkaline treatment and collected. Due to the small amount of probes that are collected by this method, amplification of the probes was carried out using primers specific to the adapter sequences present at the terminal ends of the probes. A second round of amplification was then carried out using biotinylated adapter-specific primers to generate labeled probes. Human genomic DNA was then digested with BglII endonuclease and served as the target DNA for the probes.

Hybridization-based Selection. Both the probes and target DNA were denatured, and hybridization was carried out for 24 hours at 65°C. Approximately 1 μg of target DNA was used in the hybridization. After hybridization, the biotinylated probes bound to their target were captured by streptavidin-coated magnetic beads. The captured material was washed stringently, and the bound material was then eluted from the beads under mild alkaline conditions that strip the bound target while leaving the probe attached to the magnetic beads. The stripped material was then collected and purified.

The collected material was then assayed by quantitative PCR (Q-PCR) using primers designed to amplify (1) a fragment that was selected for by the probes (selected fragment) and (2) a fragment that were not selected for by the probes (nonselected fragment). In parallel, these primers were also used on the serial dilutions of the initial BglII-digested target DNA. Figure 1 shows the results of the Q-PCR amplification of a selected fragment. The serial dilutions of the target DNA ranged from 60 ng (estimated fragment copy number of 1.71×10^4) to 6 pg (copy number of 1.71). The hybridization-recovered material was also diluted by a factor of 10 and 100 prior to amplification. Figure 2 shows the results of the Q-PCR amplification of nonselected materials.

Analysis of the C_T values of the BglII target DNA dilutions (Table 1) indicated that the ratio of the selected fragment to the nonselected fragment was almost exactly the expected 1:1 ratio, whereas in the hybridization-selected material, the ratio was nearly 25:1 (Table 2).

AMPLIFICATION OF BAR-CODED SAMPLES

Amplification of p53 exons. The ability to screen a large number of tumors for a distinct set of genomic loci at the resolution level of the nucleotide sequence is

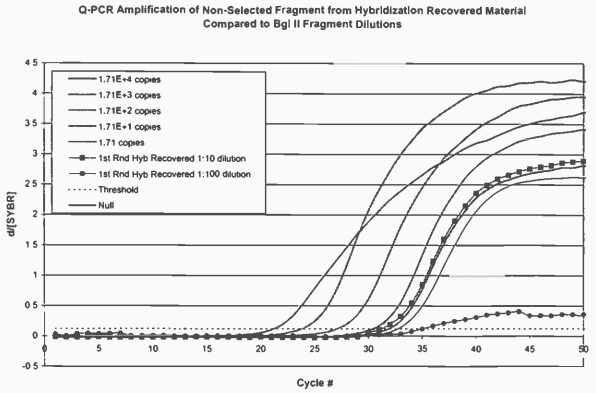

FIGURE 2 Q-PCR analysis of hybridization-nonselected material. Q-PCRs were performed using serially diluted amounts of BglII-digested human genomic DNA. The hybridization-nonselected material was also diluted and amplified for a selected fragment alongside the serially diluted BglII fragments.

invaluable in identifying novel and recurring mutations within these regions that can ultimately be linked to cancer. Although this task can be performed using currently utilized amplification and sequencing techniques, the time, labor, and cost associated with this task are considerable. A major innovation would be to perform this task on a massively parallel scale. The amplification of a distinct set of loci from a large number of patient samples in parallel is a relatively easy task; however, the ability to distinguish and link each amplified fragment to the tumor sample from which it was amplified requires consideration. The use of nucleotide "bar codes" seemed to be the ideal choice for this application. Each bar code consists of a short, unique nucleotide sequence that would be assigned to each tumor sample. The size of the bar code can vary, but, practically, a 6-base sequence would allow a unique bar code to be assigned to 4097 different tumor samples. This bar-code sequence would then be appended to the ends of an amplified fragment to identify the sample origin of the fragment. There are several ways in which amplified fragments can be bar-coded. The method chosen to test this application would entail synthesizing PCR primers specific to the loci of interest with the bar-code sequence present as a 5′ overhang region. A separate set of these pre-bar-coded primers would have to be generated for each tumor sample to be tested as part of a single group being sequenced. For instance, if we were to test 1000 tumor samples at a time, we would need 1000 sets of oligos. The same bar code could be used on multiple regions of the same tumor genome since the sequences of the target regions themselves would be different and hence easily discernible from one another. So two exons from *p53*, as an example, could be amplified from the same patient sample using one bar code, since we really only need the bar codes to distinguish between the same exon from multiple patients.

This method was tested on a small scale using ten human genomic samples from the Coriell collection. Two 100-bp regions located in exon 2 and exon 3 of the *p53* gene were used as the amplification targets. Ten pre-bar-coded primer sets were chosen for each region consisting of a 20-base complementary region and a 6-base bar-code overhang at the 5′ end. A total of 5 ng of genomic DNA from each of the ten samples was used in each amplification. The amount of amplified product was virtually equivalent in the PCRs (~10 ng/µl) without equalizing. Equimolar amounts of each product from both exon 2 and 3 were pooled together. At this stage, the pooled material was either cloned for sequence comparison on the ABI 3730xl or input directly into the 454 sample preparation work flow. For

TABLE 1 C_T Values of the BglII Target DNA Dilutions

Dilution of 1st Rnd Hyb Recovered Material	Specific Fragment Copy No.	$\log_{copy\ number}$	C_T of Selected Fragment	C_T of Non-Selected Fragment
1:10	83.10	1.919436943	27.728756	
1:10	3.13	0.495748086		31.830537
Ratio of Selected to Unselected Fragment	26.5 : 1			

TABLE 2 Extrapolated Fragment Number of Hybridization-selected Material

Amt of Bgl II Fragments	Specific Fragment Copy No.	$\log_{copy\ number}$	C_T of Selected Fragment	C_T of Non-Selected Fragment
60 ng	1.71E+04	4.233782715	21.106998	21.312162
6 ng	1.71E+03	3.233782715	23.183012	23.945086
600 pg	1.71E+02	2.233782715	26.569784	27.339945
60 pg	1.71E+01	1.233782715	30.393559	30.417076
6 pg	1.71E+00	0.233782715	32.597576	31.849861

TABLE 3 Comparison of Recovery and Identification of Primer Bar-coded *p53* Fragments

Barcode	Barcode Identifier	ABI 3730 Sequencing of cloned products*		454 Sequencing Data**	
		Frequency of p53 exon 2	*Frequency of p53 exon 3*	*Frequency of p53 exon 2*	*Frequency of p53 exon 3*
1	ACTGAC	6%	8%	6%	8%
2	ATTACA	7%	4%	19%	12%
3	CCGGCT	2%	5%	0.20%	7%
4	CTGAGG	2%	5%	11%	6%
5	GCCGTC	0%	0%	3%	5%
6	GTCCAA	7%	4%	6%	7%
7	TCATAT	7%	14%	4%	8%
8	TTACCG	49%	30%	6.80%	9%
9	AATTGC	7%	14%	17%	12%
10	AGTCAG	9%	13%	10%	11%
Ambiguous		3%	5%	17%	15%

*~400 Sequences in Analysis

**~3500 Sequences in Analysis

the cloned material, transformants were plated, and 192 clones were sampled for sequencing and separation on the ABI 3730xl. The sequences were assembled and aligned to the *p53* exon 2 and exon 3 reference sequence using the Consed software. The bar-code sequence for each sample was clearly distinguishable in each trace. The assembly was then analyzed to determine the distribution of reads from each sample. In parallel, the pooled exon products were also sequenced on a 1/4 plate on the 454 instrument. More than 20,000 sequences were generated. A sampling of 3500 fasta sequences was then examined to determine the presence of each bar code. The results are summarized in Table 3. It was apparent that sequencing of the pooled products on the 454 instrument had a much more uniform distribution of bar-code recovery when compared to the cloned products sequenced on the ABI 3730xl.

In any case, this analysis served two purposes: (1) as an exercise to determine if efficient identification of the bar-code sequence in the amplified fragments was feasible and (2) with and without cloning to determine if direct sequencing was more representative of the sample. Again, this makes it seem likely that the bias was introduced during cloning. In addition to testing bar-code distribution, we also showed that we could test the assembled fragments for polymorphisms by comparison with the human *p53* reference sequence. The results are presented in Table 4.

BAC Sequencing Utilizing Bar-code Identifiers. Two methods were designed and tested to append bar-code identifiers to randomly sheared bacterial artificial chromosome (BAC) fragments. These methods were designed to ultimately allow simultaneous sequencing of several pooled BACs on the 454 instrument and the Solexa platform as well as for testing and evaluation of the methods using the ABI 3730xl. Three *Oryza sativa* cv. Nipponbare BACs (OSJNBa0050A24, -0046G16, and -0078F08) that were previously finished at CSHL were chosen for the tests. BAC DNA from each of these clones were isolated and sheared by high-psi nebulization with a target fragment size of 300 bp.

The first bar-coding method utilized a fairly straightforward PCR-based strategy adapted from a

TABLE 4 SNPs and Deletions Detected in Samples

Coriel Sample ethinic origin	6 bp barcode	exon 2							exon 3								
		Base Position in Assembly							Base Position in Assembly								
		13	18	45	52	53	56	61	13	14	17	18	20	28	29	46	61	66
Russian	ACTGAC				A/T				C/T									
American Indian	ATTACA																	
Melanesian	CCGGCT																	
Pygmy	CTGAGG																	
Chinese	GCCGTC	DEL		A/G			A/T				A/G*		DEL			A/T		A/G
Cambodian	GTCCAA									T/G*				A/G	A/G		T/C	
French	TCATAT																	
Druze	TTACCG		T/C			A/G		A/C				DEL						
Venezuelan	AATTGC																	
Japanese	AGTCAG																	

Base position in assembly is relative to the expected product.

procedure frequently employed in CHIP-Chip experiments where small amounts of immunoprecipitated genomic representations are randomly amplified. The strategy relies on a single 26-base tagging primer, which has been named the terminal tag bar code primer, containing an 11-base "platform" region, followed by a 6-base unique bar-code identifier and a 9-base degenerate stretch at the 3′ end.

This amplification-based bar-coding procedure involves two phases. In the first phase, the degenerate region of the primer anneals to the terminal ends of the target DNA fragments, and nucleotide incorporation is initiated with two rounds of isothermal extension. In the second phase, this amplified material is used in a standard PCR utilizing a second amplification primer complementary to the "platform" region of the terminal tag bar-code primer. The extension time in this second phase was stringently controlled to amplify only smaller fragments. Agarose gel analysis of the amplification products confirmed that the product sizes were between 150 and 400 bp. The fragments were then cloned into a TA-cloning vector (pCR2.1, Invitrogen) and sequenced.

The second bar-coding method utilized a more standard ligation-based approach to append bar-code adapters to the BAC fragments. The 50-bp bar-code adapters were designed using a 44-base adapter sequence utilized by the 454 company, followed by a 6-base bar-code sequence at the 3′ end. The same sheared BAC material that was used in the first bar-coding method described above was also used to ligate the bar-code adapters. These fragments were cloned and then sequenced.

As stated previously, these methods were designed to generate bar-coded BAC fragments that would allow pooled sequencing of a large number of BACs on the 454 instrument or Solexa platform with the ability to link the resulting sequences back to the BAC of origin via the unique bar-code sequence at the ends of the reads. However, to test the effectiveness of these methodologies, we cloned the bar-coded material to sequence them using traditional methods. After cloning, approximately 600–700 reads were generated for each BAC. The expected coverage for each of these BACs with 600-700 reads was approximately 1x assuming an average insert size of 250 bp. The reads generated were aligned to the finished BAC sequence using Consed. A manual inspection of each read was performed to determine if the correct bar code assigned to each BAC was present at the terminal ends of each read. Table 5 summarizes the criteria used for the analysis as well as the results.

TABLE 5 Comparison of BAC Bar-coding Techniques

(a) Amplification-based Bar Coding

BAC name	50A24	46G16	78F08
BAC size (Kbp)	143	160	166
Number of reads	698	596	554
Avg. insert size (bp)	278	249	330
Actual coverage*	1.4x	0.9x	0.9x

**Expected Coverage = 1x*

(b) Ligation-based Bar Coding

BAC name	50A24	46G16	78F08
BAC size (Kbp)	143	160	166
Number of reads	356	346	325
Avg. insert size (bp)	321	289	302
Actual coverage*	0.8x	0.62x	0.6x

On the basis of these results, the amplification-based bar-coding method was a more efficient approach. Analysis of the ligation-based bar-coding method revealed that 40–50% of the fragments generated were not inserted into the assembly due to very small insert sizes (10–40 bp). Since this method did not rely on a traditional agarose-based size selection of fragments prior to processing, adding this size selection step in the ligation-based bar-coding procedure would readily solve this problem.

Of the fragments that were inserted into the assemblies from both methods, the insert size was in the size range expected (~300 bp). Another consideration was the distribution of reads across the assembly and the possible overrepresentation of certain sequences or regions in the reads. For both methods, the reads distributed evenly across the assembly and no apparent read "stacking" was discernible.

PCR OF DNA FROM PARAFFIN-EMBEDDED SAMPLES

Six different DNA samples were extracted from paraffin-embedded tumors using the Puregene DNA purification Kit (Gentra Systems). The samples were subsequently amplified using PCR with HotStarTaq (QIAGEN) for exons 5–8 of the *p53* gene. (Ribeiro et al., *Cancer 83*: 7 [1998]) After an initial denaturation step for 15 minutes at 95°C, samples underwent 35 cycles of PCR (30 sec 94°C; 30 sec 60°C; 1 min 72°C). This was followed by a 10-minute extension

TABLE 6 Summary of Amplification of Exons 5–8 of the *p53* Gene in Six Tumor Samples

Tumor	Exon 5 (202 bp)	Exon 6 (162 bp)	Exon 7 (184 bp)	Exon 8 (169 bp)
Liver3	amplification +	amplification +	amplification +	amplification +
Liver6 (low concentration)	no amplification –	no amplification –	amplification +	no amplification –
Liver9	amplification +	amplification +	amplification +	amplification +
Liver10	amplification +	amplification +	amplification +	amplification +
3881A2 (melanoma)	amplification +	amplification +	amplification +	amplification +
9341A2 (lungs)	no amplification –	no amplification –	no amplification –	no amplification –

at 72°C. Of the six samples, five showed amplification (Table 6). Of those five samples, four amplified very well and were sequenced with the ABI Big Dye terminator chemistry on the ABI 3730 sequencer. The sequence quality varied with the samples; however, in general, the sequences were all legible and provided usable data. Although no SNPs were found in the samples that we examined, we showed that the DNA in the paraffin-embedded samples was able to be recovered, amplified, and sequenced.

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Vivekanand Balija, Director of Technology Development

OBTAINING MECHANISTIC CLUES FROM THE EVOLUTIONARY CONSTRAINTS IMPOSED ON PROTEINS

A.F. Neuwald N. Kannan

We continue to study aspects of protein mechanisms through Bayesian statistical analysis of the functional constraints imposed during evolution. This year, our focus has been on bacterial clamp loader AAA+ ATPases and on AGC protein kinases. Clamp loaders have critical cellular roles inasmuch as a clamp must be loaded onto DNA and attached to DNA polymerase in order to keep polymerase from disassociating during DNA replication and repair. My analysis of bacterial clamp loader subunits strongly suggests that these subunits utilize a mechanism strikingly different from that of the corresponding eukaryotic clamp loader subunits. In particular, bacterial clamp loader ATPases appear to be activated through DNA-dependent repositioning of the catalytic base and of a putative *trans*-acting threonine directly involved in ATP hydrolysis. Both of these mechanisms seem to be entirely absent from eukaryotic clamp loaders. Our analysis of AGC kinases likewise suggests key aspects of their mechanisms. AGC kinases control critical cellular processes, such as cell growth, differentiation, and cell survival and thus are subject to tight spatial and temporal regulation. Our analysis suggests that features distinguishing the AGC catalytic domain from other eukaryotic protein kinases (EPKs) have coevolved with a characteristic carboxy-terminal tail to create an extended allosteric network that links the C tail to the active site. Finally, this year we have begun extending our Bayesian statistical models to include detailed three-dimensional structural features and thus to come closer to more directly modeling aspects of protein mechanisms by using structural data in addition to sequence data.

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GENOME-SCALE DATABASES OF PATHWAYS, GENETIC VARIATION, AND EVOLUTION

L. Stein S. Avraham, S. Cain, P. Canaran, P. D'Eustachio, B. Faga, T. Fiedler, M. Gillespie, G. Gopinathrao, T. Harris, L. Krishnan, C. Liang, C. Maher, L. Matthews, S. McKay, S. Michaelsen, L. Ren, C. Schmidt, W. Spooner, M. Tello-Ruiz, J. Thomason, G. Wu, C. Youens-Clark, W. Zhao

THE HUMAN HAPLOTYPE MAP

The International Human HapMap Project (www.hapmap.org) is an international project to map out regions of common genetic variability in the human genome by genotyping three major world populations across a large number of naturally variable sites. The resulting "haplotype map" will greatly reduce the cost of genetic association studies to find cancer susceptibility genes and other disorders with genetic components.

Our lab is a central participant in this project in our role as the Data Coordinating Center (DCC). We manage the central database for the project; allocate single-nucleotide polymorphisms (SNPs) to the 11 genotyping centers; coordinate data submission, quality checks, and quality control; and manage the public release of project data. The HapMap Web site, which was developed in our lab, describes the project in the four languages of the project participants (English, French, Chinese, Japanese, Yoruba) and provides access to the data both for bulk download and for interactive querying and browsing.

During 2006, we extended our phase I work on the project (published in October 2005 in *Nature*) to genotype an additional 3 million SNPs. This brings the total number of genotyped SNPs to almost 4 million, for one typed polymorphism every 750 bp of sequence—a map of unprecedented detail and resolution. The work is already bearing fruit: During the past year, the HapMap data have been used to identify a gene responsible for age-related macular degeneration (the major cause of acquired blindness in the United States) and to identify several genes that appear to contribute to type 2 diabetes.

We are also extending the features available on the HapMap Web site to bring in summary data from whole-genome association studies, as well as to allow researchers to view the locations and nature of the large number of copy-number polymorphisms that have come to light in recent months.

GRAMENE: A COMPARATIVE MAPPING RESOURCE FOR GRAINS

The Gramene database (www.gramene.org) is a comparative mapping resource for rice and other grains. Gramene allows researchers to compare the genetic and physical maps of the major monocot crops, namely, maize, barley, oats, sorghum, and wheat, to the emerging rice genomic sequence. This allows researchers to identify candidate genes in the rice genome that correspond to genetically mapped mutants and quantitative traits in the nonrice crop they are studying. Hence, the resource allows researchers studying traits in maize, barley, and so forth the benefit of genomic sequencing without waiting for the sequencing of these much larger genomes.

In addition to comparative maps, Gramene offers up-to-date genomic annotation of the rice genome, including both predicted and confirmed genes, and the current physical maps of rice and sorghum. We have mapped more than 1 million monocot expressed sequence tags (ESTs) to the rice genome, allowing gene predictions to be further refined based on cross-species comparisons.

A major milestone this year was the integration of genome mapping information on 11 wild rice species to the Gramene database. These species are adapted to a wide range of conditions, including semidesert and high-altitude sites. Our comparative maps provide a detailed view of how these genomes evolved and diversified from domestic life over a period of roughly 10 million years. By elucidating which regions were selected for during the divergence and adaptation of these species, we hope to provide information that will allow plant breeders to create new robust varieties of domestic rice.

We also used the information in the Gramene database to identify previously unknown microRNAs (miRNAs) in the rice, maize, and sorghum genomes. miRNAs are thought to be responsible for regulating

key events during development and maturation. Our work provides the first window into how these genes evolve and diversify in plants.

WORMBASE: A RESOURCE FOR *C. ELEGANS* GENOME AND BIOLOGY

Our lab continues to be a major developer and maintainer of the WormBase database (www.wormbase.org), an online information resource for the small free-living nematode, *Caenorhabditis elegans*. This organism is favored as a simple model animal because of its small genome size, experimental malleability, and well-understood cellular anatomy. WormBase is a curated model organism database developed as part of an international collaboration that includes the California Institute of Technology, Washington University at St. Louis, and the Sanger Centre. Our lab is responsible for the Web site, user interface, and software architecture for the project.

The resource, which is available to the public at www.wormbase.org, contains the complete *C. elegans* genome and key annotations, including predicted genes, alternative splicing patterns, oligonucleotide probes, and evolutionarily conserved segments. It also contains many other types of biological information, including the *C. elegans* cell pedigree, the organism's neuroanatomy, its genetic map, and the physical map from which the genomic sequence was derived.

During 2006, we added gene interaction and orthology data to WormBase. Gene interactions form a network of positive and negative regulatory reactions and are the basis for forming models of how cells regulate themselves and each other. Orthology data describe how gene families evolve and acquire new functionality. Both sets add considerable value to researchers seeking to understand the basis of life processes. We also added a new resource, called *WormBook* (www.wormbook.org), an open-access journal of *C. elegans* review articles. This resource, which already contains over 100 substantial chapters, is extensively cross-referenced with WormBase, allowing researchers to move seamlessly between highly structured database pages and human-friendly narrative text.

In 2006, we used the resources of WormBase to perform a genome-wide investigation of the functional relationship of genes that neighbor each other in the *C. elegans* genome. We found a high degree of coexpression among neighboring genes that are oriented in the same direction and among those that are transcribed in divergent directions, but no correlation of neighboring genes that are transcribed in convergent directions. This finding suggests that some neighboring genes share the same transcriptional regulatory elements and represents a novel mechanism of transcriptional coregulation.

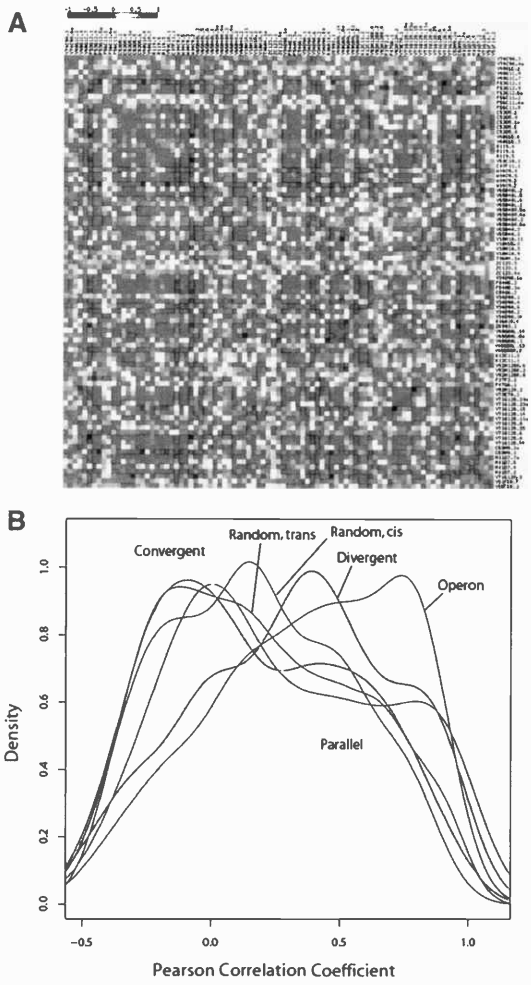

FIGURE 1 Correlation in gene expression among neighboring genes in *C. elegans*. (*A*) A heat map that shows the correlation in gene expression for genes within the region 1–2 Mb on chromosome I. Each small box represents a pairwise Pearson correlation coefficient value that indicates the level of gene expression. (*B*) Distribution of Pearson correlation coefficient values calculated based on tissue-specific SAGE tags. (Parallel) Parallel gene pairs with distance between closest coding exons <1000 bp; (Divergent) divergent neighboring gene pairs with distance between closest coding exons <1000 bp; (Convergent) convergent neighboring gene pairs between closest coding exons <1000 bp; (Operon) gene pairs within operons; (Random *cis*) random gene pairs within same chromosomes; (Random *trans*) random gene pairs in which two genes are from different chromosomes.

REACTOME

Reactome (www.reactome.org) is a collaboration with the European Bioinformatics Institute (EBI) and the Gene Ontology Consortium to develop a Web-accessible resource for curated information about biological processes.

Reactome is organized like a review journal. Bench biologists are invited to create modules that summarize a particular aspect of their field. Currently, summations include DNA replication, transcription, translation, intermediary metabolism, the cell cycle, RNA splicing, and hemostasis. Many more modules are under way. Modules are similar to minireviews, except that each paragraph of text is reduced to a series of logical assertions that is entered into a database of processes and macromolecules. The database is then used to drive a Web site that can be browsed like a textbook or searched with queries to discover pathways and connections.

During 2006, we brought the number of genes curated in Reactome to 1576 proteins, covering approximately 11% of the annotated portion of the human genome. We also added a new interface that allows users to interpret microarray data sets based on where affected genes are placed in the reaction map. A paper describing Reactome was recently accepted for publication in *Genome Biology*.

GENERIC MODEL ORGANISM DATABASE PROJECT

In collaboration with the model organism system databases FlyBase, SGD, and MGD, the Generic Model Organism Database (GMOD) project is developing a set of database schemas, applications, and interfaces suitable for creating a model organism system database. These tools significantly reduce the time and expense required to create new databases to curate genomic information coming out of various model organism system sequencing projects. 2006 saw the establishment of GMOD-based model databases for cow, honeybee, planaria, and tribolium beetle. In addition, GMOD tools were used for the analysis and publication of the largest survey of human copy-number polymorphisms to date (Redon et al., *Nature* 444: 444–454 [2006]).

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COMPUTATIONAL GENOMICS

M.Q. Zhang G. Chen, D. Das, N. Dimitrova, J. Granka, S. Kamalakaran, F. Li, J. Rosenfeld, E. Santo, D. Schones, A.D. Smith, P. Sumazin, Z. Xuan, C. Zhang, X. Zhao

In the last year, more members have left the lab and moved on to their new careers: S Kamalakaran became a staff scientist at Philip Research; G. Chen became a staff scientist at Invitrogen; and D. Schones has graduated and started a postdoc at the National Institutes of Health. Dr. F. Li, a visiting scientist, joined our DART project for 7 months, and Mr. Jeff Rosenfeld, a new New York University Ph.D. student, joined our lab.

Our main achievements last year were to (1) study large-scale structure of human brain DNA methylation patterns (Rollins et al. 2006) and develop two SVM-based prediction algorithms: one for methylation prediction in the general genomic region (Das, Dmitrova et al. 2006) [in collaboration with Columbia University groups]) and the other for methylation prediction of a CpG island (Fang et al. 2006) [in collaboration with Tsinghua University groups]); (2) demonstrate that when using the proximal promoter (~1 kb) alone, one can predict the tissue specificity of target gene expression (Smith et al. 2006). Conversely, using tissue-specific expression profiles, we are able to catalog major tissue-specific *cis*-regulatory binding-site motifs in each different tissue type (Smith et al. 2007 for heart, kidney, liver, pancreas, skeletal muscle, testis, and T cells; Martinez et al. 2006 for lung); (3) successfully develop the new algorithm *MARS_Motif_M* for mammalian promoter motif discovery using multivariate adaptive regression splines technology (Das, Nahle et al. 2006); and (4) map insulator sites in the human genome and derive the primary CTCF-binding motif for the first time (Kim et al. 2006 [in collaboration with University of California, San Diego and National Institutes of Health groups]).

Adaptively Inferring Human Transcriptional Subnetworks with MARSMotif-M

D. Das, M.Q. Zhang [in collaboration with Z. Nahle at Washington University]

Although the human genome has been sequenced, progress in understanding gene regulation in humans has been particularly slow. Many computational approaches developed for lower eukaryotes to identify cis-regulatory elements and their associated target genes often do not generalize to mammals, largely due to the degenerate and interactive nature of such elements. Motivated by the switch-like behavior of transcriptional responses, we present a systematic approach that allows adaptive determination of active transcriptional subnetworks (*cis*-motif combinations, the direct target genes, and physiological processes regulated by the corresponding transcription factors) from microarray data in mammals, with an accuracy similar to that achieved in lower eukaryotes. By extending our MARSMotif technology for mammalian transcription-factor-binding site (TFBS) motifs, our analysis uncovered several new subnetworks active in human liver and in cell cycle regulation, with functional characteristics similar to those of the known ones. We present biochemical evidence for our predictions and show that the recently discovered G_2/M-specific E2F pathway is wider than previously thought; in particular, E2F directly activates certain mitotic genes involved in hepatocellular carcinomas. Additionally, we demonstrate that this method can predict subnetworks in a condition-specific manner, as well as regulatory cross-talk across multiple tissues. Our approach allows systematic understanding of how phenotypic complexity is regulated at the transcription level in mammals and offers a marked advantage in systems where little or no prior knowledge of transcriptional regulation is available.

Understanding Tissue-specific Transcriptional Regulations in Mammals

A.D. Smith, P. Sumazin, Z. Xuan, M.Q. Zhang

For a better understanding of tissue-specific transcriptional regulation, we have further developed our classification-based *cis*-regulatory motif discovery algorithms (DME-b, and more tools, such as

STORM, in CREAD Open Source package). Using tissue-specific microarray expression data, we first showed that DNA motifs within human and mouse proximal (1-kb) promoter regions can predict tissue specificity of target gene expression with high confidence (Smith et al. 2006). Subsequently, we carried out a computational intensive characterization of specific TFBS motifs for many tissues (Smith et al. 2007). In particular, we reported on a systematic analysis of promoters controlling tissue-specific expression in heart, kidney, liver, pancreas, skeletal muscle, testis, and CD4 T cells, for both human and mouse. We integrated multiple sources of expression data to compile sets of transcripts with strong evidence for tissue-specific regulation. The analysis of the promoters corresponding to these sets produced a catalog (http://rulai.cshl.edu/tcat2/) of predicted tissue-specific motifs and modules, and *cis*-regulatory elements. Predicted regulatory interactions are supported by statistical evidence and provide a foundation for targeted experiments that will improve our understanding of tissue-specific regulatory networks. In a broader context, methods used to construct the catalog provide a model for the analysis of genomic regions that regulate differentially expressed genes.

A New Core Promoter Prediction Program *CoreBoost:* Boosting with Stumps for Predicting Transcriptional Start Sites

X. Zhao, Z. Xuan, M.Q. Zhang

Promoter prediction is a difficult but important problem in gene finding, and it is also critical for understanding the regulation of gene expression. Taking advantage of new sequences of full-length cDNAs and 5′-untranslated regions (5′UTRs), we developed a new core promoter prediction program called CoreBoost, which applies a boosting technique with stumps to select important small-scale features as well as large-scale features. CoreBoost greatly improves locating transcription start sites. We also demonstrate that by further utilizing some tissue-specific information, better accuracy can be achieved. This can also be used for correcting systematic bias in recent ChIP-chip mapping of human active core promoters/transcriptional start sites.

Analysis of the Vertebrate Insulator Protein CTCF-binding Sites in the Human Genome

A. Smith, M.Q. Zhang [in collaboration with the B. Ren Lab, University of California, San Diego, and the Lobanenkov Lab at NIH/NIAID]

Insulator elements affect gene expression by preventing the spread of heterochromatin and restricting transcriptional enhancers from activation of unrelated promoters. In vertebrates, insulator's function requires association with the CCCTC-binding factor (CTCF), a protein that recognizes long and diverse nucleotide sequences. Insulators are critical in gene regulation, but only a few have been reported. Here, we describe 13,804 CTCF-binding sites in potential insulators of the human genome, discovered experimentally in primary human fibroblasts. Most of these sequences are located far from the transcriptional start sites, with their distribution strongly correlated with genes. The majority of them fit to a consensus motif highly conserved and suitable for predicting possible insulators driven by CTCF in other vertebrate genomes. In addition, CTCF localization is largely invariant across different cell types. Our results provide resources for investigating insulator function and possible other general and evolutionarily conserved activities of CTCF sites.

A Clustering Property of Highly Degenerate TFBSs in the Mammalian Genome

C. Zhang, Z. Xuan, M.Q. Zhang [in collaboration with G. Mandel, SUNY Stony Brook]

Due to the relatively short length of TFBSs, it is important to understand how the specificity of protein-DNA interaction is achieved. We have performed a genome-wide analysis of nearby TFBS-like sequences for the transcriptional repressor, RE1 silencing transcription (REST) factor, as well as for several other representative mammalian transcription factors (c-*myc*, p53, HNF-1, and CREB). We find a nonrandom distribution of inexact sites for these transcription factors, referred to as highly degenerate TFBSs, that are enriched around the cognate binding sites. Comparisons among human, mouse, and rat orthologous promoters reveal that these highly degenerate sites are conserved significantly more than expected

by random chance, suggesting their positive selection during evolution. We propose that this arrangement provides a favorable genomic landscape for functional target site selection.

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COLD SPRING HARBOR LABORATORY FELLOWS

In 1986, Cold Spring Harbor Laboratory began a Fellows program to encourage independent research by outstanding young scientists who, during their graduate studies, displayed exceptional promise of becoming leading scientists of the future. The purpose of this program is to provide an opportunity for these young scientists to work independently at the Laboratory for a period of 3 years on projects of their own choosing. Fellows are provided with a salary, research support, and technical assistance so that they can accomplish their goals free of distraction. The interactions among research groups at the Laboratory and the program of courses and meetings on diverse topics in biology contribute to a research environment that is ideal for innovative science by these Fellows.

Previous Cold Spring Harbor Laboratory Fellows Adrian Krainer (1986), Scott Lowe (1995), and Marja Timmermans (1998) are currently members of the faculty at the Laboratory. After 9 years at the Laboratory, Carol Greider (1988) left to join Johns Hopkins University School of Medicine, where she is now the Daniel Nathans Professor and Director of Molecular Biology and Genetics. Eric Richards (1989) is now a Professor in the Department of Biology at Washington University in St. Louis. After finishing his fellowship, David Barford (1991) returned to the Laboratory of Molecular Biophysics at Oxford University and is now a Professor of Molecular Biology at the Institute of Cancer Research in London. Ueli Grossniklaus (1994) was a member of our faculty before leaving to become a Professor at the Institute of Plant Biology, Universität Zürich, Switzerland. Terence Strick (2000) left at the end of his Fellowship to become a Group Leader at the Institut Jacques Monod at the Centre National de la Recherche Scientifique and Université de Paris.

The Laboratory currently has three CSHL Fellows, Gilbert (Lee) Henry, who joined the Laboratory in 2000, and Patrick Paddison and Ira Hall, both of whom joined the Laboratory in 2004. Their reports are listed below. Lee joined us from Doug Melton's laboratory at Harvard University, where he earned his Ph.D. for studies on *Xenopus* development. Lee is studying the molecular biology of taste-receptor-cell differentiation. Patrick was a graduate student at the Watson School of Biological Sciences here at CSHL, where he worked in Greg Hannon's lab on the development of RNA interference (RNAi) libraries. Patrick has been using RNAi technology to establish the genetic requirements for embryonic stem (ES) cell self-renewal and differentiation. Ira was also a graduate student at the Watson School of Biological Sciences, where he worked in Shiv Grewal's lab on the relationship between RNA interference and chromosome dynamics. He is currently studying mammalian genomic instability and plasticity and characterizing the contribution of DNA copy-number polymorphisms to genetic variation.

I. Hall
L. Henry
P.J. Paddison

Investigation of DNA Copy-number Fluctuation Using Genomic Microarrays

I. Hall, C. Egan

We are interested in pursuing a genome-wide examination of genetic instability and plasticity and in characterizing the contribution of DNA copy-number differences to natural variation. The arrangement and copy number of chromosomal segments may vary between species, strains, and individuals, and spontaneous DNA rearrangements are recognized to be causal in the clonal evolution of cancers and in the etiology of certain human diseases. Ancestral sequence relationships within and between mammalian genomes indicate a major architectural role for duplication and deletion in shaping genomes over evolutionary time, but less is known about how these processes contribute to genetic variation across more rapid time scales within normal populations and organisms. We aim to use the laboratory mouse as a model system to investigate the fundamental properties of DNA copy-number fluctuation in mammals utilizing a form of comparative genome hybridization termed representational oligonucleotide microarray analysis (ROMA) (Lucito et al., *Genome Res. 10:* 1726 [2000]).

Internal relationships within sequenced genomes show that a significant portion (2–5%) of chromoso-

mal DNA in mammals is contained within segmental duplications (defined as stretches of DNA within a single genome >1 kb in length and >90% identical to one another), and duplicative events underlie the historical amplification and diversification of many important gene families. Sequence comparisons are essential to identify ancient copy-number change but are not well-suited to detect recent and ongoing genetic events because a complete genome sequence (1) is usually derived from a single individual, (2) relies upon assembly methods that are confounded by large identical repeats, and (3) is still impractical to attain for large genomes. Traditional molecular techniques are inevitably limited (and directed) to a small number of loci, and cytogenetic methods lack sufficient resolution to detect most changes. ROMA allows for the simultaneous detection of DNA copy-number variants (CNVs) and restriction-fragment-length polymorphisms (RFLPs) between any two related DNA samples through comparative hybridization of simplified genomic representations to high-density oligonucleotide microarrays. This technique assays a large portion of the genome in a relatively unbiased manner and requires only that a complete genome sequence exists for the organism in question. A number of recent studies have cataloged extensive copy-number differences between humans, indicating that CNVs may account for a substantial fraction of existing genetic variation.

The apparent evolutionary importance and contemporary prevalence of segmental copy-number variation raises fundamental questions: How often do new CNVs arise? Do CNVs arise through a random process? Does copy number generally reflect the ancestry of a locus? What are the prevalent mechanisms of duplication and deletion? Are different chromosomal regions or classes of DNA sequences more variable than others? Do distinct cells or cell types of an organism contain the same genetic material?

The laboratory mouse is an ideal model system for such investigations in that the human and mouse genomes appear to have been shaped by similar mutational forces, and the mouse offers the significant technical advantages of controlled crosses, experimental manipulation, and a known breeding history.

GENETIC DIVERSITY IN INBRED MICE

During the past century, a large number of phenotypically diverse inbred strains have been derived from a small number of founder mice through brother-sister mating. Because these founders were mixed descendants of Asian and European subspecies, each modern inbred line contains a unique, recombinant mixture of chromosomal segments of distinct genetic origin. These genetic differences are thought to underlie the many interesting physical and behavioral traits for which modern strains vary.

To gain an understanding of the current genetic composition of the laboratory mouse and to evaluate the utility of ROMA as a tool for mouse genetics, we have profiled the genomes of a number of commonly used inbred strains. Using a microarray containing about 83,000 probes, we have identified approximately 15,000 ROMA polymorphisms from 12 strains. More than one third of these markers can be observed in a single strain comparison, and further investigation with independent methods indicates that about one fifth of them are CNVs (the remainder are RFLPs and SNPs). These markers are distributed throughout the genome in a punctate, highly nonrandom fashion, and in collaboration with S. Sridhar (Carnegie Mellon), we have developed a segmentation algorithm to identify ancestrally divergent haplotypes and CNVs within our data (this model is based on previous work by L. Muthuswamy and M. Wigler). We have compared ROMA polymorphisms to a curated set of 3.8 million single-nucleotide polymorphisms (SNPs) and found that although the majority of ROMA differences appear to be ancient in origin and mirror the distribution of SNPs, significant genomic changes have occurred since the establishment of inbred lines in the early part of the 20th century.

GENOME STABILITY AND PLASTICITY

To assess the importance of segmental duplication and deletion in generating de novo genetic variation, we are examining genome stability through normal cycles of somatic development and germ-line transmission in the laboratory mouse.

A description of the genetic variation normally present in somatic cells is important for a few major reasons. Each cancer is thought to begin with a single cell that has diverged from its relatives, and the accumulation of mutations is thought to be a cause of aging. DNA rearrangement is also an established regulatory mechanism. Our own bodies generate a staggering diversity of immunological molecules precisely through such "mutagenic" processes, and more simple genetic systems (such as various yeasts and bacteria) utilize reversible DNA rearrangements to make heritable transcriptional decisions. It is not known how common such mechanisms are in mammals. In collabora-

tion with J. Hicks, the Wigler lab, and K. Eggan (Harvard University), we are pursuing a survey of somatic cell diversity in humans and mice. We hope that these studies result in an estimate of clonal variegation in mammals and help to identify unstable or hypervariable regions of the genome.

Mutation in the germ line is the basis for the introduction of new alleles to a species and is relevant to our understanding of evolution, population structure, and sporadic human disease. Previous estimates of the germ-line mutation rate have relied upon the observation of spontaneous loss of function at a small number of genes and do not adequately distinguish between classes of mutations. Rates of segmental duplication and deletion have been examined at just a few loci. Our analysis of genetic diversity described above utilized multiple individuals from each strain and thus represents a test of the purportedly homozygous and uniform nature of the inbred mouse genome. We found that inbred mice are indeed very inbred but are rarely identical. We did not observe residual heterozygosity of ancestral genetic variation, but we often encountered one or more spontaneous CNVs between closely related individuals of the same inbred strain. This indicated that CNVs might arise at a high rate and that it might be possible to study this process directly.

During the past year, we have examined the process of spontaneous DNA copy-number changes across the entire mouse genome. We collected pedigreed mice from many separate colonies of the C57BL/6 (B6) inbred strain, each bred independently at different institutions around the world, and constructed a genealogy spanning approximately 966 generations of inbreeding. We then used ROMA to identify 38 de novo CNVs among B6 colonies. Remarkably, based on the most parsimonious explanation for the distribution of CNV genotypes relative to the structure of the genealogy, almost half of the de novo CNVs arose multiple times within distinct lineages. Of 38 CNVs, 8 arose once within B6 colonies but were also variable among other classical inbred mouse strains, and 10 CNVs arose multiple times within different B6 colonies. Thus, significant changes in DNA copy number occur over relatively short time scales, and new segmental variation is often the product of recurrent mutation. The recurrent CNVs that we identified range in size from 4 kb to 4 mb, affect 43 known genes, and fluctuate in copy number at rates of about 1×10^{-2} to 1×10^{-3}/locus/generation. A preliminary analysis of CNV breakpoints indicates that they recur through a relatively precise mechanism. Given that we have significantly underestimated the prevalence of recurrent CNVs and that many of those we identified contain genes for which hypervariability might be beneficial to a population (i.e., factors involved in immunity, reproduction, and cognition), we believe that recurrent structural mutation will prove to be a widespread and phenotypically relevant process for creating genetic variation in mammals.

Structural and Functional Studies of the Vertebrate Taste Bud

L. Henry, M. Siddiqui

We are interested in the molecular biology of taste receptor cell differentiation. The mammalian taste bud is a dynamic structure consisting of mitotically active progenitor cells at its periphery and differentiated taste receptor cells within its core. The basic goal of our research is to understand how the daughters of the progenitors select a particular fate. As different receptor cells are known to respond to different classes of tastants, we hope that understanding this differentiation process will help to illuminate details of both taste bud structure and function. Toward this end, we have undertaken an exhaustive effort to define at the level of transcription the cellular diversity of the murine taste bud.

To do this, single-cell profiling is being used to analyze the transcriptomes of taste receptor and progenitor cells. We are currently developing a novel procedure to produce single-cell profiles that relies on the attachment of single-cell levels (picogram) of mRNA to magnetic beads. The immobolization of picogram amounts of nucleic acid allows manipulations, free from complicating purification procedures, to be preformed that would usually require microgram amounts of material. Various aspects of this procedure have been optimized over the last year, including the identification of a novel first-strand cDNA-priming scheme that solves a key background problem associated with the procedure and the design and synthesis of a novel cleavable linker that provides both flexibility for the priming oligonucleotide and a means to detach cDNA from the magnetic bead after a series of modification steps.

Finally, we have established a transgenic animal model that allows expression of green fluorescent protein (GFP) in cells of both the taste bud and the surrounding epithelium. Using this animal model, we are attempting to identify progenitor cells for the taste bud based on cell cycle criteria for future profiling experiments.

LABEL RETAINING CELLS IN THE MURINE TASTE BUD

In collaboration with Kristen Brendan and Doug Melton at Harvard University, we have used a transgenic label retention system to identify a population of slow-cycling cells in the lingual epithelium surrounding the anterior fungiform taste bud. The transgenic model uses a Tet-repressor system to drive expression of a GFP-histone H2B fusion protein. At anytime during prenatal or postnatal development, the transgene is turned off by withdrawal of the inducing drug from the animals drinking water. Defined chase periods of no expression then allow one to track the half-lives of GFP-labeled cells. In the case of our experiments, the ubiquitous expressed ROSA26 promoter is used to drive expression of the transgene from embryonic day zero (E0) to postnatal day 42 (P42).

Analysis of the tongues of mice chased with no expression for between 1 and 6 months indicates a number of interesting points. First, it is widely cited in the literature that the average lifetime of a cell in the taste bud is 7–10 days. We however, detect label-retaining cells in the bud for up to 2 months. The identity of these long-lived cells is currently under investigation. Of more interest is the identification of a group of label-retaining cells located around the apical margin of the fungiform taste bud. These cells retain label for as long as 3 months, and they form a continuous border around the bud with more basally located Ki67-positive mitotically active cells. Both the Ki67 and label-retaining cells express Sox2, a molecular marker known to be expressed in other types of stem/progenitor cells. Moreover, in both the skin and hematopoietic systems, the same transgenic animal shows label retention in cells known to exhibit a stem/progenitor function. In the future, we will attempt to isolate these cells and determine the exact nature of their transcriptomes via the single-cell amplification techniques that we have developed.

Overview

P. Paddison

Among the emerging themes of cancer research during the last few decades is the notion that cancer cells hijack molecular pathways involved not only in promoting cell proliferation and survival, but also in developmental decisions such as cell-fate determination. The recent discovery of cancer stem cells, which seem to have vital roles in tumor progression, maintenance, and recurrence, further underscores this notion, suggesting that cancers arise from maligned developmental programs. Because many of these co-opted developmental pathways (e.g., Jak/Stat, epidermal growth factor [EGF], fibroblast growth factor [FGF], Smad, etc.) are also important for the self-renewal of embryonic and adult stem cells, it is vital that we understand how dependencies on these pathways for growth, survival, and potency arise during development and if such functional dependencies are restructured in cancer cells. Thus, understanding how molecular pathways promote or constrain "stemness" may provide critical insight into cancer biology as well as inroads for novel therapeutic strategies.

General Research Goals

1. Identify genes required for self-renewal and lineage specification in embryonic and adult stem cells in humans and rodents using in vitro functional genetic approaches.
2. Determine if these genes function in stem-cell-driven cancers.
3. Apply RNAi (RNA interference) technology for the creation of desirable cell types for cell replacement therapies.
4. Continue to develop RNAi screening technologies.

Currently I have begun to apply RNAi in mouse embryonic stem cells to functionally dissect self-renewal and lineage specification using focused gene sets. We are now expanding our screens for genome-wide coverage and incorporating human embryonic stem (ES) cells and neural stem cells.

Current Research

MODIFIERS OF NANOG EXPRESSION IN ESCS

ESCs are cell lines derived from the inner cell mass (ICM) of blastocyst-stage mammalian embryos (Evans and Kaufman, *Nature 293:* 154 [1981]; Martin, *Proc. Natl. Acad. Sci. 78:* 7634 [1981]; Thomson et al., *Science 282:* 1145 [1998]). They can grow indefinitely in culture and give rise to cells of all three embryonic germ layers as well as germ cells (Keller, *Genes Dev. 19:* 1129 [2005]). For this reason, ESCs hold great promise for regenerative medicine, where human ESC derivatives might be used in cell replacement therapies.

Nanog is an atypical homeobox domain transcription factor that is one of several known key regulators that are required to maintain pluripotency of ESCs (Chambers et al., *Cell 113:* 643 [2003]; Mitsui et al., *Cell 113:* 631 [2003]). Current models posit that

FIGURE 1 RNAi screen for modifiers of Nanog expression in mouse ES cells

Nanog, along with factors such as Oct4 and Sox2, functions in cross-regulatory networks that collectively repress differentiation-inducing genes and activate those that are required for self-renewal. To find regulators of self-renewal and lineage specification, we carried out a loss-of-function genetic screen designed to find modifiers of Nanog expression during the differentiation of mouse ESCs (Fig. 1). We used an RNAi approach in a Nanog-GFP (green fluorescent protein) ESC line, targeting genes involved in chromatin modification, signal transduction, and transcription.

We identified 35 that act to repress Nanog expression during retinoic acid (RA)-induced differentiation (Figs. 2 and 3). These gene products represent at least five distinct pathways or complexes. In addition, we identied 47 gene products that act to down-regulate Nanog expression levels. We functionally characterized screen hits using transcript microarrays, combinatorial short hairpin RNA (shRNA) experiments, and other differentiation assays (trophectoderm differentiation). Among the novel finding from this screen were the following:

1. Swi/Snf complex members repress Nanog and Oct4 expression during ES cell differentiation.
2. Elac2, a novel prostate cancer susceptibility gene, represses Nanog expression through negative regulation of Rif1, a novel telomere-binding protein involved in self-renewal.
3. Other Nanog repressors including Hira, Ikbkb, Erk1, Tcf3, and Socs3, counteract LIF-Stat3 function during differentiation, either directly or indirectly.
4. Myst1/Mof, Myst2/Hbo1, Oct4, and Stat3 converge to regulate genes required for pluripotency and self-renewal.
5. Oct4 involvement in self-renewal is reversibly dependent on the presence of serum in ESC culture medium.

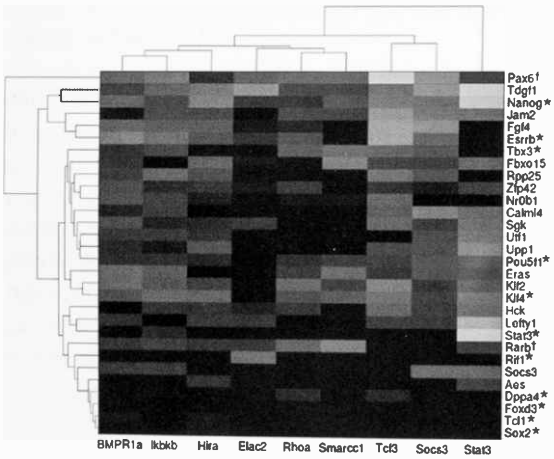

FIGURE 2 Clustering of microarray expression profiles of mouse ES cells transfected with shRNAs and treated with 2 μM RA (48 hrs). Each column represents knockdown of indicated gene (*bottom*), and rows represent ES cell marker genes (*right*). Relative expression levels are shown from low to high. (*Asterisks*) Genes known to be required for self-renewal ($n = 3$; t-test, $p < .05$).

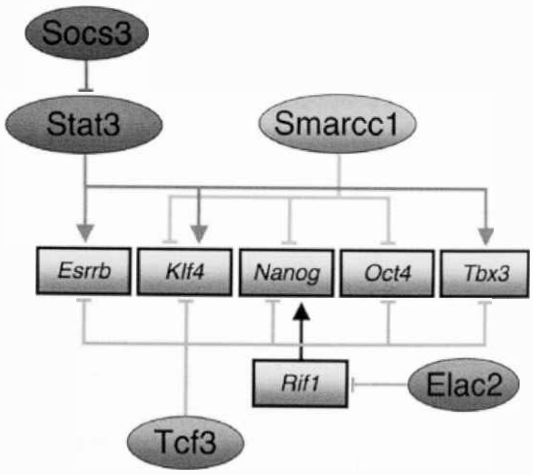

FIGURE 3 Model for how various RNAi suppressors function with respect to self-renewal gene expression during RA-induced differentiation. *Lines* and *arrows* indicate phenotypic outcomes.

While we are gearing up for genome-wide screens, we are pursuing some of the underlying biology produced by this initial screen, including Swi/Snf-dependent chromatin remodeling of self-renewal gene promoters and the identity of specificity factors required for Myst1 and Myst2 function. Additionally, we are exploring in vivo phenotypes using Dox-inducible shRNA expression cassettes.

Future Work

PATHWAY ADDICTION IN STEM CELLS

The idea of "oncogene addiction," first put forward by Bernard Weinstein, applies to scenarios where an oncogene drives a cell toward malignancy through bypassing molecular constraints imposed on cells during development for growth, cell division, and/or survival. Thereby, malignant cells become "addicted" to an oncogene's activity such that they become more vulnerable to its loss than normal cells. During development, similar scenarios arguably arise in stem cells. Instead of a rogue gene activity, however, we find natural "addiction" developing to signaling pathways that promote stem cell proliferation, survival, and "potency." Similar to oncogene addiction, when these pathway activities are withdrawn, cells either differentiate or die.

Some examples include LIF-Stat3 and BMP-Smad signaling in mouse ESCs (Chambers, *Cell 113:* 643 [2003]; Ying et al., *Cell 115:* 281 [2003]); EGF and FGF dependency for neural stem/progenitor cells (Conti et al., *PLoS Biol. 3:* e283 [2005]); FGF4-ERK signaling in trophoblast stem cells (Yang et al. 2006); c-Kit and gp130 signaling in hematopoietic stem cells (Sui et al., *Proc. Natl. Acad. Sci. 92:* 2859 [1995]); and the FBS-dependent self-renewal of mesenchymal stem cells (Shahdadfar et al., *Stem Cells 23:* 1357 [2005]). In each case, these dependencies are required for the in vitro derivation and expansion of these cells. One goal of my research is to build molecular genetic frameworks to determine how different pathway dependencies arise in stem cell systems.

Initially, I will focus on RNAi-based screens in mouse ESCs and neural stem cells (NSCs) to identify genes required for (1) differentiation and lineage specification upon withdrawal of a self-renewal factor or introduction of a differentiation inducer and (2) self-renewal and potency under normal growth conditions.

CANCER STEM CELLS

Stem cell tumor models posit that tumors are hierarchically organized, such that only a subset of cells, i.e., the "stem" or initiating cells, are capable of initiating tumor development. To date, there is evidence for the existence of subpopulations of tumor initiating cells in several cancers, including: colon (O'Brien et al. 2007; Ricci-Vitiani et al. 2007), prostate (Patrwala et al. 2006), breast (Al-Hajj et al., *Proc. Natl. Acad. Sci. 100:* 3983 [2003]), brain (glioblastoma and medulloblastoma) (Singh et al., *Cancer Res. 63:* 5821 [2003]; Hemmati et al., *Proc. Natl. Acad. Sci. 100:* 15178 [2003]), and certain leukemias (Lapidot et al., *Nature 367:* 645 [1994]). To be considered a stem cell per se, however, cells must display the ability to self-renew and the potential for full or partial differentiation. To my knowledge, this criterion has been met for glioblastoma, medulloblastoma, and leukemias.

For experimentation with cancer stem cells (CSCs), I will take advantage of a mouse model for glioblastoma using genetically defined, tumorigenic mouse NSCs. Ron DePinho's group published that Ink4a/Arf$^{-/-}$ NSCs expressing the activated EGFR gene (EGFR*) induce tumors resembling high-grade gliomas (Bachoo et al., *Cancer Cell. 1:* 269 [2002]). In human glioblastomas, Ink4a/Arf loss of function and activation of EGFR are "signature" lesions (Vogelstein and Kinzler, *Nat. Med. 10:* 789 [2004]; Wiltshire et al., *Neuro-oncol. 2:* 164 [2000]). Thus, these cells may well recapitulate many of the important features of glioma cells.

Through performing parallel screens in tumorigenic and wild-type NSCs I hope to identify self-renewal and differentiation genes that are required by both wild-type and tumorigenic NSCs and specifically required by one or the other but not both. Among these sets of genes, we should find genetic lesions that induce wild-type NSCs to become tumorigenic in mice and genetic lesions that are specifically lethal to tumorigenic NSCs.

SELF-RENEWAL, CELL DIVISION, AND GROWTH IN ESCS, NSCS, AND CSCS

Mouse ESCs have unusual proliferative properties, which may be related to their ability to maintain the undifferentiated state. They devote more than half of their cell cycle to S phase and have short G_1 and G_2 phases (Savatier et al., *Oncogene 9:* 809 [1994]; Stead et al., *Oncogene 21:* 8320 [2002]), resulting in short

doubling times (~12 hours) compared to somatic cells. They also constitutively express many cell cycle factors, including cyclin A2, cyclin B1, cyclin E1, Cdc6, and geminin (Fujii-Yamamoto et al., *J. Biol. Chem. 280:* 12976 [2005]), and Cdk activities, which normally oscillate, remain robust throughout the cell cycle with the exception of cyclin B/Cdc2 (Savatier et al., *Oncogene 12:* 309 [1996]; Stead et al., *Oncogene 21:* 8320 [2002]). Moreover, unlike somatic cells, cyclin D1 is expressed independently of mitogenic signals (Burdon et al., *Trends Cell. Biol. 12:* 432 [2002]), and cyclin E/Cdk2 activity appears to be leukemia inhibitory factor (LIF)-dependent (Savatier et al., *Oncogene 12:* 309 [1996]). How factors responsible for pluripotency also promote ESC proliferation remains an open question.

Similarly, with NSCs, there are few links known between potency and cell cycle regulation. NSC differentiation is associated with the lengthening of G_1 phase (Lukaszewicz et al., *J. Neurosci. 22:* 6610 [2002]) and knocking out retinoblatoma (Rb) family genes, which inhibit G_1/S progression, in NSCs results in an enhanced capacity for self-renewal ($p107^{-/-}$) (Vanderluit et al., *J. Cell Biol. 166:* 853 [2004]) or significant delay in terminal differentiation ($Rb^{-/-}$) (Callaghan et al., *Dev. Biol. 15:* 257 [1999]). NSCs require EGF and basic FGF for proliferation, survival, and potency and have a doubling time of 24–30 hours, similar to other somatic cell types (Lukaszewicz et al., *J. Neurosci. 22:* 6610 [2002]).

Our initial RNAi studies in mouse ESCs have revealed potential linkages between genes required for cell division, growth, and genome integrity and those promoting the expression of pluripotency genes (e.g., Stat3). Moreover, we found that nonmitogen signaling pathways, e.g., β-catenin and Smad4, activate genes rate-limiting for cell cycle progression, including ribonucleotide reductase and components of the anaphase-promoting complex. We also observed that Stat3 uniquely maintains expression of cyclin E1, whereas Oct4 is uniquely required for cyclin D1 expression, providing possible explanations for LIF-dependent cyclin E/Cdk2 activity and mitogen-independent cyclin D1 expression in ESCs.

By the nature of our in vitro screens in stem cells (especially where outgrowth is the primary phenotype), we may uncover some of the fundamental links between stem cell cycle dynamics and the regulation of developmental programs.

RNAi SCREENS IN HUMAN ESCS

One clinical goal of the stem cell research community is the creation of desirable cell types that could one day be used in cell replacement therapies. The manipulation of human ESCs (hESCs) may represent the best approach to realize this goal. However, despite the "hype" surrounding them, hESCs are difficult to culture and cannot be grown at clonal cell densities. Moreover, there are therapeutic concerns with the coculturing hESCs with feeder cells. Thereby, instead of initially attempting "biological" screens in hESCs, I have decided first to perform a screen for preventing their feeder-free, clonal expansion in vitro. This work is being carried out in collaboration with Jamie Thomson's lab at University of Wisconsin (Madison). The hope is that once the pathways are identified that are rate-limiting for hESC expansion in culture, their activities can be manipulated by modifying culture conditions, generating knockout lines, or stably expressing shRNAs. Once we have the growth parameters down for hESCs, we will move on to more sophisticated biology screens. Part of the goal of my collaboration with Jamie's lab is for members of my lab to gain training and experience for future work in this system.

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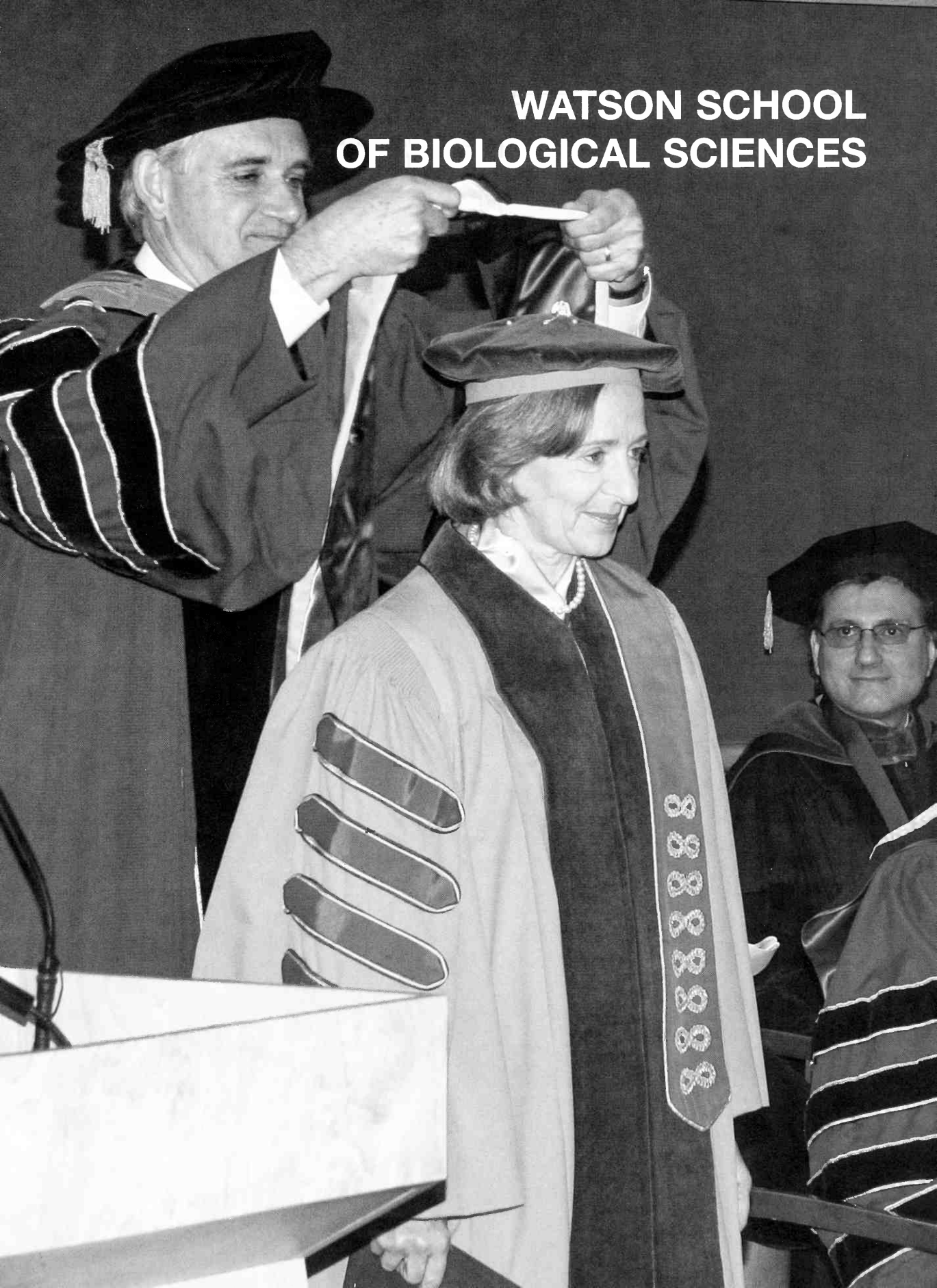
AUTHOR INDEX



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Alyson Kass-Eisler, Ph.D., *Postdoctoral Program Officer and Curriculum Administrator*
Dawn Meehan, B.A., *Admissions, Recruitment, and Student Affairs Manager*
Eva Radeck, *Administrative Assistant and Assistant to the Dean*

EXECUTIVE COMMITTEE

Chair
Lilian Clark

Members
Hollis Cline (January–November)
Terri Grodzicker
W. Richard McCombie
Scott Lowe
Lincoln Stein
William Tansey (Vice Chair)
Nicholas Tonks

Student Representatives
Hiroki Asari, Watson School (January–March)
David Simpson, Watson School (from April)
Despina Siolas, Stony Brook University

Secretary
Alyson Kass-Eisler

ADMISSIONS COMMITTEE

Chair
Gregory Hannon

Members
Josh Dubnau
Leemor Joshua-Tor
Adrian R. Krainer
Robert Lucito
David L. Spector
Nicholas Tonks
Linda Van Aelst

Secretary
Dawn Meehan

Ex Officio
William Tansey

QUALIFYING EXAM COMMITTEE

Chair
Linda Van Aelst

Members:
W. Richard McCombie
Anthony Zador

CURRICULUM DEVELOPMENT AND INTEGRATION COMMITTEE

Chair
William Tansey

Members
Lilian Clark
Z. Josh Huang
David L. Spector
Nicholas Tonks

EXTERNAL ADVISORY COMMITTEE

Keith Yamamoto (Chair)
Executive Vice Dean, School of Medicine
University of California, San Francisco

Victor Corces
Professor, Department of Biology, The Johns Hopkins University

Gail Mandel
Professor, Department of Neurobiology and Behavior, Stony Brook University (to November); Senior Scientist, Vollume Institute, Oregon Health and Science University (from November)
Investigator, Howard Hughes Medical Institute

Marguerite Mangin
Academic Programs Director, Office of the President
Senior Research Associate, The Rockefeller University

Barbara Meyer
Professor of Genetics and Development, University of California, Berkeley
Investigator, Howard Hughes Medical Institute

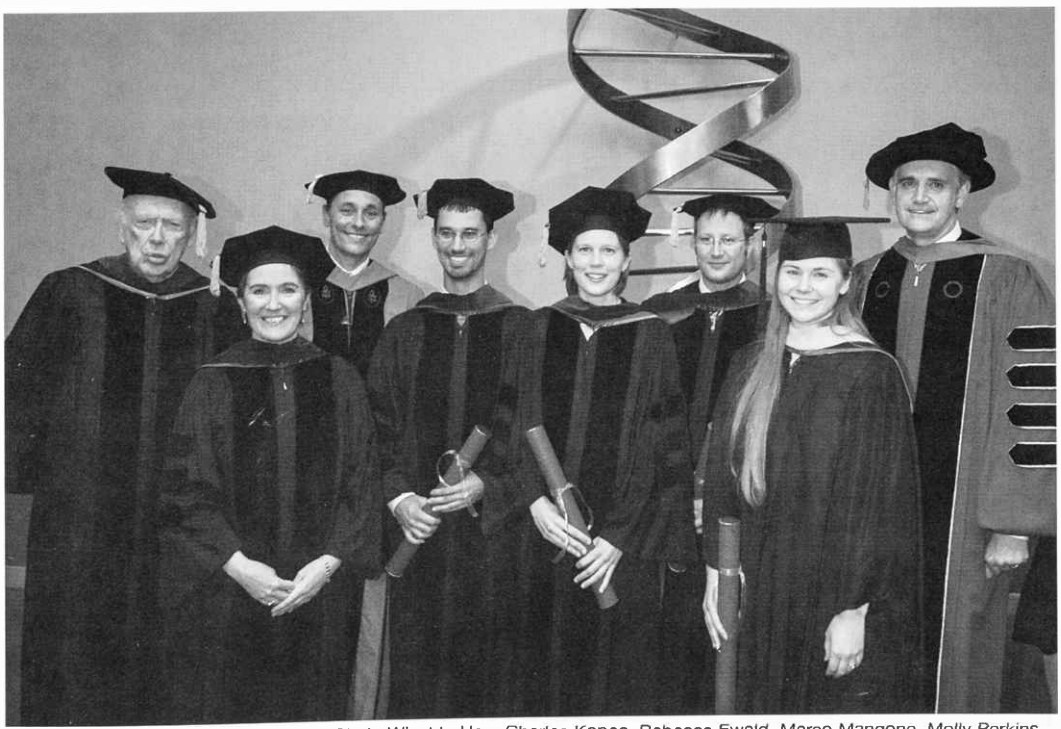
Frank Solomon
Professor, Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology

WATSON SCHOOL OF BIOLOGICAL SCIENCES DEAN'S REPORT

Yet another year has flown by here at the Watson School—it is hard to believe that we are now in our eighth year! "Team Watson" (Alyson Kass-Eisler, Dawn Meehan, Eva Radeck, and Bill Tansey) continue to ensure that we deliver world-class programs and that we are all taken care of—you are the best!

The Third Watson School Graduation

April 30 saw the Watson School's third graduation ceremony. The graduating class of 2006 comprised Santanu Chakraborty, Rebecca Ewald, Charles Kopec, and Marco Mangone, who were awarded the Ph.D. degree, and Molly Perkins and Izabela Sujka, who were awarded Masters degrees. Both Molly and Izabela left the program early—Molly relocated to Oxford, England, and Izabela accepted a position at OSI Pharmaceuticals as a research assistant in Cancer biology. Both had successfully completed the first year curriculum and as such received Masters degrees. The graduation was once again a wonderful occasion where honorary degrees were bestowed on Drs. Susan Hockfield, Tom Maniatis, and Matt Ridley. Dr. Ridley gave the Commencement address. We were further honored that the parents and family of Rebecca Ewald and Marco Mangone traveled from Germany and Italy, respectively, to attend the graduation ceremony.



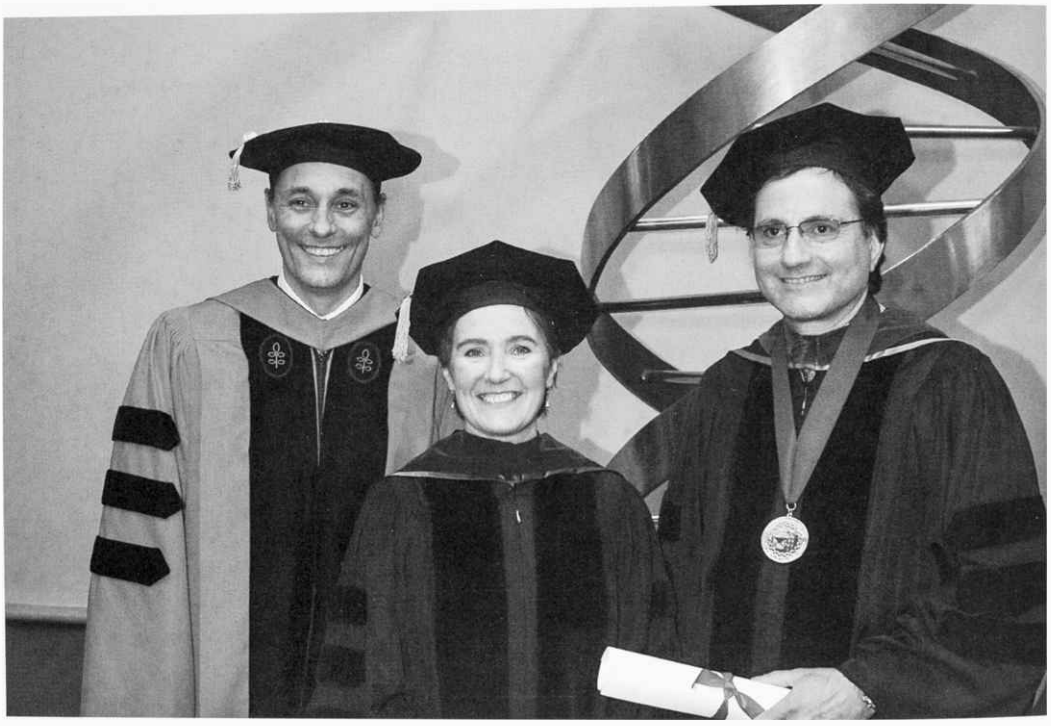
(*Left to right*) James D. Watson, Lillian Clark, Winship Herr, Charles Kopec, Rebecca Ewald, Marco Mangone, Molly Perkins, Bruce Stillman

Student	Thesis advisor	Current Postdoctoral Position
Santanu Chakraborty	Carlos D. Brody	With Dr. Michael Wigler, CSHL
Rebecca Ewald	Hollis Cline	With Dr. Hollis Cline, CSHL
Charles Kopec	Roberto Malinow	With Dr. Carlos Brody, Princeton University
Marco Mangone	Winship Herr	With Dr. Fabio Piano, New York University

Our First Teaching Award

At this year's graduation ceremony, it was my honor to present the first annual Winship Herr Faculty Teaching Award to Dr. David Spector, lead instructor of the Specialized Disciplines course on *Cellular Structure and Function*. This award, named in honor of our founding dean, recognizes the excellence and creativity in teaching Watson School courses. The students chose the recipient of this award and the following is some of what was said about the recipient:

> *Though being a Principal Investigator is demanding of time and energy, the lead instructor of the Cellular Structure and Function Course showed a level of dedication, preparation, and enthusiasm for the subject and course itself that was most infectious. This level of enthusiasm was also seen in the guest instructors, due to their respect for David Spector. The discussions fostered by this course often continued outside the classroom, usually around the kitchen table at the Knight house. The quality of our final presentations was a testament to the outstanding leadership and instruction of David "Speckles" Spector.*



(*Left to right*) Winship Herr, Lilian Clark, David Spector

THESIS DISSERTATION DEFENSES

ENTERING CLASS OF 2000

Rebecca C. Ewald, April 7, 2006

NMDA receptor subtypes and dendritic arbor morphology.

Thesis Examining Committee

Chair:	**Anthony Zador**
Research Mentor:	**Hollis Cline**
Academic Mentor:	**Bruce Stillman**
Committee Member:	**Linda Van Aelst**
Committee Member:	**Roberto Malinow**
External Examiner:	**Kimberly McAllister,** *University of California, Davis*

Marco Mangone, March 30, 2006

Analysis of the HCF-1 basic region and its role in sustaining cell proliferation.

Thesis Examining Committee

Chair:	**Michael P. Myers**
Research Mentor:	**Winship Herr**
Academic Mentor:	**Linda Van Aelst**
Committee Member:	**David L. Spector**
Committee Member:	**Lincoln Stein**
External Examiner:	**Angus Wilson,** *New York University*

ENTERING CLASS OF 2001

Charles Kopec, April 3, 2006

AMPA receptor trafficking and their effect on spine enlargement during long-term potentiation.

Thesis Examining Committee

Chair:	**Linda Van Aelst**
Research Mentor:	**Roberto Malinow**
Academic Mentor:	**Anthony Zador**
Committee Member:	**Hollis Cline**
Committee Member:	**David L. Spector**
External Examiner:	**John Lisman,** *Brandeis University*

Catherine Y. Cormier, November 13, 2006

Alternative apoptotic pathways in cancer cells.

Thesis Examining Committee

Chair:	**Adrian Krainer**
Research Mentor:	**Yuri Lazebnik**
Academic Mentor:	**David Stewart**
Committee Member:	**Gregory Hannon**
Committee Member:	**William Tansey**
External Examiner:	**Marcus E. Peter,** *University of Chicago*

Alumni Spotlight

Ji-Joon Song, Ph.D., received a B.S. from Seoul National University and an M.Sc. from Kwangju Institute of Science and Technology. Dr. Song is conducting postdoctoral research with Dr. Robert E. Kingston on chromatin modification at Massachusetts General Hospital, Harvard University, in Boston, Massachusetts. Dr. Song is the recipient of the Jane Coffin Childs Postdoctoral Fellowship.

I remember when I came to Cold Spring Harbor Laboratory for my interview. I immediately felt the unique and amazing scientific atmosphere, which I did not appreciate before, beyond its reputation. I was impressed by how much I would be cared about as an individual by being a student at the Watson School, as opposed to a larger institute. I became an explorer of science at the Watson School, with endless support and encouragement from the faculty and colleagues. I also had the opportunity to participate in many of the CSHL meetings and courses and to interact with many scientists from all over the world simply by walking along Bungtown Road. My enthusiasm for science lingers on, as do my memories of the Watson School at Cold Spring Harbor Laboratory.

Ji-Joon Song

Alumni Highlights

We are very proud of the continuing success of our alumni. Three of our graduates were recognized at the international level in 2006. Ira Hall, from the graduating class of 2004, received a Burroughs Wellcome Fund Career Award in the Biomedical Sciences. Elena Ezhkova, from the graduating class of 2005, is the recipient of a Life Sciences Research Foundation Postdoctoral Fellowship and is their New York Stem Cell Foundation Fellow. Masafumi Muratani, also from the graduating class of 2005, was awarded the Human Frontier of Science Program Long-term Fellowship.

The Fall Term Curriculum

Our faculty continues to do an outstanding job developing and delivering the curriculum. As ever, the curriculum development and integration committee (CDIC), headed by William Tansey, continues to carefully monitor and develop the curriculum. The CDIC comprises William Tansey (chair), Lilian Clark, Z. Josh Huang, David Spector, and Nicholas Tonks. For Fall 2006, the Scientific Reasoning and Logic (SRL) core course has undergone a major re-vamp. Gregory Hannon has taken over again as lead instructor and has recruited several other senior faculty as instructors. The instructors for Fall 2006 are Gregory Hannon, Leemor Joshua-Tor, David Jackson, Scott Lowe, Robert Martienssen, and Anthony Zador.

For 2006, the contents of Scientific Exposition and Ethics (SEE) core course remained essentially the same, but instructors William Tansey and Yuri Lazebnik stepped down. They were replaced by Lilian Clark and Alea Mills with Jan Witkowski as lead instructor. This course continues to attract outstanding guest instructors who this year included the following.

Bruce Budowle	Senior Scientist, FBI
Robert Charrow	Greenberg Traurig, LLP
Mariette Di Christina	Executive Editor, *Scientific American*
Angela Eggleston	Senior Editor, *Nature*
Kay Fields	Office of Research Integrity, HHS
Kathy Hudson	Director, Genetics and Public Policy Center

Two of the three Specialized Disciplines courses, *Genetics* and *Cellular Structure and Function*, have largely remained the same. The third course, *Systems Neuroscience*, has been completely revamped. Zachary Mainen has taken over from Carlos Brody as lead instructor, but Carlos has stayed on as his coinstructor.

Recruiting Efforts

Recruitment of the graduate program's entering class of 2007 and of participants for our summer 2007 Undergraduate Research Program (URP) was once again managed by Ms. Dawn Meehan, the School's admissions, recruitment, and student affairs manager. Throughout 2006, Dawn traveled the length and breadth of the Country to recruit students to our graduate and undergraduate programs. She has done an outstanding job at representing Cold Spring Harbor Laboratory and the Watson School and I am deeply indebted to her efforts. The Recruitment table on page 200 details the different recruitment fairs and conferences the School has participated in, together with the names of faculty, students, and administrators who represented the School on these occasions.

Academic Mentoring

The Watson School takes great pride in the level of mentoring that it offers to its students. One of the very special aspects in this regard is its academic mentoring program, led by William Tansey. In this program, entering students select by mutual agreement a member of the research or nonresearch faculty to serve as an academic mentor—a watchful guardian to look over and encourage the student through the sometimes-trying process of a doctoral education. This program continues to receive much support from the faculty who volunteer to be academic mentors, and it has rightfully become a vital ingredient in the Watson School's success. This year's new academic mentors for the entering class of 2006 are John Inglis (Claire Biot and Yaniv Erlich), Nicholas Tonks (Eyal Gruntman), Jan A. Witkowski (Adrienne Jones), David Stewart (Colin Malone), William Tansey (Amy Rappaport), and Gregory Hannon (Claudio Scuoppo).

Entering Class of 2006

On August 28, 2006, the Watson School opened its doors for the eighth time to welcome yet another new class. Seven students—Claire Biot, Yaniv Erlich, Eyal Gruntman, Adrienne Jones, Colin Malone, Amy Rappaport, and Claudio Scuoppo—comprise the Entering Class of 2006. Yaniv Erlich was a par-

ticipant in our summer Undergraduate Research Program in 2005, and Claire Biot came to us after having spent two months in Gregory Hannon's lab in the late summer of 2005.

The School is also deeply indebted to its graduate program's Admissions Committee, who review, interview, and select candidates for our doctoral program. The Admissions Committee for the 2006 entering class comprised Gregory Hannon (chair), Josh Dubnau, Leemor Joshua-Tor, Adrian R. Krainer, Robert Lucito, David L. Spector, Nicholas Tonks, Linda Van Aelst, Anthony Zador, and William Tansey (*ex officio*). They are a truly remarkable team!

ENTERING CLASS OF 2006

Claire Biot, École Polytechnique, France
George A. and Marjorie H. Anderson Fellow

Yaniv Erlich, Tel Aviv University
Goldberg-Lindsay Fellow

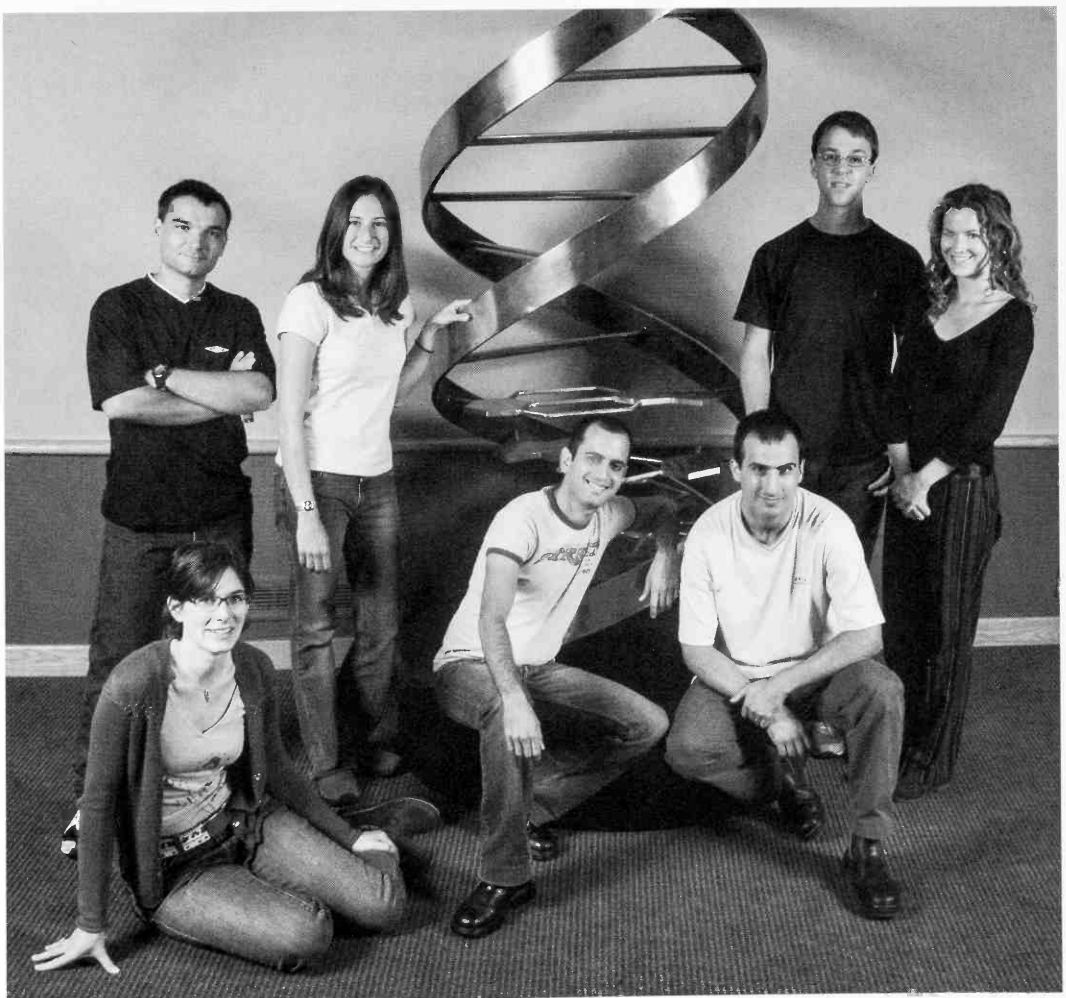
Eyal Gruntman, Tel Aviv University
Elizabeth Sloan Livingston Fellow

Adrienne Jones, University of Toronto
George A. and Marjorie H. Anderson Fellow

Colin Malone, Washington University, St. Louis
Beckman Graduate Student

Amy Rappaport, Cornell University
Barbara McClintock Fellow

Claudio Scuoppo, University of Turin
Engelhorn Scholar



(*Standing, left to right*) Claudio Scuoppo, Amy Rappaport, Colin Malone, Adrienne Jones. (*Sitting, left to right*) Claire Biot, Yaniv Erlich, Eyal Gruntman

2006 WATSON SCHOOL OF BIOLOGICAL SCIENCES RECRUITMENT SCHEDULE

Event	Location	Date	WSBS Attendees/Titles
Ronald E. McNair Scholars Conference	University of Maryland, College Park	March 16–18	Dawn Meehan, Admissions, Recruitment, and Student Affairs Manager
Roger Williams University 10th Annual Career and Graduate School Fair	Roger Williams University	April 5	Dawn Meehan
Stony Brook University Summer Undergraduate Research Program Information Session	Cold Spring Harbor Laboratory	July 10	Dr. Lilian Clark, Dean, Dawn Meehan, Dr. William Tansey, Director of Graduate Studies
Ronald E. McNair Scholars Conference	State University of New York, Buffalo	July 13–16	Dawn Meehan, Dr. William Tansey
Princeton University Summer Undergraduate Research Program Career Symposium	Princeton University	July 20	Katherine McJunkin, Graduate Student
The Johns Hopkins University Information Session	The Johns Hopkins University	September 19	Dawn Meehan, Dr. William Tansey
University of Maryland, Baltimore County, Meyerhoff Scholarship Program Visit	University of Maryland, Baltimore County	September 20	Dawn Meehan, Dr. William Tansey
Massachusetts Institute of Technology Career Fair	Massachusetts Institute of Technology	September 21	Dr. Lilian Clark, Dawn Meehan
Big 10+ Graduate School Expo	Purdue University	September 25	Galen Collins, Graduate Student
Cornell University Information Session and Graduate and Professional School Day	Cornell University	September 26–27	Dawn Meehan, Dr. William Tansey
Washington University, St. Louis Information Session	Washington University, St. Louis	October 2	Dr. Lilian Clark
Georgetown University Information Session	Georgetown University	October 3	Jeremy Wilusz, Graduate Student
Massachusetts Institute of Technology Information Session	Massachusetts Institute of Technology	October 6	Beth Chen, Graduate Student
University of California, Berkeley, Graduate School Fair and Information Session	University of California, Berkeley	October 11–12	Dawn Meehan, Dr. William Tansey
Brandeis University Graduate School Fair	Brandeis University	October 11	Fred Rollins, Graduate Student
Tufts University Career and Graduate School Fair	Tufts University	October 11	Fred Rollins
Stony Brook University "A Gathering of Science Scholars: Access for Underrepresented Students in the Biological, Engineering, and Applied Sciences" Graduate School Fair	Stony Brook University	October 13	Dr. Alyson Kass-Eisler, Curriculum Administrator and Postdoctoral Program Officer
Carnegie Mellon University Information Session	Carnegie Mellon University	October 13	Shraddha Pai, Graduate Student
California Institute of Technology Career Fair	California Institute of Technology	October 18	Dr. Leemor Joshua-Tor, Professor
Duke University Graduate and Professional School Day	Duke University	October 19	Dr. David Jackson, Professor, Dawn Meehan
Dartmouth College Information Session	Dartmouth College	October 23	Angelique Girard, Graduate Student
Five College Graduate and Professional Schools Information Day (University of Massachusetts, Amherst, Amherst College; Mt. Holyoke College; Smith College; Hampshire College)	University of Massachusetts, Amherst	October 25	Dawn Meehan
University of North Carolina, Chapel Hill, Information Session	University of North, Carolina, Chapel Hill	October 26	Dr. Vivek Mittal, Assistant Professor
Society for Advancement of Chicanos and Native Americans in Science (SACNAS)	Tampa, Florida	October 26–28	Dawn Meehan
University of California, Irvine, Graduate and Professional School Day	University of California, Irvine	October 30	Vicky Zhou, 2006 URP
Notre Dame University Information Session	Notre Dame University	October 31	Oliver Tam, Graduate Student
University of Pennsylvania Information Session	University of Pennsylvania	November 1	Amy Leung, Graduate Student
Colgate University Information Session	Colgate University	November 6	Dr. Alea Mills, Associate Professor
Annual Biomedical Research Conference for Minority Students (ABRCMS)	Anaheim, California	November 8–11	Oliver Fregoso, (Graduate Student), Keisha John (Graduate Student), Dawn Meehan, Dr. William Tansey
Princeton University Graduate and Professional School Fair and Information Session	Princeton University	November 9–10	Galen Collins, Dr. Adrian Krainer, Professor
Pomona, Scripps, Harvey Mudd, Pitzer, and Claremont-McKenna Colleges Information Session	Pomona College	November 13	Dr. Alea Mills
Emory University Information Session	Emory University	November 13	Jeremy Wilusz
Wesleyan University Information Session	Wesleyan University	November 13	Patrick Finigan, Graduate Student
Yale University Information Session	Yale University	November 14	Dr. David Jackson
Northwestern University Information Session	Northwestern University	November 15	Dr. Patrick Paddison, CSHL Fellow and Watson School Alumnus
Rice University Information Session	Rice University	November 17	Dr. Jan Witkowski, Executive Director, Banbury Center
Harvard University Information Session	Harvard University	November 29	Dr. James D. Watson, Chancellor, Dawn Meehan
Swarthmore College Undergraduate Research Opportunities Presentation	Swarthmore College	December 5	Information sent for presentation
Haverford College Undergraduate Research Opportunities Presentation	Haverford College	December 5	Information sent for presentation
Vassar College Genetics and Bioinformatics Course Visit and Information Session	Cold Spring Harbor Laboratory	December 11	Dr. Rob Lucito, Assistant Professor, Dawn Meehan, Dr. Vivek Mittal, Dr. William Tansey

DOCTORAL THESIS RESEARCH

Student	Academic Mentor	Research Mentor	Thesis Research
ENTERING CLASS OF 2000			
Rebecca C. Ewald *Engelhorn Scholar* **Thesis Defense: April 2006**	Bruce Stillman	Hollis Cline	NMDA receptor subtypes and dendritic arbor morphology
Marco Mangone *Charles A. Dana Foundation Fellow* **Thesis Defense: March 2006**	Linda Van Aelst	Winship Herr	Analysis of the HCF-1 basic region and its role in sustaining cell proliferation
ENTERING CLASS OF 2001			
Catherine Y. Cormier *Beckman Graduate Student* *NSF Graduate Research Fellow* **Thesis Defense: November 2006**	David J. Stewart	Yuri Lazebnik	Alternative apoptotic pathways in cancer cells
Claudia E. Feierstein *George A. and Marjorie H. Anderson Fellow*	Linda Van Aelst	Zachary Mainen	Odor coding and neural correlates of behavioral choice in the olfactory cortex
Tomás Hromádka *Engelhorn Scholar*	William Tansey	Anthony Zador	Stimulus optimization in the auditory cortex
Charles D. Kopec *Goldberg-Lindsay Fellow* *NRSA Graduate Research Fellow* **Thesis Defense: April 2006**	Anthony Zador	Roberto Malinow	AMPA receptor trafficking and their effect on spine enlargement during long-term potentiation
Dougal G.R. (Gowan) Tervo *George A. and Marjorie H. Anderson Fellow* *Howard Hughes Medical Institute Predoctoral Fellow*	Carlos D. Brody	Karel Svoboda	An inducible and reversible lesion of the corticothalmic projection
ENTERING CLASS OF 2002			
Allison L. Blum *Barbara McClintock Fellow*	Hollis Cline	Josh Dubnau	Genetic, behavioral, and anatomical characterization of Radish-dependent memory
Darren Burgess *Engelhorn Scholar*	Nicholas Tonks	Scott Lowe	Mammalian RNAi genetic screens: Discovery and characterization of genes mediating the response to cancer therapy
Beth L. Chen *Beckman Graduate Student*	Senthil K. Muthuswamy	Dmitri Chklovskii	Neuronal network of *C. elegans*: From anatomy to behavior
Shu-Ling Chiu *Elisabeth Sloan Livingston Fellow*	Alea A. Mills	Hollis Cline	The role of insulin receptors in the development of neuronal structure and function
Jonathan Kui *Alfred Hershey Fellow*	David Jackson	Tim Tully	Identification and characterization of candidate memory genes in Arleekin, a *Drosophila* memory mutant
Elizabeth Murchison *Engelhorn Scholar*	John Inglis	Gregory Hannon	The role of Dicer in mammalian development
ENTERING CLASS OF 2003			
Hiroki Asari *Farish-Gerry Fellow*	Z. Josh Huang	Anthony Zador	Sparse overcomplete representation as a principle for computation in the brain
Rebecca Bish *David H. Koch Fellow*	Linda Van Aelst	Michael Myers	A proteomics approach to the study of ubiquitylation
François Bolduc *William R. Miller Fellow*	Hollis Cline	Tim Tully	Role of dFMR1 and the RNAi pathway in *Drosophila* learning and memory
Monica Dus *Engelhorn Scholar*	John Inglis	Gregory Hannon	Characterization of the biological roles of the PIWI subfamily
Angélique Girard *Florence Gould Fellow*	Jan Witkowski	Gregory Hannon	The role of the Piwi family and Piwi-associated small RNAs in mammalian spermatogenesis
Christopher Harvey *David and Fanny Luke Fellow*	Adrian Krainer	Karel Svoboda	Visualization of MAPK activity in neurons
Wei Wei *George A. and Marjorie H. Anderson Fellow*	Jan Witkowski	Roberto Malinow	Activity-dependent modulation of APP processing and Aβ production in rat hippocampal neurons

DOCTORAL THESIS RESEARCH (continued)

Student	Academic Mentor	Research Mentor	Thesis Research
ENTERING CLASS OF 2004			
Daniel Chitwood *George A. and Marjorie H. Anderson Fellow*	Alea A. Mills	Marja Timmermans	The contribution of small RNAs to positional signaling and the establishment of adaxial-abaxial polarity in leaves
Galen Collins *Beckman Graduate Student*	Marja Timmermans	William Tansey	Role of ubiquitin ligases and activator destruction in transcription
Oliver Fregoso *Seraph Foundation Fellow* *William Randolph Hearst Scholar*	Adrian R. Krainer	Michael P. Myers	Identifying the mechanism of adeno-associated virus (AAV) integration and gene targeting and development of a novel molecular biology tool based on AAV
Keisha John *William Randolph Hearst Fellow*	Josh Dubnau	Linda Van Aelst	Identification of the molecular determinants contributing to DOCK7's role in neuronal polarity
Shraddha Pai *Charles A. Dana Fellow*	Anthony Zador	Carlos D. Brody	Determining the neuroanatomical loci and electrical correlates of duration discrimination in the rat
David Simpson *Beckman Graduate Student*	Scott Lowe	William Tansey	Revealing insights into cancer biology with tumor-derived mutations in c-Myc
ENTERING CLASS OF 2005			
Patrick Finigan *Beckman Graduate Student*	Senthil K. Muthuswamy	Bruce Stillman	ORC1 degradation in the cell cycle
Amy Leung *Beckman Graduate Student*	David L. Spector	William Tansey	Role of H2B-ubiquitylation in chromatin localization
Sarahjane M. Locke *George A. and Marjorie H. Anderson Fellow*	Josh Dubnau	Robert Martienssen	Histone H3 lysine 9 methylation and RNA processing in RNAi-mediated heterochromatin formation in *Schizosaccharomyces pombe*
Hiroshi Makino *Elisabeth Sloan Livingston Fellow*	Hollis Cline	Roberto Malinow	Optical determination of the spatial distribution of experience-dependent bidirectional synaptic plasticity
Katie McJunkin *Robert and Teresa Lindsay Fellow* *Leeds Family Scholar*	Terri Grodzicker	Scott Lowe	Using negative-selection RNAi screens to identify novel treatment strategies for hepatocellular carcinoma
Frederick D. Rollins *Cashin Fellow*	Jan Witkowski	Gregory Hannon	An RNAi screen for modifiers of cellular response to the targeted therapeutic erlotinib
Oliver Tam *Bristol-Myers Squibb Fellow*	David Jackson	Gregory Hannon	The role of RNAi machinery in oocyte maturation and embryonic development of the mouse
Jeremy Wilusz *Beckman Graduate Student*	John Inglis	David L. Spector	Identification and functional characterization of large nuclear retained noncoding RNAs misregulated in breast cancer

Graduate Student Seminar Series

One of the most important but often overlooked elements of the graduate student experience at Cold Spring Harbor Laboratory is the weekly Graduate Student Seminar Series. The Laboratory is special in that it has a diverse multiinstitutional graduate student community, especially mixing Stony Brook University and Watson School students. The graduate student seminars, which are held each September to May, enable all graduate students studying at the Laboratory to hear seminars from their colleagues.

Students present their research once a year, with two students presenting each week moderated by a graduate student chair. In attendance are the graduate students and two members of a core set of six faculty mentors whose attendance rotates each week. The seminar series, which is open to the entire Laboratory community, serves three important roles: (1) It provides students with an opportunity to hone their oral presentation skills; (2) it gives students a chance to defend their research without the assistance of their research mentors, such that they experience standing on their own two feet as they will have to throughout their careers; and (3) students in the audience have an opportunity to ask ques-

tions within their own peer group, thus learning the important roles of audience participation for the advancement of research. At the end of the evening, two faculty mentors provide each presenting student a critique of the seminar. In addition, members of the audience complete (anonymously) seminar evaluation forms, which are given to the presenting students. Josh Dubnau, Terri Grodzicker, David Jackson, Patrick Paddison, Arne Stenlund, and myself served as faculty mentors and brought a breadth of knowledge and experience to the seminar series.

Interinstitutional Academic Interactions

It is important to always bear in mind that many of the graduate students studying at Cold Spring Harbor Laboratory are not Watson School students. Indeed, the largest percentage of students are from Stony Brook University, via the programs we have shared with them for some 30 years. The Watson School provides an on-site "home" for all of these students to ensure that they feel part of the Laboratory community and to help them as much as possible with the complexities of performing doctoral research away from the parent institution. This year, the students listed below joined us.

NEW STUDENTS FROM SHARED GRADUATE PROGRAMS

Student	CSHL Research Mentor	Affiliation
Canan Aksoy	Leemor Joshua-Tor	Stony Brook, Molecular and Cellular Biology
Yen-Yu Tina Chen	Tim Tully	Stony Brook, Neuroscience
Wonchang Choi	Jonathan Sebat	Stony Brook, Applied Mathematics and Statistics
Michael Cressy	Josh Dubnau	Stony Brook, Genetics
Yu Fu	Josh Huang	Stony Brook, Neuroscience
Miao He	Josh Huang	Stony Brook, Genetics
Mary Kusenda	Jonathan Sebat	Stony Brook, Genetics
Wanhe Li	Josh Dubnau	Stony Brook, Molecular and Cellular Biology
Elena Lum	Robert Lucito	Stony Brook, Molecular and Cellular Biology
Nick Navin	Michael Wigler	Stony Brook, Molecular Genetics and Microbiology
Ruei-Ying Tzeng	Hollis Cline	Stony Brook, Genetics
Yang Yang	Anthony Zador	Stony Brook, Neuroscience
Jianping Zhang	Scott Powers	Stony Brook, Biomedical Engineering
Rui Zhao	David L. Spector	Stony Brook, Molecular and Cellular Biology

Faculty Changes

Three new research faculty members joined the Watson School this year. Raffaella Sordella joined us as an assistant professor. Raffaella came to the Watson School from Harvard Medical School and the Massachusetts General Hospital where she was an instructor in Medicine and assistant in Genetics, respectively. Raffaella's research will focus on the phenomenon of "oncogene addiction" the process by which cancer cells become dependent on specific oncogenes. Hiro Furukawa also joined the School this year as an assistant professor. Hiro was a postdoctoral fellow at the Vollum Institute in Oregon before joining the Laboratory. Hiro's research will use a number of techniques including structural biology, biochemistry, and electrophysiology to focus on the signal transduction process of regulated intramembrane proteolysis. Our third new assistant professor is Glenn Turner. Glenn came to the Laboratory after completing his postdoctoral studies at the California Institute of Technology. Glenn will be focusing on neural coding using smell and taste in the fruit fly, *Drosophila*, as model systems. We are very excited to have them here and look forward to their participation in Watson School activities.

This year, we also saw the departure of four faculty members: Eli Hatchwell, who served as a thesis committee chair and a guest lecturer in a Specialized Disciplines course on *The Genome*, moved his

laboratory to the Pathology Department at Stony Brook University; Andrew Neuwald, who was a guest lecturer in Specialized Disciplines courses *Fundamentals of Bioinformatics* and *The Genome* and served as a member of the admissions committee, moved his laboratory to The Institute for Genome Research; Rui-Ming Xu, who served as a thesis committee member and chair and also lectured in the core *Scientific Reasoning and Logic* course, moved his laboratory to the Department of Pathology at New York University; Carlos Brody, who has been an integral part of the Watson School, moved his laboratory to Princeton University this year. Carlos served on the School's Admissions Committee, was an instructor in the Scientific Reasoning and Logic core course, the *Systems Neuroscience* specialized disciplines course, and led a Specialized Disciplines course, on *Neuroscience: Systems and Behavior*. Carlos served as a laboratory rotation advisor, an academic mentor, and a thesis committee member and chair. Carlos will continue to be a part of the Watson School family as an adjunct faculty member.

We are deeply indebted to all for their efforts and wish them well in their new "homes."

Graduate Students and Postdoctoral Fellow Departures

With each year comes not only new arrivals, but also departures. The following graduate students and postdoctoral fellows departed from the Laboratory during 2006:

Graduate Students

Santanu Chakraborty
Rebecca Ewald
Teresa Haire (deceased)
Jill Hemish
Helen Hsieh
Cem Kuscu
Michael Kwofie
Pei-Chun Lin
Shi-Chie (Jack) Lin
Chris Maher
Marco Mangone
Anagha Matapurkar
Jasmin Roohi
Dustin Schones
Khalid Siddiqui
Aleksander Sobczyk
Kathryn Tworkowski
Seugtai (Chris) Yoon
Min Yu
Chih-Chi Yuan

Postdoctoral Fellows

Eric Allemand
Amy Altick
Jannik Andersen
Michelle Carmell
Stephanie Chow
Laurence Denis
Michael Deweese
Graziella di Cristo
Ingrid Ehrlich
Jeffrey Erlich
Michael Golding
Noah Gray
Michael Hemann
Hao-Chi Hsu
Ying Huang
Kanae Iijima
Koichi Iijima
Vijay Iyer
Kendall Jensen
Sitharthan Kamalakaran
Jidong Liu
Christian Machens
Alexis Maizel
Tianyi Mao
Masashi Narita
Leopoldo Petreanu
Tara Phelps-Durr
Amit Puniyani
Yijun Qi
Michael Quirk
Michael Ronemus
Simon Rumpel
Takashi Sato
Volker Scheuss
Christian Speck
Naoshige Uchida
Mitsuko Uchida
Santosh Vadivelu
Robby Weimer
Hans Guido Wendel
Bin Xiang
Zhong Yao
Haining Zhong
Jack Zilfou
Karen Zito

Executive Committee

A large measure of the Watson School's success can be traced to the sage advice, guidance, and governance of the School's Executive Committee. In 2006, Hollis Cline, a valuable member of the committee since 2002, departed the committee as *ex officio* member, a position she held in her role as Director of Research. As happens each year, there was also turnover among the student representatives. The Watson School representative, Hiroki Asari was replaced by David Simpson. Despina Siolas continued as the Stony Brook University representative. The School is indeed thankful for their frank, honest, and thoughtful advice.

External Advisory Committee

An outstanding External Advisory Committee (EAC) led by Dr. Keith Yamamoto, Professor and Executive Vice Dean at the School of Medicine, University of California, San Francisco, guides the Watson School. The members of the Committee are noted for their leadership in graduate education

in the biological sciences and are recognized leaders in their fields of research. The EAC visited the School on May 18–19, 2006. As usual, the team met with students, faculty, and administrators, and the overall feeling was that our "grand experiment" was indeed working. This year, with the help of EAC members, we will be exploring and putting into practice ways of directly targeting undergraduates at the top Schools and Liberal Arts Colleges. In addition, we have been invited to participate in the *National Research Council Assessment of Research-Doctorate Programs*, which is being conducted by the National Academies of Science. This study, which is carried out every 10 years, has three main purposes:

- Help universities improve their doctoral programs through benchmarking.
- Expand the talent pool through accessible information, easily available to potential doctoral students, about doctoral programs.
- Benefit the nation's research capacity by improving the quality of doctoral programs and their students.

The EAC's continued commitment to the success of the Watson School has been a humbling experience—thank you so much guys!

Site Visits to the School

On May 3, the School was site-visited by Mr. Gerry Gallwas, a trustee of the Arnold and Mabel Beckman Foundation (the Watson School Graduate Program is now in receipt of its second five-year grant of $1.75 million from the Beckman Foundation). This was an unexpected visit, but as ever, the students and faculty rose to the occasion by putting together a series of meetings and a mini-symposium, which were extremely well received. Mr. Gallwas was also able to visit the other educational divisions of the Laboratory, including the Dolan DNA Learning Center and the Banbury Conference Center.

The Watson School Graduate program has been in receipt of a training grant from the National Institutes of Health (NIH) since 2002, which currently supports five American students. In May, the School submitted a competitive renewal application for this grant. As part of the peer-review process, three reviewers, Drs. Timothy Formosa, Roger Chalkley, and Dennis McKearin, a scientific review administrator, Dr. Carole Latker and the training grant program director, Dr. Marion Zatz, site-visited the School. The purpose of this visit was to gather more information regarding the program. The site-visit team met with the Laboratory's president, chief financial officer, faculty, and Watson School students. We have received a priority score in the outstanding range, and the review committee has recommended that we be funded at the level we requested. Below is some of what the site visit team had to say about the Watson School.

> *Other strengths include an internationally renowned faculty with well-funded members participating in training and a very strong pool of graduate students. While this combination alone would be expected to produce a graduate education experience of high caliber, the real strength of this program has been the number of innovative features that have been incorporated into the design of the Watson School curriculum. The faculty of the School has taken full advantage of the opportunity to design a graduate program from the ground up and as a result have created a very strong system of graduate education. Innovative features include the use of the teaching professionals at the CSHL DNA Learning Center to provide instruction and experience in teaching to the graduate students beginning in their first year of study and an excellent system of mentoring that provides experience giving presentations with formal feedback at many points during training. Each student has both a formal research advisor and a dedicated academic mentor. This gives each of the students at least two faculty mentors, which provides students the opportunity to get advice on a range of problems quickly and easily. Remarkably, students report that they feel the advice they get from their advisors is more reliable and informed than the advice they get from other students. Although true in most places, it is highly unusual for students to have this perception and indicates how well this system is working at the Watson School.*

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 the Laboratory an eminent researcher and educator to give the Gavin Borden Lecture, which is dedicated to the graduate students at the laboratory. Dr. Michael Levine, Professor of Genetics and Development at the University of California, Berkeley, was this year's Gavin Borden Fellow. His lecture *"Gene Networks for Fly Gastrulation and Heart Formation in Sea Squirts"* was thoroughly enjoyed as evidenced by the standing-room-only audience. In addition, Michael shared his experiences as a scientist at a roundtable discussion with the students the following day.

Student Awards

The students at the Watson School continue to impress us all with their accomplishments. Our students continue to publish their research findings in prestigious international journals. Additionally, our students have received international recognition by obtaining fellowships to pursue their research interests. Rebecca Bish was awarded a fellowship from the American Foundation for Aging Research. David Simpson was awarded a fellowship from the U.S. Army Medical Research and Material Command Breast Cancer Research Program Predoctoral Fellowship. Elizabeth Murchison was awarded an American Australian Association Education Fund Fellowship, which she will use for her postdoctoral studies.

The WSBS Family Events

On a lighter note, I am pleased to announce that August saw two marriages in the School. Darren Burgess of the entering class of 2002 married Fiona Holloway, and David Simpson of the entering class of 2004 married Erin Dann. Our congratulations and best wishes to all of them.

Lilian Clark
Dean

SPRING CURRICULUM

Topics in Biology

ARRANGED BY **Alyson Kass-Eisler and Jan A. Witkowski**

FUNDED IN PART BY **The Daniel E. Koshland, Jr. Visiting Lectureship; The David Pall Visiting Lectureship; The Fairchild Martindale Visiting Lectureship; The Lucy and Mark Ptashne Visiting Lectureship; The Michel David-Weill Visiting Lectureship**

Each year, one or a team of invited instructors offer 7-day courses at the Banbury Conference Center to explore specialized topics outside the expertise of the Cold Spring Harbor Laboratory faculty. These courses include morning or evening lectures as well as afternoon sessions during which students read assigned papers. These intensive courses are modeled on the Cold Spring Harbor Laboratory Lecture Courses held each summer at the Banbury Conference Center. In Spring 2006 there were two such courses: *Immunology* and *Microbial Pathogenesis*.

Immunology

Attended by the entering classes of 2002 and 2005

INSTRUCTOR **Hidde Ploegh,** Whitehead Institute for Biomedical Research

TEACHING FELLOWS **Marianne Boes,** Brigham and Women's Hospital, Harvard University
Roberto Bonasio, The CBR Institute for Biomedical Research
Cristina Costantino, Harvard Medical School

Immunology is a discipline that stretches across a number of distinct disciplines: genetics, cell biology, microbiology, biochemistry, to name just a few. The immune system comprises a set of elements (cells, molecules, tissues/organs) whose function and mode of action are not only unique, but also essential to a successful defense against pathogenic microbes (antigens). The cells of the immune system are readily obtained from experimental animals as well as humans, and hence, many aspects of their function can be studied in vitro. Several classes of immunologically important molecules are serum proteins, and they are likewise available in quantities that allow their full characterization, in terms of both structure and function (antibodies, complement). The diversity of antigen-specific receptors (antibodies, T-cell receptors) is the result of a somatic recombination process unique to lymphocytes. Because the vertebrate immune system is not essential for survival, gene ablation and knock-in approaches have been particularly informative. Especially questions pertaining to lymphocyte development (fate decisions), cell survival, and programmed cell death have benefited from this approach. All of these elements justify detailed study of the immune system as an evolutionary successful solution to a problem that confronts all metazoans: how to preserve organismal integrity in the face of microbial aggression. There is a second, more practical element

(*Top left to bottom right*) Darren Burgess, Shu-Ling Chiu, Jeremy Wilusz, Allison Blum, Jonathan Kui, Katherine McJunkin, Hidde Ploegh, Fred Rollins, Oliver Tam, Amy Leung, Sarahjane Loke, Maria Chen, Suying Sun, Patrick Finigan, Nicholas Navin, Cristina Costantino, Hiroshi Makino, and Roberto Bonasio

that justifies a detailed examination of the immune system. A number of disease states result from inappropriate activation of the immune system. Successful therapies can emerge only if the underlying basis for the disease is fully understood. At the applied level, antibodies (the antigen-specific receptors on and secreted by B lymphocytes) are extremely versatile reagents that can be used to detect, isolate, or modify the function of almost any protein. How does an immune response lead to the production of antibodies that would be useful for these applications?

This course introduced the elements of the immune system that make it a unique object of biological studies, with cross-references to some of the more applied aspects. Its challenge was to convey a sense of what the immune system is about. The course ran from Sunday to Saturday, February 26–March 4, and was organized and largely taught by Hidde Ploegh. It integrated lectures by the instructors, daily quizzes, directed readings of research papers, and seminars by the instructors. The students, many of who had little prior awareness of the subject, rated the course very highly.

Microbial Pathogenesis

Attended by the entering classes of 2003 and 2004

INSTRUCTORS **Stanley Maloy,** Center for Microbial Sciences, San Diego
Ronald K. Taylor, Dartmouth Medical School

GUEST LECTURERS **Darren Higgins,** Harvard Medical School
Linda Kenney, University of Illinois, Chicago
Paula R. Sundstrom, Dartmouth Medical School
Michele Swanson, University of Michigan

Throughout recorded history microbial pathogens have been a major cause of human disease and mortality. However, with the advent of effective antibiotics, it seemed like the war on microbes had been won. Hence, for several decades health-related research shifted to topics such as cancer, heart disease, and genetic diseases. Although research in microbial pathogenesis slowed, the microbes demonstrated the efficacy of evolution. Microbial resistance to antibiotics developed faster than new antibiotics could be made available, and the resistance spread throughout the microbial world. The global expansion of food distribution networks has increased the incidence of common microbial pathogens. Simultaneously, emerging microbial pathogens filled new ecological niches, such as indwelling medical devices that provide a surface for biofilms and the growing population of patients who are immunocompromised due to primary infections such as HIV or due to therapies for chronic diseases. Furthermore, recent discoveries have demonstrated that some diseases (e.g., ulcers) previously believed to be caused by a genetic predisposition or environmental conditions are actually caused by microbes. This microbial offensive has summoned a renewed counterattack on microbial pathogens that has intensified during the last several years. Meanwhile, a variety of new tools have become available that make it possible to dissect the molecular basis of pathogenesis from both the microbial and host perspectives. Recently, the complete DNA sequence of bacterial pathogens has provided valuable insights into how microbial pathogens evolve and the extent of gene transfer between pathogens. These advances have revealed new ways to control infection, including the identification of novel targets for antimicrobials and novel approaches for vaccine development. Nevertheless, many more questions remain unanswered and many pathogens are still poorly understood.

Understanding bacterial pathogenesis demands a detailed knowledge of the host response as well as the pathogen itself. Both of these perspectives provide potential strategies for solving important clinical problems. To elucidate these distinct aspects of microbial pathogenesis requires an interdisciplinary approach, integrating the fields of microbiology, eukaryotic cell biology, immunology, and genomics.

This course focused on mechanisms of microbial pathogenesis and the host response, and the scientific approaches that are used to investigate these mechanisms. How do microbes adhere to host cells? How do environmental cues direct the response of microbial pathogens? How do microbial pathogens modulate host cells to expedite virulence? How do host cells respond to microbial pathogens? How does

(*Left to right*) François Bolduc, Monica Dus (and Cupcake), Christopher Harvey, Ronald Taylor, Rebecca Bish, Galen Collins, Keisha John, Daniel Chitwood, Oliver Fregoso, David Simpson, Shraddha Pai, Stanley Maloy, Angelique Girard, Hiroki Asari

the host immune system react to microbial pathogens? What does genomics tells us about how microbial pathogens evolve? How do emerging pathogens take advantage of new ecological niches? Although there are numerous microbial pathogens, the answers to these questions indicate that many pathogens use similar approaches to solve common problems. The course integrated lectures by the instructors, directed readings of research papers, and seminars by the instructors plus four invited speakers who specialize in various aspects of bacterial pathogenesis. The course ran from Monday, April 17 through Sunday, April 23. As in previous years, the course was highly rated by all of the students.

Teaching Experience at the Dolan DNA Learning Center

DIRECTOR	**David A. Micklos**
INSTRUCTORS	**Uwe Hilgert (Lead instructor, high school)** **Erin McKechnie (Lead instructor, middle school)** **Greg Chin** **Jeanette Collette** **David Gundaker** **Amanda McBrien** **Bruce Nash**
ADMINISTRATOR	**Carolyn Reid**

As science has 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, 18 faculty members served as rotation mentors, some mentoring more than one student.

ROTATION MENTORS

Hollis Cline
Gregory Hannon
David Jackson
Leemor Joshua-Tor
Scott Lowe
Wolfgang Lukowitz
Zachary Mainen
Roberto Malinow
Robert Martienssen
Alea A. Mills
Senthil K. Muthuswamy
David L. Spector
Lincoln Stein
Bruce Stillman
William Tansey
Marja Timmermans
Nicholas Tonks
Michael Wigler

FALL COURSE CURRICULUM

CORE COURSES

The Leslie C. Quick, Jr. Core Course on Scientific Reasoning and Logic

FUNDED IN PART BY **The Arnold and Mabel Beckman Foundation; The William Stamps Farish Lectureship**

INSTRUCTORS
Gregory Hannon (Lead)
David Jackson
Leemor Joshua-Tor
Scott Lowe
Robert Martienssen
Anthony Zador

GUEST LECTURERS
Josh Dubnau
Eric Enemark
Grigori Enikolopov
Alexander A.F. Gann
Adrian R. Krainer
Wolfgang Lukowitz
Patrick Paddison
Scott Powers
Bruce Stillman
William Tansey

VISITING LECTURERS
David Allis, The Rockefeller University
Michael Hengartner, University of Zurich
William Merrick, Case Western Reserve University
Daniella Nicastro, Brandeis University

A fundamental aspect of earning the Ph.D. is training in the pursuit of knowledge. In this core course, which forms the heart of the curriculum, students (1) acquired a broad base of knowledge about the biological sciences, (2) learned the scientific method, and (3) learned how to think critically. This course consisted of six biweekly modules, each of which had a different theme. Each week, students read an assigned set of research articles and, at the end of the module, provided written answers to a problem set that guided them through several of the articles. Twice weekly, students attended lectures related to the week's topic, which 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. The module topics for this course were as follows:

Module 1 Macromolecular Structure and Function
Module 2 Cell Proliferation and Cancer
Module 3 Gene Expression
Module 4 Signaling in Development
Module 5 Neuroscience and Quantitative Reasoning
Module 6 NIH Study Section

The Darrell Core Course on Scientific Exposition and Ethics

FUNDED IN PART BY **The Arnold and Mabel Beckman Foundation; The John P. and Rita M. Cleary Visiting Lectureship; The Seraph Foundation Visiting Lectureship; The Susan T. and Charles E. Harris Visiting Lectureship**

INSTRUCTORS
Jan A. Witkowski (Lead)
Lilian Clark
Alea A. Mills

GUEST LECTURERS
Walter Goldschmidts
William Tansey

VISITING LECTURERS
Bruce Budowle, Federal Bureau of Investigation
Robert P. Charrow, Greenberg Traurig LLP
Mariette Di Christina, *Scientific American*
Angela Eggleston, *Nature*
Kay Fields, Office of Research Integrity
Kathy Hudson, Genetics and Public Policy Center

This core course offered instruction in the fundamental elements of scientific exposition—writing skills and public speaking—and ethics. The ability to communicate effectively and to appreciate the intricacies of ethical issues are essential skills for biologists; both subjects were taught in a series of example-based lectures and discussion groups. Writing skills included the fundamentals of modern scientific English and the organization and preparation of papers, research abstracts, and grant applications. Oral presentation skills were taught by instructors with different modes of presentation. Together with instructors, students critiqued formal seminar presentations at the Laboratory. Instruction and discussions about ethics included the ethical implications of biological discovery on society as well as the nature and boundaries of ethical behavior of scientists and their rights and responsibilities. A primary objective of the course was that students consider exposition and ethics an integral part of scientific research.

Research Topics

ORGANIZERS
Eva Radeck
Lilian Clark

This core course provided students with an in-depth introduction to the fields of research that Laboratory scientists investigate. Students and faculty attended a weekly Research Topics seminar, at which faculty members presented their current research topics and methods of investigation each Wednesday evening over dinner. The students learned how to approach important problems in biology. These seminars, together with the annual fall Laboratory In-House symposium, provided students with a basis for selecting laboratories in which to do rotations. The weekly speakers were:

Hollis Cline
Josh Dubnau
Grigori Enikolopov
Hiro Furukawa
Gregory Hannon
Z. Josh Huang
David Jackson
Leemor Joshua-Tor
Alexei Koulakov
Adrian R. Krainer
Robert Lucito
Scott Lowe

Wolfgang Lukowitz
Zachary Mainen
Robert Martienssen
W. Richard McCombie
Alea A. Mills
Partha P. Mitra
Vivek Mittal
Senthil K. Muthuswamy
Michael P. Myers
Scott Powers
Jonathan Sebat
Raffaella Sordella
David L. Spector
Lincoln Stein
Arne Stenlund
Bruce Stillman
William Tansey
Marja Timmermans
Nicholas Tonks
Lloyd Trotman
Glenn Turner
Linda Van Aelst
Doreen Ware
Anthony Zador
Michael Q. Zhang
Yi Zhong

SPECIALIZED DISCIPLINES COURSES

Genetics

FUNDED IN PART BY **The Edward H. and Martha F. Gerry Lectureship; The Pfizer Lectureship; The George B. Rathmann Lectureship; The Edward H. Gerry Visiting Lectureship**

INSTRUCTORS **Josh Dubnau (Lead)**
Lincoln Stein
Tim Tully

GUEST LECTURER **Ira Hall**

VISITING LECTURER **Bambos Kyriacou,** University of Leicester, United Kingdom

In the past, "gene discovery" and association between gene and phenotype were accomplished in model organisms. Our understanding of human disease then was advanced by identification of human orthologs associated with disease and by interventionist experiments using animal models of human disease. The completion of the human genome sequence and the remarkable advances in molecular biological techniques have initiated a paradigm shift in genetics. Associations between gene variants and disease can now be directly discovered in humans. Gene-to-phenotype functional associations can thus be discovered in humans as well as in model organisms. Causal mechanistic relationships between gene and phenotype can then be established using interventionist genetic experiments in animal models. This permits both a "vertical integration" to understand how molecular mechanisms influence functional output across various levels of biological organization and a "horizontal integration" to understand how genetic pathways have been conserved evolutionarily.

This course placed modern human genetics and genomics into the context of classical organismal genetics. History, perspective, and technique were described around four levels of analysis: naturally occurring variation, association studies, genome evolution, and genetic screens. How do gene mutations help to define biological processes? How are more complex traits genetically dissected into simpler (underlying) components? What concepts and techniques are used to organize genes into pathways and networks? How are genes mapped, cloned, and engineered to identify functional domains of proteins? What gene variation exists in natural populations? What are the functional consequences of gene variation? How is it detected? How are genomes organized and coordinately regulated? How can genomic information be cataloged, organized, and mined? These questions and concepts were discussed using examples from the literature.

Cellular Structure and Function

FUNDED IN PART BY **The Mary D. Lindsay Lectureship; The Sigi Ziering Lectureship; The Martha F. Gerry Visiting Lectureship**

INSTRUCTORS **David L. Spector (Lead)**
Linda Van Aelst

VISITING LECTURERS **Michael Caplan,** Yale University
Gregg Gundersen, Columbia University
Alexy Khodjakov, Wadsworth Center
Marc Symons, The Feinstein Institute for Medical Research
Graham Warren, Yale University

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.

Systems Neuroscience

FUNDED IN PART BY **The George W. Cutting Lectureship; The Klingenstein Lectureship**

INSTRUCTORS **Zachary Mainen (Lead)**
Carlos D. Brody

GUEST INSTRUCTORS **Hollis Cline**
Alexei Koulakov
Roberto Malinow

VISITING LECTURER **Marta Moita,** Instituto Gulbenkian de Ciência, Portugal

This course introduced students to neuroscience, with a focus on learning and plasticity from its cellular basis, through development, to systems and behavior. Both experimental and theoretical viewpoints were explored. The course started with the basics of electrical signaling in neurons: ion channels, action potentials, and synaptic transmission. The cellular basis of learning including Hebb's postulates, LTP (long-term synaptic potentiation), was discussed. The course explored the consequences of synaptic learning rules by examining how experience shapes the wiring of the nervous system during development and investigated how such building blocks translate into whole-organism behavior. The course then examined classical conditioning and asked how changes in synaptic transmission could underlie such behavior. Associative learning computational models, of the learning process, were also discussed.

From behaviors that focus on simple memories, the course turned to behaviors that require making perceptual decisions. To do this, some basic concepts of perceptual neuroscience were covered, such as neuronal "receptive fields," and these were used to discuss current results and models of perceptual decision-making. Finally, the course turned to the learning of behaviors through reward and punishment, known as reinforcement learning. The course concluded with a discussion at 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. The Laboratory currently employs approximately 160 postdoctoral fellows working in the labs of 40 principal investigators. Current fields of research expertise include genetics; molecular, cellular, and structural biology; neuroscience; cancer; plant biology; and genomics and bioinformatics. The postdoctoral community at CSHL is diverse and international, arriving at the Laboratory with many different backgrounds and with needs that change during the course of the postdoctoral years. To enhance the postdoctoral experience at CSHL, an office for postdoctoral education (the Postdoctoral Program Office) was established to work closely with the postdoctoral fellows and the administration to coordinate and organize educational and career development activities.

The Postdoctoral Program Office was established within the Watson School of Biological Sciences in January 2003, with the appointment of Dr. Alyson Kass-Eisler, to provide support and programmatic elements to postdoctoral training at the Laboratory. The Office provides support for current and arriving postdoctoral fellows as well as educational and career development programs. CSHL has an active Postdoctoral Association (PDA) whose purpose is to represent the interests of the CSHL postdoctoral community. In addition, the Postdoctoral Program Office also maintains a resource center containing career information, job opportunities, and fellowship opportunities; they organize workshops and career development seminars, job search forums, and grant-writing workshops and provide networking opportunities for postdocs.

In 2006, the Watson School was fortunate enough to attract a wonderful array of speakers for its career development seminar series. John Tooze, Vice President for Scientific and Facility Operations at The Rockefeller University, described his fascinating career path in "Beyond the Bench." Michael Stebbins, a former graduate student from the Laboratory and currently Director of Biology Policy at the Federation of American Scientists, spoke about "Career? What career? Knowing when to seize your next opportunity." Matt Ridley, an internationally renowned science author and honorary degree recipient from the Watson School in 2006, spoke about "Nature versus Nurture." Dave Jensen, Managing Director of CareerTrax Inc. and the host of the *Science* magazine careers forum, provided his "Insiders' Secrets to the Job Market." Alex Rai, also a former graduate student and currently an Attending Staff member at Memorial Hospital of Memorial Sloan-Kettering, finished the year by talking to postdocs about a special training program in Clinical Chemistry that he participated in.

The Postdoctoral Program Office also hosted a visit from Dr. Jing Li from the Merck Research Laboratories (MRL). CSHL has been a member of a special ambassador recruitment program from MRL since 2005. This program provides the postdocs and students at CSHL an inside connection to a scientist working at MRL. In the morning, Dr. Li hosted a discussion on what it is like to work at Merck and spent the afternoon holding one-on-one appointments with individuals interested in working at Merck. As a result of his visit, three of the Laboratory's trainees had their resumes submitted to the hiring managers at MRL!

The Dean holds quarterly "Dean's teas" with postdoctoral trainees newly arrived at CSHL. These sessions are designed to assist new postdoctoral fellows with making the most of their postdoctoral experience at CSHL. During these sessions, postdoctoral fellows are provided information on the services available through the Postdoctoral Program Office and were given copies of the excellent books by Kathy Barker, *At the Bench: A Laboratory Navigator* and *At the Helm: A Laboratory Navigator*, pub-

lished by Cold Spring Harbor Laboratory Press. In addition, Laboratory President, Bruce Stillman, holds a "Town Hall" meeting specifically for postdoctoral fellows in November of each year. At this year's Town Hall meeting, the postdoctoral fellows were treated to Dr. Stillman's personal insights into how to proceed with an academic job search.

In Spring 2003, all postdoctoral fellows and graduate students at the Laboratory were enrolled in a new initiative of the New York Academy of Science, 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 the New York Academy of Sciences. 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. In 2006, we were fortunate to cohost a Science Alliance event on our own campus. Dr. Philip Clifford, Professor of Anesthesiology and Physiology and Associate Dean for Postdoctoral Education at the Medical College of Wisconsin, came to talk about Individual Development Plans for postdoctoral fellows. These plans are organizational tools to assist postdoctoral fellows in planning their future and their career path. Dr. Clifford was also a member of The Federation of American Societies for Experimental Biology (FASEB) committee that drafted the model Individual Development Plan for postdoctoral fellows. The talk was well attended, extremely interactive, and highly praised.

Nationally, CSHL continues to help shape the postdoctoral experience. In 2004, CSHL became a founding institutional member of the National Postdoc Association (NPA). The NPA was founded by a dynamic group of postdoctoral fellows who attended the 2002 National Postdoc Network meeting. The 2006 National Postdoc Association meeting was held on the National Institutes of Health campus in Bethesda, Maryland. Alyson Kass-Eisler attended this year's meeting, which highlighted postdoctoral offices and postdococtoral associations. Lori Conlan, Manager of the Science Alliance, wrote in the Summer 2006 issue of the NPA newsletter, "*As a member of the NPA, I gathered information on postdoc issues that are now invaluable. I witnessed many institutions change their postdoc policies, and many of these are now our members. Knowing a bit of history on how Stanford has changed, or the newly implemented system in California, or the strength of the postdoc office at Cold Spring Harbor gives me an advantage*."

One measure of our postdoctoral program's success is the ability of a postdoc to secure permanent positions at the end of their training. In 2006, the Laboratory's departing postdocs went on to positions at the Cambridge Research Institute–Cancer Research UK, Cold Spring Harbor Laboratory Press, Harvard University, Memorial Sloan-Kettering Cancer Center, Merck Research Laboratory, Massachusetts Institute of Technology, Medical Research Center Clinical Sciences Centre–Imperial College London, National Institute of Biological Sciences–Beijing, Radford University, Research Institute of Molecular Pathology–Vienna, Thomas Jefferson University, University of California, Davis, Université de Montréal, to name but a few.

UNDERGRADUATE RESEARCH PROGRAM

Program Directors David Jackson
Lincoln Stein

Program Administrator Dawn Meehan

Each summer, 25 undergraduates from around the world and across the country participate in the CSHL summer Undergraduate Research Program (URP). More than 700 students have participated in the URP since the program was founded in 1959.

The fundamental objective of the program is to give students an opportunity to conduct first-rate research. Participants learn about scientific reasoning, laboratory methods, theoretical principles, and scientific communication. The specific objectives of the program are to (1) give college undergraduates a taste of conducting original research at the cutting edge of science, (2) encourage awareness of the physical and intellectual tools necessary for modern biological research, (3) foster an awareness of the major questions currently under investigation in the biomedical and life sciences, and (4) promote interactions with laboratory scientists through an immersion in the research environment.

During the 10-week program, URPs work with senior Laboratory staff members on independent research projects, specifically in the areas of cancer biology, neuroscience, plant biology, cellular and molecular biology, genetics, macromolecular structure, and bioinformatics.

URPs work, live, eat, and play among CSHL scientists. They have a very busy academic and social calendar for the summer. They attend lectures in the Goldberg Faculty Lecture Series from CSHL faculty members and outside faculty members. The 2006 URPs were fortunate to hear talks by Dr. Keith Yamamoto, University of California San Francisco, and Dr. James Konopka, Stony Brook University. URPs also attend a seven-part Bioinformatics Workshop Series and special lectures designed for them, including lectures by Drs. James Watson (10 Rules for Science), Jan Witkowski (Ethics), and Bill Tansey (How to give a talk). URPs also are invited to join Dr. and Mrs. Watson for a pizza party and Dr. and Mrs. Stillman for dinner, have BBQs and pool parties, design an URP tee shirt, run in the annual CSHL plate race and scavenger hunt, and participate in the ever-famous URP vs. PI volleyball match. The URPs have never won!

(*Top row, left to right*) Christopher Quinn, Joseph Calarco, Kathryn Schmidt, Vicky Zhou, Kipp Weiskopf, Wenke Li, Ryan Devenyi, Julie Granka, William Kruesi, Caroline Leeds, Tal Shamia, Paloma Guzzardo, Oleg Dmytrenko, Lincoln Smith. (*Bottom row, left to right*) Katie Amodeo, Bryan Schmidt, Joshua Siegle, Adam Lowe, Nandita Garud, Lilian Ho, Brittany Sternard, Lenore Barhak, Silvia Caballero, Angelica Contero

At the beginning of the summer, all of the URPs write an abstract and present a talk on their proposed research for the summer. At the URP Symposium in August, they give a 15-minute talk on their results and also prepare a final report. The following students, selected from 634 applicants, took part in the 2006 program:

Katherine Amodeo, Marist College
Advisor: **Dr. Vivek Mittal**
Sponsor: Jephson Educational Trust
Role of tumor growth factor VEGF in bone-marrow-dependent angiogenesis-mediated tumor growth.

Lenore Barhak, The Cooper Union
Advisor: **Dr. Leemor Joshua-Tor**
Sponsor: National Science Foundation and Department of Defense
A molecular view of transcriptional repression.

Silivia Caballero, Hunter College
Advisor: **Dr. Alea A. Mills**
Sponsor: Hunter College Fund
Gene targeting of a novel tumor suppressor gene *chd5* in mouse embryonic stem cells.

Joseph Calarco, University of Toronto
Advisor: **Dr. Senthil K. Muthuswamy**
Sponsor: Former URP Fund
The relationship between Erb-B2, the Par polarity complex, and apoptosis.

Angelica Contero, Swarthmore College
Advisor: **Dr. Hollis Cline**
Sponsor: William Townsend Porter Foundation
The effect of visual stimulation on GABA expression patterns in the optic tectum of *Xenoplus laevis* tadpoles.

Ryan Devenyi, Bowdoin College
Advisor: **Dr. Wolfgang Lukowitz**
Sponsor: National Science Foundation and Department of Defense
Investigations in the Yoda MAP kinase pathway in *Arabidopsis*.

Oleg Dmytrenko, International University of Bremen
Advisor: **Dr. David P. Jackson**
Sponsor: Former URP Fund
Analysis of *gat2*$^{-}$ mutants with reduced plasmodesmata size-exclusion limit (*Arabidopsis thaliana*).

Nandita Garud, Cornell University
Advisor: **Dr. Doreen Ware**
Sponsor: National Science Foundation and Department of Defense
Weeding for phenotypes and motifs in a weed, in the field, and within genomes.

Julie Granka, Cornell University
Advisor: **Dr. Michael Q. Zhang**
Sponsor: National Science Foundation and Department of Defense
Characterizing the binding specificity of CTCF.

Paloma Guzzardo, University of Puerto Rico
Advisor: **Dr. Adrian R. Krainer**
Sponsor: Former URP Fund
Characterization of a new S6 kinase 1 isoform.

Lilian Ho, Vassar College
Advisor: **Dr. Cordula Schultz**
Sponsor: National Science Foundation and Department of Defense
Novel protein lucky luke and cellular integrity.

William Kruesi, Carleton College
Advisor: **Dr. Marja Timmermans**
Sponsor: National Science Foundation and Department of Defense
The AS1/AS2 and ta-siRNA pathways regulate *MIR166* gene expression in *Arabidopsis*.

Caroline Leeds, Amherst College
Advisor: **Dr. Patrick Paddison**
Sponsor: Former URP Fund
Knockdowns and neurons: Using RNAi to specify cell fate in mouse embryonic stem cells.

Wenke Li, Stevens Institute of Technology
Advisor: **Dr. Ravi Sachidanandam**
Sponsor: National Science Foundation and Department of Defense
How old are introns?

Adam Lowe, Salisbury University
Advisor: **Dr. William Tansey**
Sponsor: Steamboat Foundation
Role of the *Saccharomyces cerevisiae* gene *Sc11* in proteasome-mediated transcriptional pathways.

Christopher Quinn, Cornell University
Advisor: **Dr. Alexei Koulakov**
Sponsor: National Science Foundation and Department of Defense
An improved neural spike-clustering approach.

Bryan Schmidt, Indiana University, Bloomington
Advisor: **Dr. Gregory J. Hannon**
Sponsor: Former URP Fund
Developing a direct biochemical method to identify the targets of microRNAs.

Kathryn Schmidt, Yale University
Advisor: **Dr. Linda Van Aelst**
Sponsor: Former URP Fund
The role of the X-linked mental retardation protein oligophernin-1 in glutamate receptor signaling.

Tal Shamia, Tel Aviv University
Advisor: **Dr. Robert Martienssen**
Sponsor: Former URP Fund
RNAi and gene silencing effects on *Arabidopsis* development.

Joshua Siegle, Brown University
Advisor: **Dr. Partha Mitra**
Sponsor: National Science Foundation and Department of Defense
Oscillatory brain dynamics of working memory: A simultaneous MEG and EEG study.

Lincoln Smith, Wabash College
Advisor: **Dr. W. Richard McCombie**
Sponsor: National Science Foundation and Department of Defense
BglII fragment selection in the human genome using different hybridization-based selections.

Britni Sternard, Bethel College
Advisor: **Dr. Yi Zhong**
Sponsor: Former URP Fund
Construction of RNAi for neurofibromatosis type 1.

Kipp Weiskopf, Amherst College
Advisor: **Dr. Bruce Stillman**
Sponsor: Former URP Fund
Role of Orc2 in mitotic checkpoint assembly.

Vicky Zhou, University of California, Irvine
Advisor: **Dr. Lincoln Stein**
Sponsor: National Science Foundation and Department of Defense
Visualization of *C. elegans* gene expression data in WormBase.

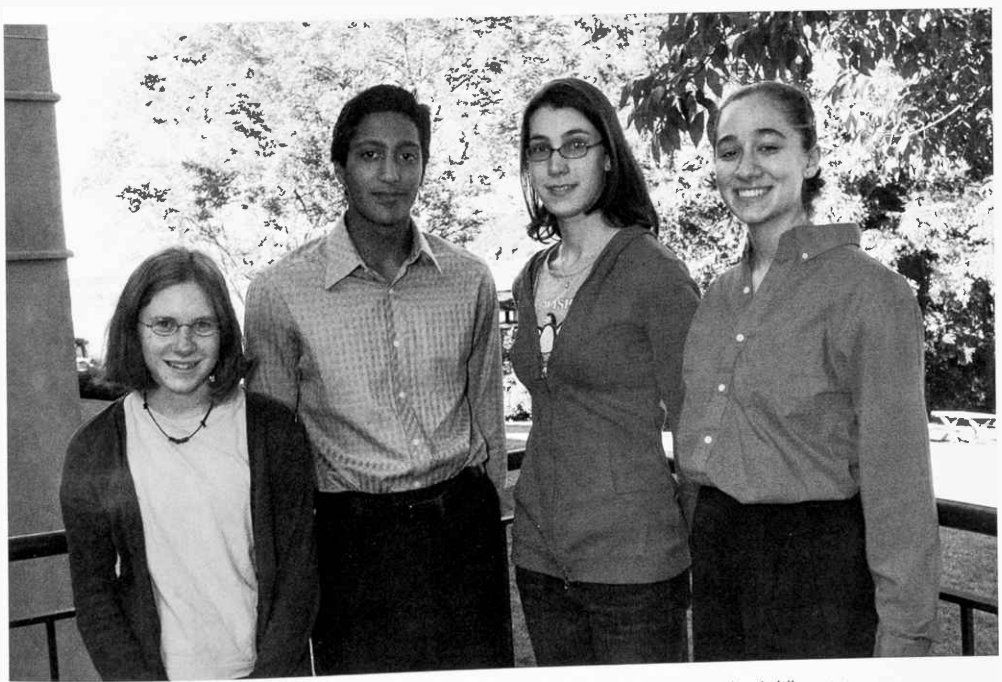
PARTNERS FOR THE FUTURE

Program Director: Yuri Lazebnik

Program Administrator: Lynn Hardin

The Partners for the Future Program, established by Dr. James Watson in 1990, brings Long Island high school students into Cold Spring Harbor Laboratory and gives them a taste of the real world of biomedical research. The 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, and ten to twelve student semifinalists are then interviewed by CSHL scientists. The selected students spend a minimum of 10 hours per week, September through March of their senior year, doing original research under the watchful eye of a scientist mentor. At the conclusion, the students give oral presentations of their research projects to an enthusiastic audience of the students' scientific mentors, Lab 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 in exposing the students to day-to-day life in a working lab. The students are introduced to a world of relatively young scientists and their interactive support staff in a relaxed, problem-solving atmosphere. The 2006–2007 Partners for the Future are

Partner	High School	CSHL Mentor	Laboratory
Leah Libresco	The Wheatley School	Ken Chang	Gregory Hannon
Souvik Paul	Syosset High School	Anindya Bagchi	Alea A. Mills
Hannah Payne	Huntington High School	James Demas	Hollis Cline
Catherine Schlingheyde	Oyster Bay High School	Fabiola Rivas	Gregory Hannon



(*Left to right*) Catherine Schlingheyde, Souvik Paul, Hannah Payne, Leah Libresco

COLD SPRING HARBOR LABORATORY MEETINGS AND COURSES

ACADEMIC AFFAIRS

The academic program at the Laboratory serves to communicate new discoveries, concepts, and methodologies to an international community of scientists. The program consists of advanced laboratory and lecture courses, short courses at our Woodbury campus, as well as large meetings and biotechnology conferences that are held almost year-round. More than 8000 scientists, ranging from graduate students and postdoctoral fellows to senior faculty, come from around the world to attend these events. A growing international program complements the main program of meetings and courses.

In 2006, 27 laboratory and lecture courses were held. These covered a diverse array of topics in molecular biology, neurobiology, structural studies, and bioinformatics. The primary aim of the courses remains to teach students the latest advances in technologies and concepts that can be immediately applied to their own research. Courses are always being evaluated and updated to include the latest concepts and approaches. For example, the course on microarrays has now evolved into a course on *Integrated Data Analysis for High-throughput Biology,* since the majority of investigators are increasingly interested in the experimental design and analysis of the large data sets generated by these kinds of experiments. New Banbury courses/workshops were started on the biology of brain tumors and the biology of social cognition.

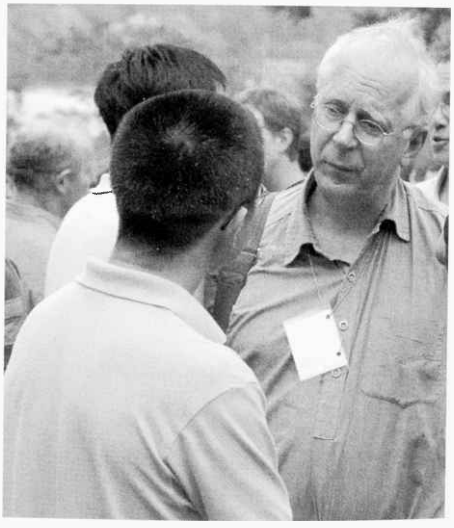
Instructors, course assistants, and course lecturers come from universities, medical schools, research institutes, and companies around the world to teach at Cold Spring Harbor. Their excellence and dedication make the course program work so well. The full program of 2006 courses and instructors are listed below. We would especially like to thank Drs. Judith Bender, Blanche Capel, Lawrence Hobbie, Thomas Hughes, Michel Labouesse, Hong Ma, Sheila McCormick, Michael Shen, Nelson Spruston, Jeff Strathern, Jason Swedlow, and Laszlo Tora, who all retired this year after many years of service.

Grants from a variety of sources support the courses. These include multiple awards from the National Institutes of Health and the National Science Foundation. We also have a valuable large education grant from the Howard Hughes Medical Institute for the support of neurobiology courses and new courses. The courses also depend on equipment and reagents that are loaned or donated by a large number of companies. These are invaluable for making it possible to keep up with the latest technologies.

The Laboratory held 24 meetings this year, which brought together more than 7100 scientists from around the world to discuss their latest research. A prime feature of the meetings is that there are very few invited speakers. Meetings organizers select talks from abstracts that are submitted. This format ensures that the latest findings will be presented and that young scientists will have the chance to describe their work.

As often happens, many meetings were oversubscribed, including *The Biology of Genomes, Retroviruses, Mechanisms and Models of Cancer and Translational Control,* as well as the annual Symposium. Many of the meetings have become essential for those in the field and are held on a biannual basis. Partial support for individual meetings is provided by grants from NIH, NSF, Department of Energy, foundations, and companies. Core support for the meetings program is provided by the Laboratory's Corporate Patrons, Benefactors, Sponsors, Affiliates, and Contributors.

The Symposium—now in its 71st year—continues to be the flagship conference of the meetings program. This year's meeting on Regulatory RNAs addressed the regulation of cellular processes by RNA-based mechanisms and featured 70 talks and 209 poster presentations. Opening night speakers included Susan Gottesman, David Baulcombe, Gary Ruvkun, and Greg Hannon, and Elizabeth Blackburn presented the Reginald Harris lecture on "Effects of Telomerase RNP Knockdown." Ronald Plasterk enlightened a mixed audience of scientists and lay



D. Baulcombe

friends and neighbors with his Dorcas Cummings lecture on "The Emerging World of Small RNAs" and Joan Steitz ended the meeting with a masterful and eloquent summary.

The joint Cold Spring Harbor/ Wellcome Trust conference series held at the genome campus south of Cambridge, England, was expanded to include meetings on *Interactome Networks, Genomic Perspectives to Host-pathogen Interactions, Genome Informatics, and Integrative Approaches to Brain Complexity.* These conferences follow the Cold Spring Harbor model in that the majority of talks are selected from the abstracts; they attracted a total of 479 participants.

The success of the very large number of meetings and courses is also due to the skilled work of many Cold Spring Harbor staff and faculty who contribute their expertise, efforts, and good humor to the program.

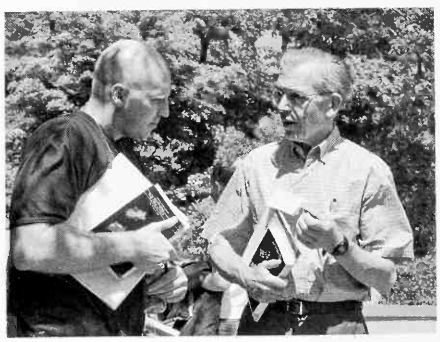
Terri Grodzicker
Assistant Director for Academic Affairs

David Stewart
Executive Director, Meetings and Courses Program

Dining on the terrace overlooking Cold Spring Harbor at the *Symposium on Regulatory RNAs*

A leisurely lunch on the lawn at the *Structural Biology* meeting

Members of the *Integrated Data Analysis for High-throughput* course

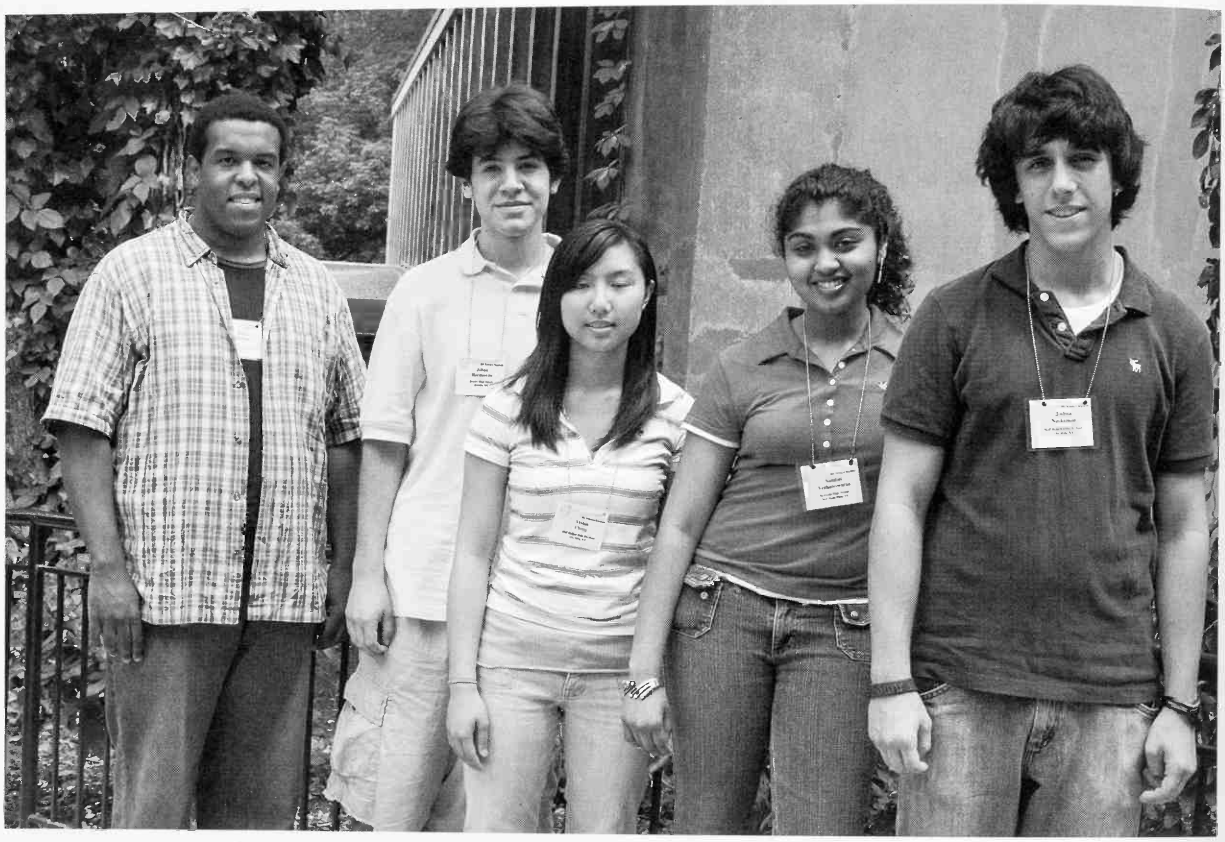


J. Pomerening and D. Thron at *The Cell Cycle* meeting

PROFESSOR FOR A DAY PROGRAM

Cold Spring Harbor Laboratory's "Professor for a Day" program introduces a select group of juniors and seniors drawn from high schools throughout Long Island who show real interest in pursuing a career in science to experience a day at a Cold Spring Harbor meeting. A small group of students (5–10 maximum) sit in on platform sessions, listen to the talks, attend one of the meeting's poster sessions, take a tour of the Laboratory, and meet some of the scientists who congregate at Cold Spring Harbor to exchange the latest concepts and methods in their field of science.

This first year, students participated in the *Channels, Receptors, and Synapse* meeting; *The Cell Cycle* meeting; and the *71st Symposium on Regulatory RNAs*.



J. Williams, Program Mentor; J. Hershowitz, V. Cheng, N. Venkateswaran, J. Nackenson at the *71st Symposium on Regulatory RNAs*

71th COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

Regulatory RNAs

May 31–June 5 469 participants

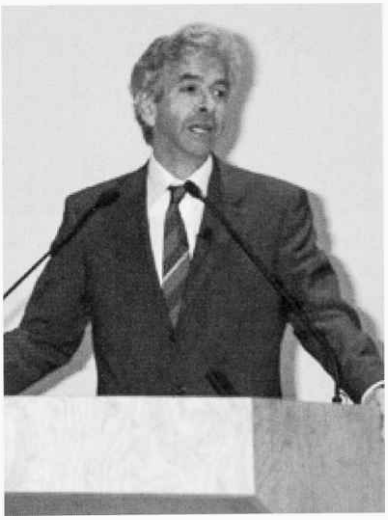
ARRANGED BY **Bruce Stillman and David Stewart**, Cold Spring Harbor Laboratory

The role of DNA as the primary information carrier and RNA as the interpretative system for that information has been the key tenet of modern biology—the central dogma of molecular biology. This was modified by the discovery of reverse transcription and presented at this Symposium in 1970. The large majority of enzymatic reactions are carried out by proteins, although the discovery of catalytic RNA and its emerging centrality in certain highly conserved cellular processes such as protein synthesis and eukaryotic mRNA splicing have increasingly suggested that RNA has a more diverse and complex role in the cell than initially proposed.

The discovery of RNA interference (RNAi) and the ubiquity of microRNAs in diverse systems have clearly established that this polymer regulates gene transcription and translation in hitherto unexpected ways, as well as providing cellular defense mechanisms against viruses. How many of these regulatory processes are relics of an ancient RNA-based world, as has been suggested for the riboswitches, remains unclear, but their growing importance in both prokaryotic and eukaryotic biology is fueling research and new ideas in laboratories around the world. Transcriptional and translational control by RNA, epigenetic phenomena such as RNA-directed DNA methylation and RNAi-directed heterochromatin formation, meiotic silencing, and cellular defense mechanisms against viruses are just some of the current areas under intense investigation through a variety of genetic, genomic, biochemical, cell biological, and structural approaches.

It was therefore deemed timely to focus the annual Symposium broadly on the theme of regulation of cellular processes by RNA-based mechanisms. It is increasingly clear that many of these processes may be harnessed to perturb biological function in a variety of biological systems, work that has reached its most widespread application with the use of large-scale RNAi screens. The Symposium explored how the application of these technologies, combined with a growing understanding of the molecular basis of many of these processes, is yielding new insights into the treatment of many human diseases.

In organizing this Symposium with considerable help from Terri Grodzicker, we relied on the assistance of our colleagues Greg Hannon and Adrian Krainer for suggestions for speakers while Susan Gottesman provided useful guidance in the prokaryotic field. We also wish to thank the first evening speakers—Susan Gottesman, David Baulcombe, Gary Ruvkun, and Greg Hannon—for providing an overview of the areas to be covered. This year's Reginald Harris Lecture was delivered by Elizabeth Blackburn on telomere ribonucleoprotein biology. Thanks to Joan Steitz, with help from Greg Hannon, for delivering a thoughtful and realistic summary of the current state of the field, and to Ronald Plasterk, who conveyed the excitement and surprises of the emerging world of small RNAs in his Dorcas Cummings Lecture to the local community and the attending scientists. We also thank Val Pakaluk and Mary Smith in the Meetings and Courses Program office for their efficient help in organizing the Symposium.

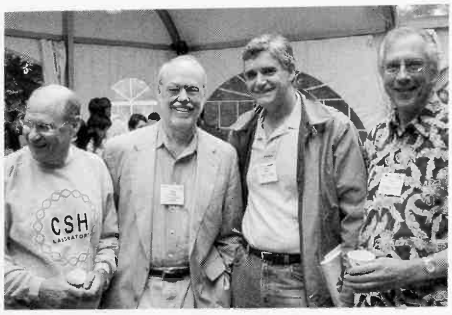


R. Plasterk, Dorcas Cummings Lecture

J. Steitz giving the Summary

This Symposium was attended by 469 scientists from more than 25 countries, and the program included 70 oral presentations and 209 poster presentations.

Essential funds to run this meeting were obtained from the National Cancer Institute, a branch of the National Institutes of Health. In addition, financial help from the corporate benefactors, sponsors, affiliates, and contributors of our meetings program is essential to the continued success of these Symposia and we are most grateful for their continued support. *Corporate Patrons* include Pfizer Inc. *Corporate Benefactors* include Amgen Inc.; GlaxoSmithKline; and Novartis Institutes for BioMedical Research. *Corporate Sponsors* include Abbott Laboratories; Applied Biosystems; BioVentures, Inc.; Bristol-Myers Squibb Company; Diagnostic Products Corporation; Forest Laboratories, Inc., GE Healthcare Bio-Sciences; Genentech, Inc.; Hoffmann-La Roche Inc.; Johnson & Johnson Pharmaceutical Reseach and Development, LLC; Kyowa Hakko Kogyo Co., Ltd.; Merck Research Laboratories; New England BioLabs, Inc.; OSI Pharmaceuticals, Inc.; Pall Corporation; Sanofi-Aventis; and Schering-Plough Research Institute.



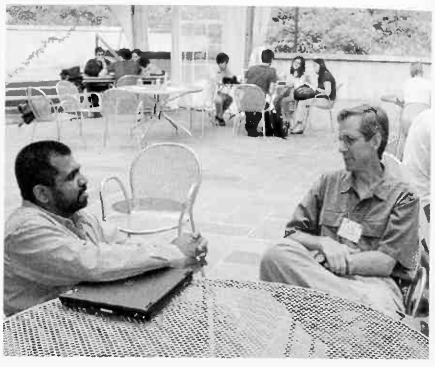
N. Sonenberg, P. Sharp, B. Stillman, T. Cech

M. Terns, J. Dahlberg, J. Steitz

S. Grewel, D. Moozed

N. Sacchi, J. Watson

C. Sharma, R. Giegerich



S. Jayesena, J. McSwiggen

R. Jorgensen, D. Baulcombe, M. Timmermans, R. Plasterk

H. Sussman

Plant Corporate Associates include ArborGen; Monsanto Company; and Pioneer Hi-Bred International, Inc. *Corporate Affiliates* include Abcam and Agilent Technologies. *Corporate Contributors* include Aviva Systems Biology; Bethyl Labs; Cell Signaling Technology; Epicentre Biotechnologies; inGenious Targeting Laboratory Inc.; Illumina; and IRx Therapeutics. *Foundations* include Albert B. Sabin Vaccine Institute, Inc. and Hudson Alpha Institute for Biotechnology.

PROGRAM

Introduction
T. Grodzicker, *Cold Spring Harbor Laboratory*

Mechanism and Biology of RNAi
Chairperson: N. Proudfoot, *University of Oxford, United Kingdom*

Genome-Wide Approaches
Chairperson: B. Bass, *HHMI/University of Utah School of Medicine, Salt Lake City*

Small RNAs in Development
Chairperson: G. Storz, *NICHD/NIH, Bethesda, Maryland*

Telomeres and Cancer
Chairperson: B. Stillman, *Cold Spring Harbor Laboratory*

Reginald B. Harris Lecture: Effects of Telomerase RNP Knockdown
Speaker: E.H. Blackburn, *University of California, San Francisco*

End Regulation of Transcripts
Chairperson: T. Nilsen, *Case Western Reserve University, Cleveland, Ohio*

RNPs and RNA Editing
Chairperson: J. Doudna, *University of California, Berkeley*

Biology of Short RNAs
Chairperson: C. Greider, *Johns Hopkins University School of Medicine, Baltimore, Maryland*

Control of Gene Expression by Noncoding RNAs
Chairperson: D. Spector, *Cold Spring Harbor Laboratory*

Dorcas Cummings Lecture: The Emerging World of Small RNAs
Speaker: Ronald Plasterk, *Hubrecht Laboratory*

Heterochromatin
Chairperson: M. Kuroda, *Harvard University, Boston, Massachusetts*

Quality Control, mRNA Turnover, and Translational Control
Chairperson: P. Zamore, *University of Massachusetts Medical School, Worcester*

Summary
J. Steitz, *Yale University School of Medicine*

Viewing the Dorcas Cummings Lecture

MEETINGS

Neuronal Circuits: From Structure to Function

March 9–12 164 participants

ARRANGED BY **Edward Callaway,** Salk Institute for Biological Sciences
Dmitri Chklovskii, Cold Spring Harbor Laboratory
Liquin Luo, Stanford University

In the quest to understand the brain, neuronal circuits represent a central level of description. Establishing connectivity in neuronal circuits seems to be as essential for solving the brain as having a geographic map for planning 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. Included at this meeting were six broad slide sessions covering the olfactory system, the visual system, methods, behavior learning and memory, and motor systems and the cortex, as well as a very interactive poster session.

For a first meeting of this kind, the response of the field was very enthusiastic. The meeting brought together participants from all over the world, most of whom made either oral or poster presentations. Invited talks were given by D. Anderson, C. Bargmann, L. Buck, T. Clandinin, W. Denk, B. Dickson, C. Dulac, M. Goulding, W. Kristan, K. Martin, I. Meinertzhagen, A. Bernard, K. Kindt, P. Somogyi, S. Smith, P. Sterling, and K. Svoboda. 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.

The meeting program included a Larry Katz memorial, where Liqun Luo, Ed Callaway and Cori Bargmann shared their reminiscences with the audience.

This meeting was funded in part by The Swartz Foundation.

D. Chklovskii, D. O'Malley

L. Buck, L. Luo

PROGRAM

Olfactory/Gustatory System
Chairpersons: C. Dulac, *HHMI/Harvard University, Cambridge, Massachusetts;* D. Anderson, *HHMI/California Institute of Technology, Pasadena*

Visual System I
Chairpersons: P. Sterling, *University of Pennsylvania, Philadelphia;* T. Clandinin, *Stanford University, California*

Visual System II
Chairpersons: P. Sterling, *University of Pennsylvania, Philadelphia;* T. Clandinin, *Stanford University, California*

Methods
Chairperson: W. Denk, *Max-Planck Institute for Medical Research, Heidelberg, Germany*

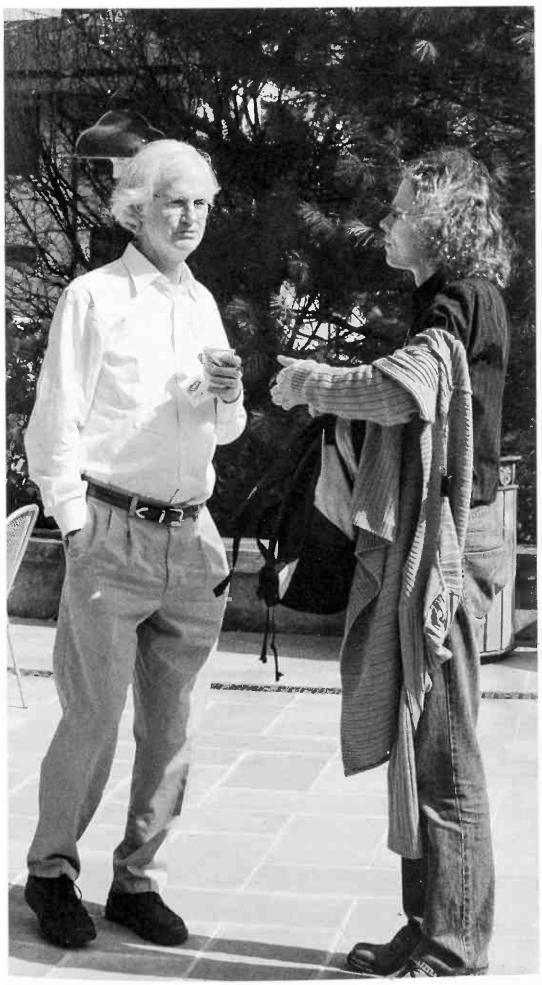
Behavior, Learning, and Memory I
Chairpersons: C. Bargmann, *HHMI/The Rockefeller University, New York;* B. Dickson, *Institute of Molecular Pathology, Vienna, Austria*

Behavior, Learning, and Memory II
Chairpersons: C. Bargmann, *HHMI/The Rockefeller University, New York;* B. Dickson, *Institute of Molecular Pathology, Vienna, Austria*

Motor and Oscillations
Chairperson: M. Goulding, *Salk Institute, La Jolla, California*

Hippocampus
Chairperson: P. Somogyi, *Medical Research Council, Oxford, United Kingdom*

Cortex
Chairpersons: K. Svoboda, *HHMI/Cold Spring Harbor Laboratory;* K. Martin, *Swiss Federal Institute of Technology, Zürich*



D. Hall, R. Kurtzler

Y. Jin, K. Brose, J. Simpson

J. Gray, S. Ashraf

PTEN Pathways

March 15–19 135 participants

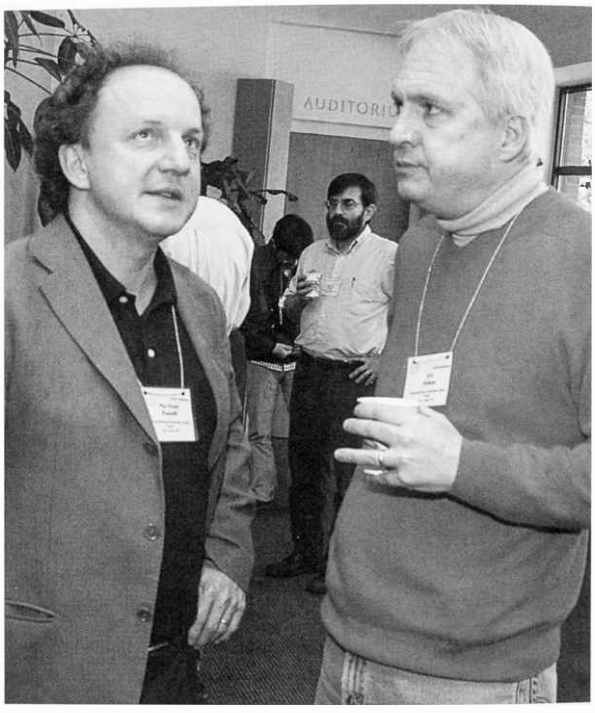
ARRANGED BY **Carlos Cordon-Cardo,** Memorial Sloan-Kettering Cancer Center
Pier Paolo Pandolfi, Memorial Sloan-Kettering Cancer Center
Ramon Parsons, Columbia University
William Sellers, Dana-Farber Cancer Institute

This first conference was enormously successful. It provided many scientists in the top of this field—who are involved in the study of molecular, genetic, and biochemical approaches to the analysis of the PTEN pathway in cancer, diabetes, and neurological disorders such as Parkinson's disease–the opportunity to meet with 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 human cancer pathogenesis, disease models, and therapy.

A total of 26 investigators presented in the nine scientific sessions, with nearly 55 platform and poster presentations and 135 registered attendees. The nine 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. Topics at the meeting are listed below under "Program."

Essential funding for the meeting was provided by the National Cancer Institute, a branch of the National Institutes of Health, and the National Science Foundation.

L. Cantley, C. Cordon-Cardo



P. Paolo Pandolfi, E. Holland

PROGRAM

PTEN/PI-3 Kinase Pathway Alterations in Human Cancer
Chairperson: G. Mills, *University of Texas M.D. Anderson Cancer Center, Houston*

PTEN Regulation
Chairperson: M.-M. Georgescu, *University of Texas M.D. Anderson Cancer Center, Houston*

PI-3 Kinase, IRS, PDK1, and AKT Signaling
Chairperson: L. Cantley, *Harvard Medical School, Boston, Massachusetts*

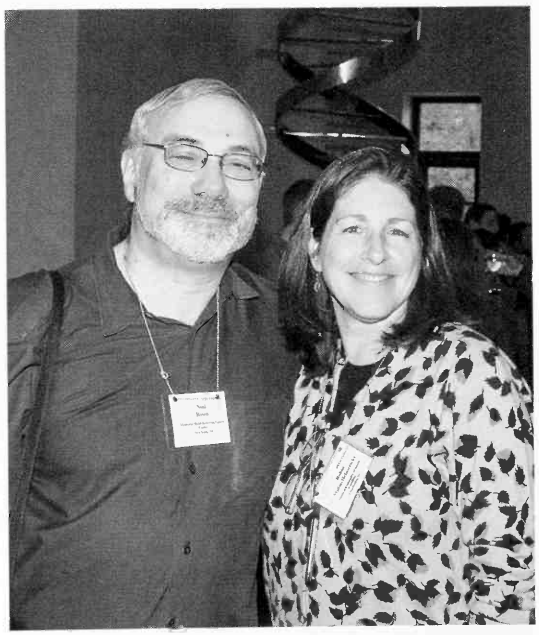
AKT & FOXO Signaling
Chairperson: A. Brunet, *Stanford University, California*

TSC and mTOR Signaling
Chairperson: S. Baker, *St. Jude Children's Research Hospital, Memphis, Tennessee*

PTEN Pathways, Development, and Metabolic Diseases
Chairperson: D. Accili, *Columbia University College of Physicians & Surgeons, New York*

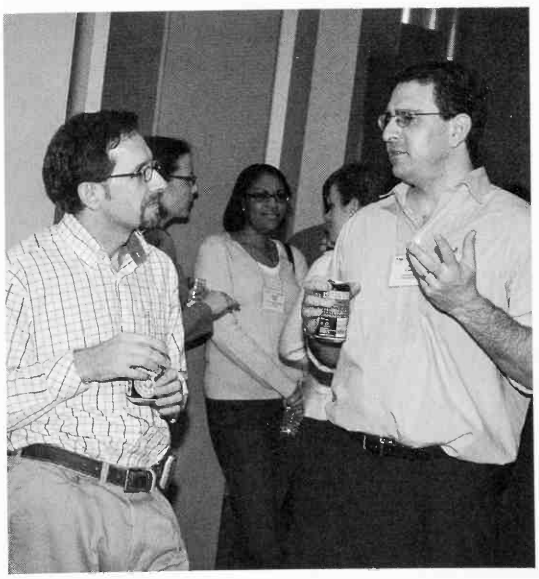
Therapy
Chairperson: S. Lowe, *HHMI/Cold Spring Harbor Laboratory*

E. Janssen, C. Knobbe



N. Rosen, R. Muise-Helmericks

T. Lingraj, J. Thomas, A. Fischl



J. Jackson, J. Kahana

Systems Biology: Global Regulation of Gene Expression

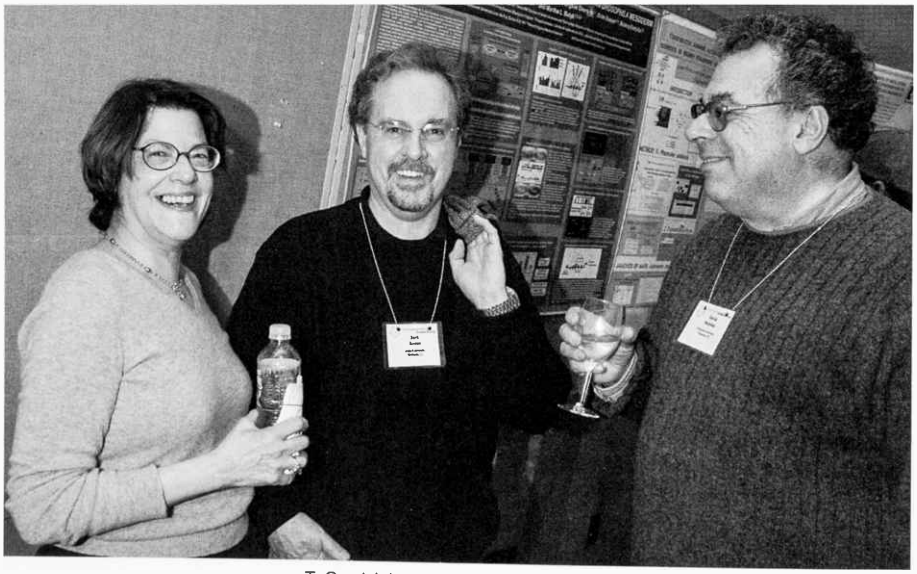
March 23–26 275 Participants

ARRANGED BY **Jack Keene,** Duke University Medical Center
Peggy Farnham, University of California, Davis
Nir Friedman, Hebrew University

Gene expression is one of the most important processes determining the functional characteristics of organisms. Investigating the mechanisms that regulate gene expression is essential to understating all biological processes and diseases. Most knowledge of the regulation of gene expression in the past has been derived by investigations of single interactions and outcomes. However, the onset of the genomic revolution has for the first time allowed the ability to investigate many events in gene expression simultaneously and in parallel. Much progress has been made in the study of transcriptional regulation, but the global regulation of gene expression involving posttranscriptional mechanisms as well is especially challenging. Therefore, the global analysis of gene expression involving both transcription and posttranscription was the subject of this fourth meeting on Systems Biology.

It was unique in that it brought together researchers using three major approaches to global gene expression and systems biology, including transcription, posttranscription (RNA splicing, localization, stability, and translation), and computational analysis. The sessions were structured with an opening keynote speaker whose research overlaps all of these three areas and was followed in the first session by representative presentations form each of the three areas. The subsequent sessions considered first computational, then transcriptional, then posttranscriptional, and then comparative genomics, followed on the last day with a session on emerging technologies. Poster sessions that represented these different approaches to understanding global gene expression were well attended, allowing direct interactions among investigators with these common interests.

Two changes were made to the format of this 2006 meeting based on discussion with the participants of the previous 2005 meeting. First, the poster session was split into two parts, allowing presenters to participate in discussion of the posters at the session at which they were not presenting. Second, two workshops were held prior to the main meeting sessions. The purpose of these workshops was to give brief tutorials of bioinformatics-related approaches to bench scientists and to pro-



T. Grodzicker, J. Keene, D. Botstein

vide to the computational researchers overviews of the experimental approaches that are employed by the bench scientists. Many participants commented on the quality of the meeting and in a planning session; enthusiasm was expressed to continue this meeting series in its current format, including the incorporation of the workshops.

Partial funding was provided by the National Science Foundation.

PROGRAM

Keynote Speaker: A Systems Approach to Dissecting Immunity
A. Aderem, *Institute for Systems Biology, Seattle, Washington*

Overture
J. Keene, *Duke University, Durham, North Carolina*

Computational Approaches to Identifying *cis*-Regulatory Elements
Chairperson: N. Friedman, *Hebrew University, Jerusalem, Israel*

Advances in Detection of Transcription Factor/DNA Interactions
Chairperson: P. Farnham, *University of California, Davis*

Transcriptional and Posttranscriptional Network Modeling
Chairperson: P. Benfey, *Duke University, Durham, North Carolina*

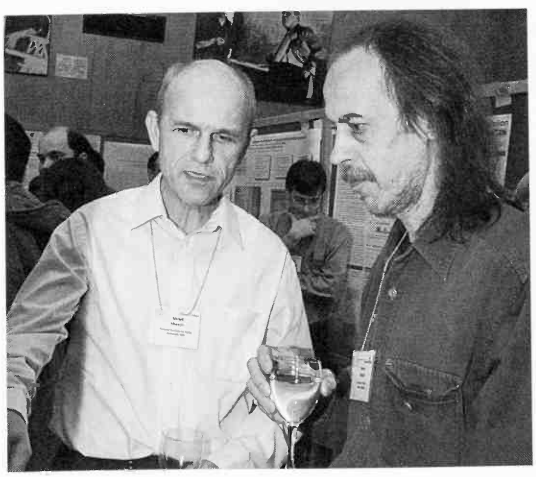
Comparative Genomics of Global Gene Regulation
Chairperson: S. Mango, *University of Utah, Salt Lake City*

Emerging Technologies and Concepts in Systems Biology
Chairperson: P. Silver, *Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusett*

R. McCord, J. Warner

W. Agnew, N. Clarke

M. Brauer, M. Torkar



A. Sharov, J. Boberg

Channels, Receptors, and Synapses

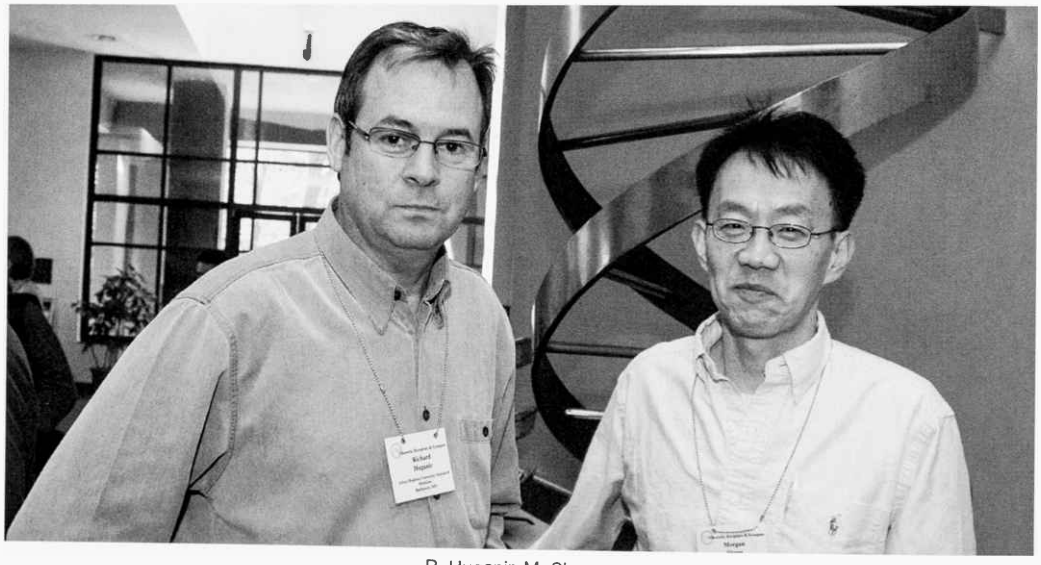
April 18–22 190 participants

ARRANGED BY **Richard L. Huganir,** HHMI/Johns Hopkins University School of Medicine
Lily Jan, HHMI/University of California, San Francisco
Morgan Sheng, HHMI/Massachusetts Institute of Technology

The brain consists of a vast network of excitable cells (neurons) that conduct electrical impulses and communicate with each other via specialized junctions (synapses). Information is processed and stored in the nervous system through patterns of electrical activity and via changes in the strength and structure of synapses. All aspects of nervous system function, including perception, cognition, and action, depend on proper information processing by synapses. As master regulators of neuronal excitability and synaptic communication, ion channels and receptors lie at the heart of neurobiology. In recent years, the molecular and cell biological analyses of neuronal ion channels and receptors have revolutionized our understanding of the basic mechanisms that control electrical signaling and synaptic function in the nervous systems. The convergence of advances in biochemistry, molecular genetics, microscopic imaging, and electrophysiology has made synaptic biology one of the most exciting and rapidly growing fields in neuroscience. Increasingly, scientists are moving to in vivo systems to investigate the synaptic basis of behavior in living animals via the genetic engineering of channels, receptors, and other synaptic proteins in mice and other model organisms.

L. Jan, K. Brose

The entire field is poised for further breakthroughs that will not only illuminate basic workings of the brain, but also shed light on neurological and psychiatric diseases that stem from abnormal neuronal excitability and synaptic dysfunction. Indeed, genetic association studies already point to genes for synaptic structure and function as being involved in neuropsychiatric illnesses. Future advances will be facilitated by cross-fertilization of ideas and technologies among scientists studying channels, receptors, and synapses at all levels, in diverse organisms and using different methodological approaches. To provide a forum that unites this exciting multidisciplinary area, this meeting was convened for a second time in April 2006 (inaugural meeting was held in April 2004).



R. Huganir, M. Sheng

The second "Synapses" meeting was extremely successful, bringing together nearly 200 participants from the United States, Europe, and Asia in an atmosphere of social and scientific exchange. A wide range of topics were discussed (see Program below). Compared to the first meeting in 2004, the 2006 meeting had less emphasis on the structure and function of ion channels and more on the cell biology and development of synapses and on the in vivo significance of synaptic function and plasticity. More than 50 attendants were selected to give oral presentations of their work, and the majority of the rest presented posters. Outstanding plenary lectures were given by Tom Sudhof (University of Texas, Southwestern) on presynaptic mechanisms and by Rob Malenka (Stanford) on molecular mechanisms of synaptic plasticity.

The overall response to the meeting was extremely positive, cementing the reputation of this Cold Spring Harbor conference in the field. Attendees enjoyed the breadth of subjects covered and the chance to interact with investigators in related but distinct fields. The unusual opportunity for junior investigators (including postdocs and students) to present their own work was welcomed. Based on its continuing success, this conference will take place again in one year (April 2007), but under the modified name "Synapses: From Molecules to Circuits and Behavior."

Lily Jan and Morgan Sheng have stepped down as organizers of the meeting. Tom Sudhof (University of Texas Southwestern) and Holly Cline (Cold Spring Harbor Laboratory) will take over and work with Richard Huganir. We expect that this Cold Spring Harbor meeting will become a "must-attend" conference for learning the latest developments in molecular and cellular physiology of the brain.

Funding for this meeting was provided by the National Institutes of Mental Health and the National Institute of Neurological Diseases and Stroke.

A. Vincent, S. Waxman

H. Beale, H. Carlisle

PROGRAM

Presynaptic Function
Chairperson: P. DeCamilli, *Yale University School of Medicine, New Haven, Connecticut*

Keynote Speaker I: How Ca^{2+} Triggers Neurotransmitter Release
Thomas Sudhof, *University of Texas Southwestern Medical Center*

Synapse Formation
Chairperson: A.M. Craig, *University of British Columbia, Vancouver, Canada*

Neuronal Signaling Mechanisms
Chairperson: M. Greenberg, *Harvard Medical School, Boston, Massachusetts*

Modulation of Channel and Receptor Function
Chairperson: I. Levitan, *University of Pennsylvania School of Medicine, Philadelphia*

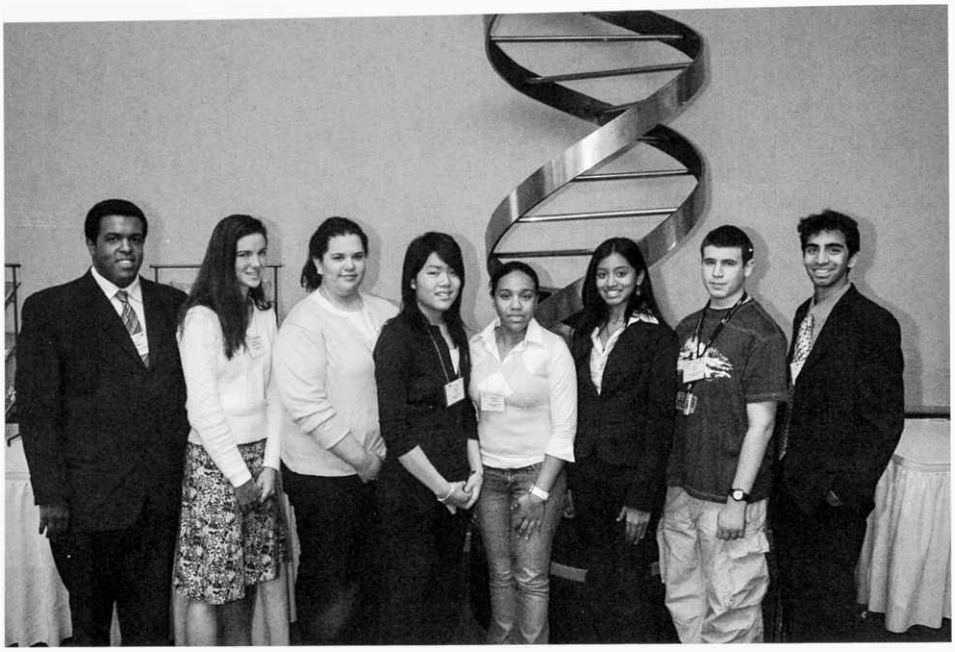
Synaptic and Nonsynaptic Plasticity
Chairperson: D. Linden, *Johns Hopkins University School of Medicine, Baltimore, Maryland*

Keynote Speaker II: Roles of PSD-95 and SAP97 in the Control of Synaptic Strength
Robert Malenka, *Stanford University School of Medicine*

Synaptic Plasticity and Development
Chairperson: H. Cline, *Cold Spring Harbor Laboratory*

Synaptic Plasticity and Behavior
Chairperson: A. Silva, *University of California, Los Angeles*

Diseases of Channels, Receptors, and Synapses
Chairperson: L. Ptacek, *University of California, San Francisco*



Professor for a Day Program students and teachers from Elmont, Manhasset, Roslyn, Uniondale, and John F. Kennedy High Schools

Gene Expression and Signaling in the Immune System

April 26–30 426 participants

ARRANGED BY **Doreen Cantrell,** University of Dundee, United Kingdom
Richard Flavell, HHMI/Yale University, New Haven, Connecticut
Rudolf Grosscheldl, Max-Planck Institute of Immunobiology, Germany
Stephen Smale, HHMI/University of California School of Medicine, Los Angeles

This year's meeting, the third in the series, continued its focus on rigorous mechanistic studies that succeed in advancing our understanding of gene regulation and signal transduction circuitry during immune development and immune responses. A new addition to this year's meeting was a session dedicated to the regulation of innate immune responses. This session broadened the meeting beyond its previous focus on adaptive immunity. The innate immunity session attracted major figures in the field, including Shizuo Akira, Ruslan Medzhitov, Sankar Ghosh, and David Baltimore, and was exceptionally well-received. One theme of this session was the mechanisms responsible for negative regulation of inflammatory gene activation.

A highlight of the meeting was the use of an RNA-interference-based screen in *Drosophila* to identify elusive, evolutionarily conserved regulators of calcium influx, which promotes the activation and nuclear translocation of NFAT transcription factors. Kinases responsible for NFAT deactivation were also identified in this screen and a separate presentation provided a link between one of these kinases and the genetic abnormalities responsible for Down's syndrome. Another major focus was the regulatory cascades involved in the maintenance of hematopoietic stem cells in an undifferentiated state, and in commitment to lymphocyte lineages. Considerable attention was also directed toward the roles of lymphocyte commitment factors in maintaining the differentiated state after lymphocyte maturation.

S. Tundip, S. Smale

This meeting was supported in part by funds provided by Abbott Bioresearch; B&D Biosciences; Elan Pharmaceuticals; Genentech, Inc.; Merck & Co.; Research and Diagnostic Systems, Inc.; Point Therapeutics, Inc.; Cell Signaling Technology, Inc.; and National Institute of Allergy and Infectious Diseases, a part of the National Institutes of Health.

R. Grosschedl, L. Goyal

PROGRAM

Stem Cells and Early Developmental Decisions
Chairperson: R. Grosschedl, *Max-Planck Institute of Immunobiology, Freiburg, Germany*

Regulation of Immune Cell Development
Chairperson: L. Glimcher, *Harvard School of Public Health, Boston, Massachusetts*

Chromatin Structure and Epigenetic Regulation
Chairperson: R. Flavell, *Yale University School of Medicine, New Haven, Connecticut*

Antigen Receptor Gene Assembly, Somatic Hypermutation, and Class Switching
Chairperson: M. Schlissel, *University of California, Berkeley*

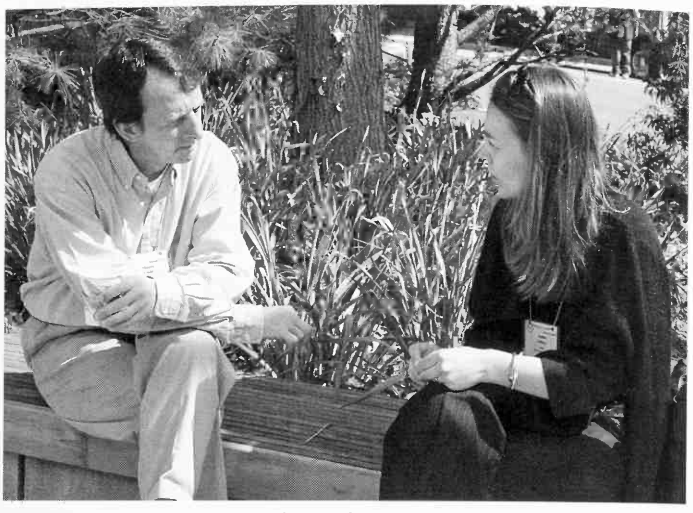
Signal Transduction in Immune Cells
Chairperson: D. Cantrell, *University of Dundee, United Kingdom*

Regulation of Lymphocyte Function I
Chairperson: G. Griffiths, *University of Oxford, United Kingdom*

Regulation of Lymphocyte Function II
Chairperson: D. Mathis, *Joslin Diabetes Center, Harvard Medical School, Boston, Massachusetts*

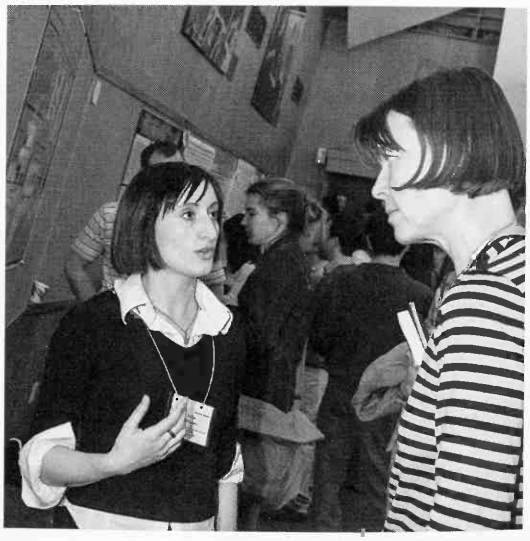
Innate Immunity
Chairperson: S. Smale, *HHMI/University of California School of Medicine, Los Angeles*

H. Yoon, J. Boss



T. Coche, S. Delhaye

M. Green, R. Schlimgen



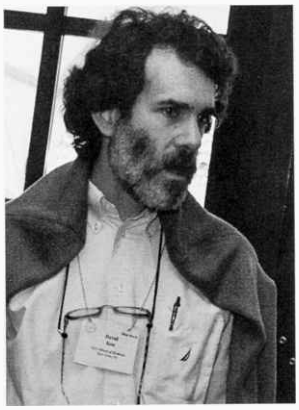
L. Cimmino, A. Spurkland

Molecular Chaperones and Heat Shock Response

May 3–7 279 participants

ARRANGED BY **James Bardwell,** HHMI/University of Michigan
David Ron, New York University School of Medicine
Jonathan Weissman, HHMI/University of California, San Francisco

This meeting—24 years after the inaugural meeting—featured advances in the areas of structure and mechanism of action of molecular chaperones, mechanisms of induction of unfolded protein stress responses in various compartments, the role of chaperones in protein degradation and in cell signaling, and chaperone function in disease and aging. In the first area, detailed structural and biophysical studies on well-characterized chaperones, such as GroEL/ES, the small heat shock factors, Hsp70, 90, and 100 classes, were presented. Structural data on the relationship between the ribosome and chaperones were presented here for the first time. New insight into regulation of oxidative protein folding in the endoplasmic reticulum was provided in a study describing allosteric regulation of the oxidase ERO1. In a session devoted to regulation of the heat shock response the involvement of a noncoding RNA were described for the first time and data suggesting that it may be a heat sensor was presented. A novel role for the endoplasmic reticulum stress-activated kinase/endonuclease IRE1 in mRNA metabolism was described for the first time.

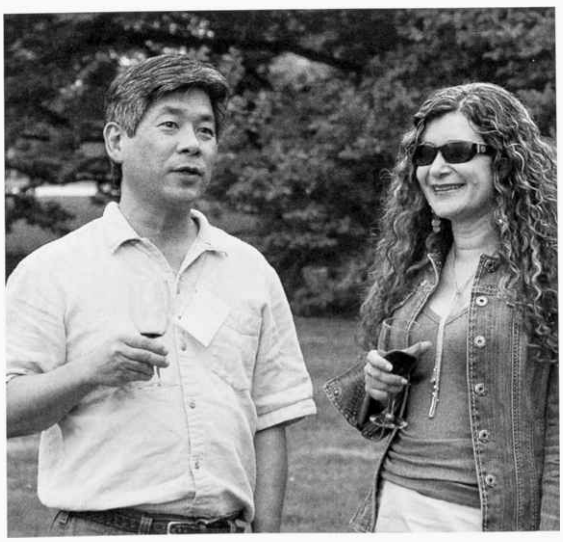


D. Ron

Several talks addressed recent developments in quality control mechanisms and signals involved in the identification and tagging of misfolded proteins for degradation.The session on protein degradation included structural analysis of the protein degradation and unfolding machines in prokaryotes (ClpB) and eukaryotes (the proteasome). Several talks described new developments in the application of model organisms to study the implications of protein misfolded to human diseases. Notable in this regard is the presentation of experimental tools to correlate the physical state of misfolded proteins with aging (in worms). Major advances in our understanding of the biophysical basis of prion diseases were presented, notably studies of prion speciation and fiber growth and fiber structure and their correlation with phenotypic outcome.

This conference was supported in part by funds provided by National Institutes of Neurological Diseases and Stroke, a part of the National Institutes of Health.

J. Heikkila, D. Mosser



R. Morimot, L. Joshua-Tor

PROGRAM

Chaperone Biochemistry and Protein Folding
Chairperson: E. Craig, *University of Wisconsin, Madison*

Quality Control and Protein Trafficking
Chairperson: W. Neupert, *University of Munich, Germany*

Cellular Response to Stress
Chairperson: A. Horwich, *Yale University School of Medicine, New Haven, Connecticut*

Chaperone Function in Disease and Development
Chairperson: F.U. Hartl, *Max-Planck Institute for Biochemistry, Martinsried, Germany*

Regulation of the Stress Response
Chairperson: R. Morimoto, *Northwestern University, Evanston, Illinois*

Chaperones and Proteolysis
Chairperson: B. Sauer, *Massachusetts Institute of Technology, Cambridge*

Diseases of Protein Misfolding
Chairperson: J. Kelly, *Scripps Research Institute, La Jolla, California*

T. Serio, V. Grimminger

K. Van Wijk, A. Breiman

D. Smith

Workshop on Chicken Genomics and Development

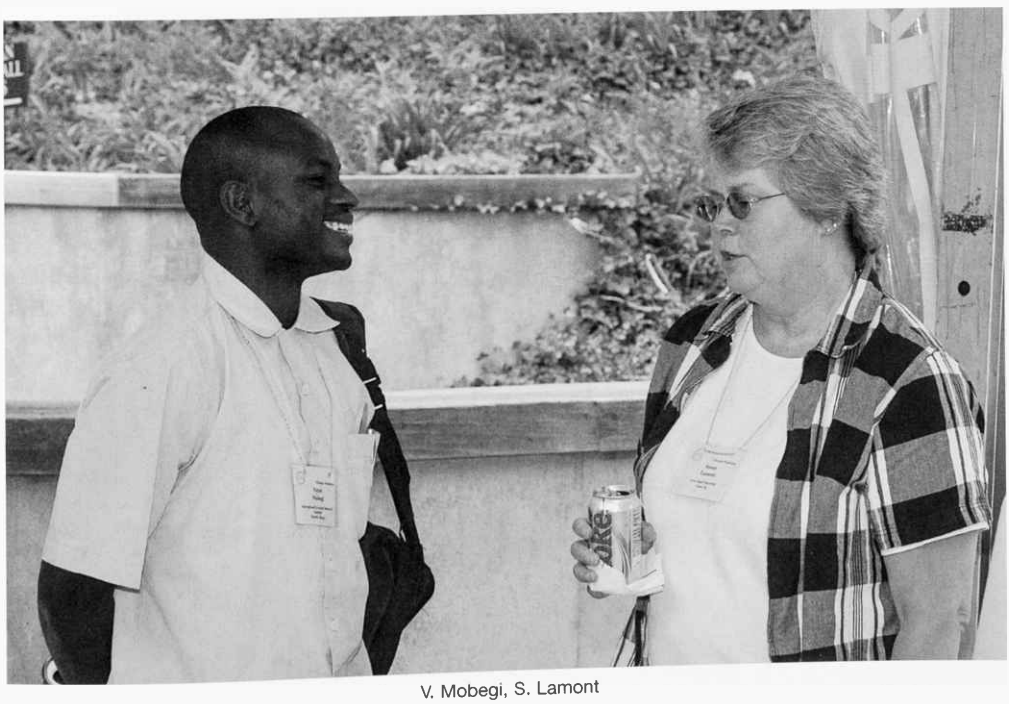
May 7–10 91 participants

ARRANGED BY **David Burt,** Roslin Institute
Olivier Pourquié, HHMI/Stowers Institute for Medical Research

This meeting was well attended by researchers representing 15 countries. There were 60 abstracts of papers presented during the sessions. The workshop began with a keynote presentation by Cliff Tabin on insights into the development of vertebrate limb and gut. Scientific sessions are listed below under "Program." Additional discussions centered on ways to improve the chicken genome, such as new release and annotations, and on the current sequencing of the zebra finch. Presentations also highlighted some successful techniques to make transgenic chicken. A wrap-up session included an exploration of "what now" and "what next" with plans to hold yearly meetings alternating between Europe and the United States to allow more people to attend this meeting and build a strong "chicken community."

This meeting was supported in part by funds provided by a grant from the Society for Developmental Biology and proceedings from *Developmental Biology*, the SDB official journal.

D. Burt



V. Mobegi, S. Lamont

PROGRAM

Opening Remarks/Plenary Session
O. Pourquié, *Stowers Institute for Medical Research, Kansas City, Missouri*

Keynote Speaker: Utilizing the Chick System to Gain Insights into the Development of the Vertebrate Limb and Gut
C. Tabin, *Harvard Medical School*

Emerging Disease and Immunity
Chairperson: D. Burt, *Roslin Institute, Edinburgh, United Kingdom*

Development I
Chairperson: C. Tabin, *Harvard Medical School, Boston, Massachusetts*

QTL and Population Biology
Chairperson: L. Andersson, *Uppsala University, Sweden*

Avian Genomes and Bioinformatics I
Chairperson: M. Groenen, *Wageningen University, The Netherlands*

Avian Genomes and Bioinformatics II
Chairperson: M. Groenen, *Wageningen University, The Netherlands*

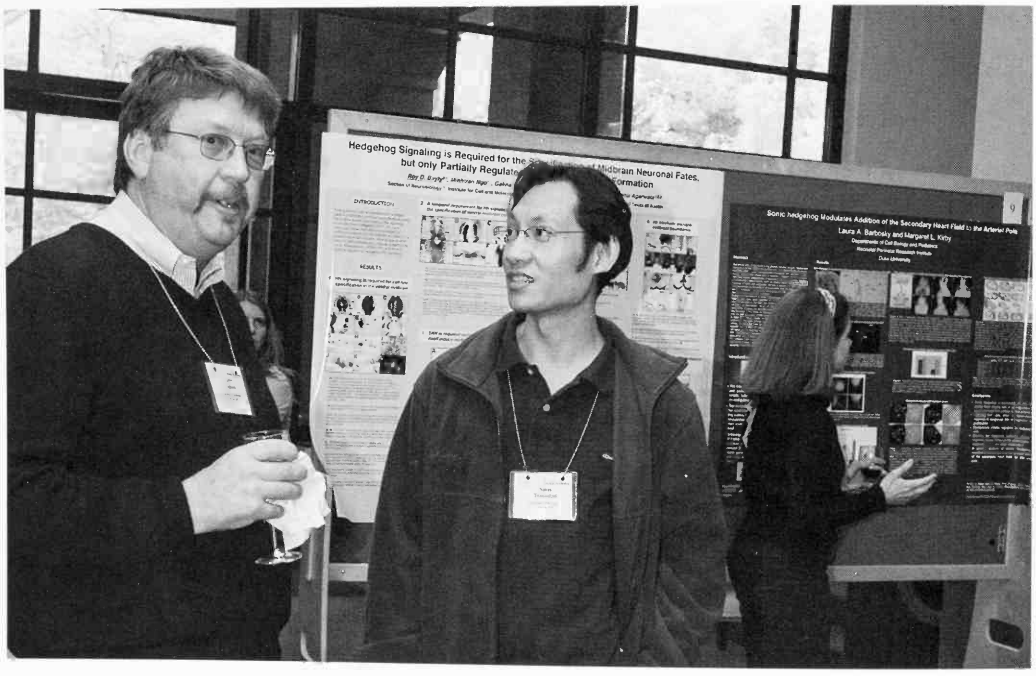
Evolution and Biodiversity
Chairperson: J. Dodgson, *Michigan State University, East Lansing*

Development II
Chairperson: P. Antin, *University of Arizona, Tucson*

What's Next?
Summary and Plans for the Future
D. Burt, *Roslin Institute*

Closing Remarks
O. Pourquié, *Stowers Institute for Medical Research*

H. Sang, J. Inglis



L. Cogburn, N. Trakooljul

The Biology of Genomes

May 10–14 524 participants

ARRANGED BY **Kelly Frazer**, Perlegen
Thomas Hudson, McGill University
Svante Pääbo, Max-Planck Institute
Richard Wilson, Washington University

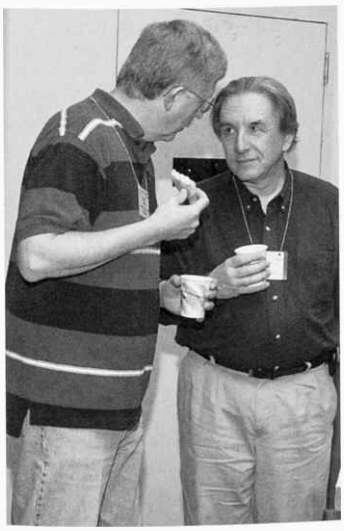
This meeting marked the 17th annual gathering of genome scientists in this setting. The past decade or more has seen remarkable progress in the mapping, sequencing, and annotation of the genomes of many "model organisms" and publication of finished and draft sequences of the human, mouse, rat, and dog genomes, in addition to several model organisms and hundreds of bacteria. Just over 500 people from around the world attended the meeting, with more than 300 abstracts presented describing a broad array of topics relating to the analysis of genomes from a number of different organisms.

T. Hudson, K. Frazer

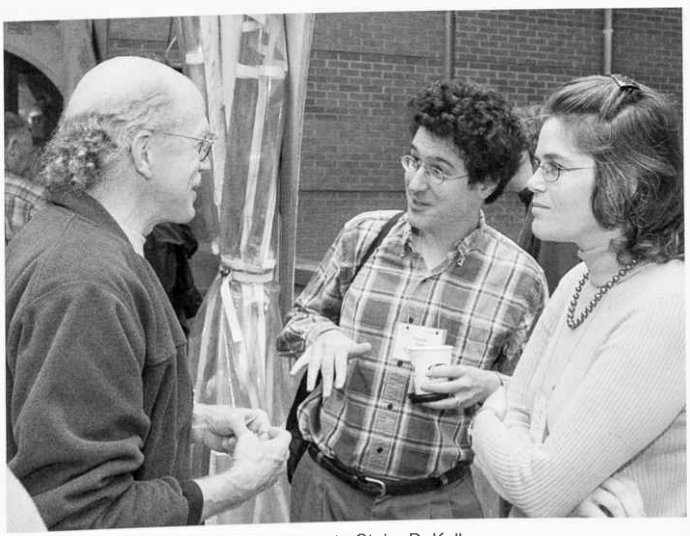
The session topics included areas such as biological insights from computational genomics, functional genomics, genomics of nonhuman species, evolutionary genomics, population genomic variation, statistical genomics and association studies, and high-throughput genomics. The International HapMap program announced the completion of the project successfully genotyping 4 million SNPs in 270 individuals. There was considerable enthusiasm and discussion for how the HapMap data would enable large-scale association studies to occur over the upcoming year. The ELSI (Ethical, Legal, and Social Implications) panel chaired by Francis Collins focused on issues surrounding data release and intellectual property challenges in the age of large-scale human genotyping and sequencing.

The major themes of the meeting included the analysis of genetic variation across the human genome, statistical analysis approaches for understanding whole-genome scans, and several new imaging and large-scale molecular approaches for understand complex biological processes. The Saturday afternoon keynote talks were delivered by David Cox, and Nancy Wexler.

Funding for this meeting was provided by National Human Genome Research Institute.



F. Collins, D. Cox



R. Waterston, L. Stein, D. Keller

PROGRAM

Computational Genomics
Chairpersons: T. Hubbard, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*; M. Kellis, *Broad Institute of MIT and Harvard, Cambridge, Massachusetts*

Functional Genomics
Chairpersons: L. Pennachio, *Lawrence Berkeley National Laboratory, California*; P. Fraser, *Babraham Institute, Cambridge, United Kingdom*

Genomics of Nonhuman Species
Chairpersons: L. Andersson, *Uppsala University, Sweden*; P. Andolfatto, *University of California, San Diego*

Evolutionary Genomics
Chairpersons: J. Graves, *Australian National University, Canberra*; H. Kaessmann, *University of Lausanne, Switzerland*

ELSI Panel Discussion: Data Release and Intellectual Property Challenges in the Age of Large-scale Human Genotyping and Sequencing
Moderator: F. Collins, *National Human Genome Research Institute*

Panelists: G. Duyk, *TPG Ventures*; W. Lowrance, *Independent Consultant*; R. Cook-Deegan, *Duke University*

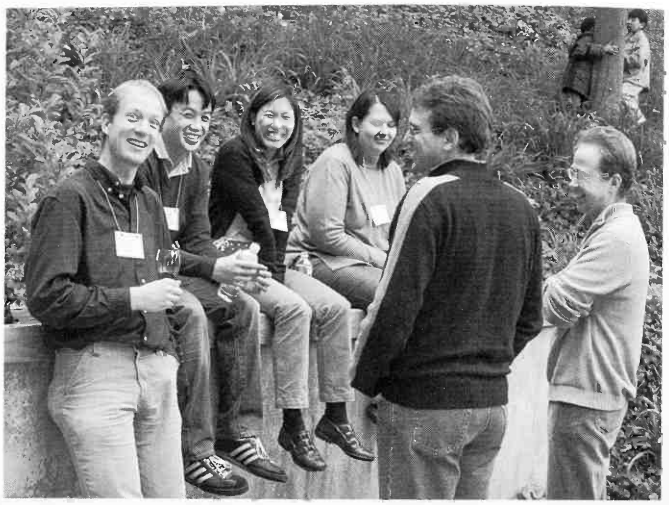
Population Genomic Variation
Chairpersons: J. Pritchard, *University of Chicago, Illinois*; J. Sebat, *Cold Spring Harbor Laboratory*

Statistical Genomics and Association Studies
Chairpersons: K. Roeder, *Carnegie Mellon University, Pittsburgh, Pennsylvania*; L. Cardon, *University of Oxford, United Kingdom*

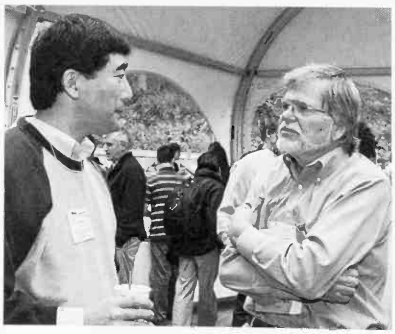
Guest Speakers
D. Cox, *Perlegen Sciences*; N. Wexler, *Columbia University*

High-throughput Genomics
Chairpersons: J. Rogers, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*; M. Uhlen, *Royal Institute of Technology, Stockholm, Sweden*

A. Baross, S. Sunkin



G. Lunter, D.-Q. Nguyen, T. Oh, Z. Lombard, A. Heger, C. Ponting



J. Ng, J. Hudson

R. Guigo, H. Sussman

S. Bhattacharya, S. Chatterjee

The Cell Cycle

May 17–21 382 participants

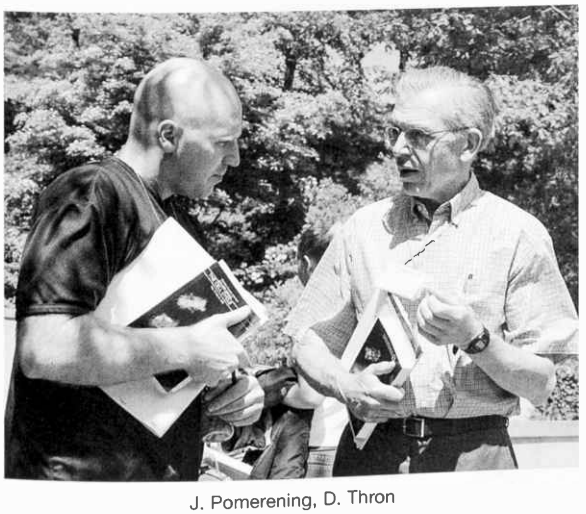
ARRANGED BY **Orna Cohen-Fix,** NIDDK/National Institutes of Health
Nicholas Dyson, Massachussetts General Hospital Cancer Center
David Morgan, University of California, San Francisco

The eighth biannual Cell Cycle meeting was held this year at Cold Spring Harbor. This conference is internationally recognized for its ability to bring together scientists who study cell cycle regulation in eukaryotes ranging from yeast to humans. As usual, the meeting began with a keynote talk from a leader in the field, and on this occasion, we were fortunate to have Dr. Marc Kirschner, Chair of the Department of Systems Biology at Harvard Medical School, tell us about his exciting new studies of molecular mechanisms controlling the vertebrate cell cycle. The remainder of the meeting was organized around eight sessions focused on the major stages of the cell division cycle, with an emphasis in each session on the molecular mechanisms that govern cell cycle progression.

Many of these sessions focused on long-standing problems in cell cycle control, including the transcriptional mechanisms controlling entry in the cell cycle, the mechanisms that initiate chromosome duplication and limit it to once per cell cycle, the mechanisms that trigger the complex events of chromosome segregation in mitosis, and the numerous checkpoint systems that ensure that cell cycle events occur in the correct order. The mechanics of mitosis, and in particular the operation of the mitotic spindle, received considerably more attention at this meeting than in the past, thanks to a surging interest in the complex processes that lead to assembly of the spindle and govern the behavior of chromosomes during mitosis.

For the first time, the meeting also included a half-session on the exciting field of cytokinesis, the process by which the cytoplasm is divided at the end of the cell cycle. Other emerging areas, including the control of meiosis and the coordination of cell division with cell growth, were also represented. As always, every major model system for cell cycle analysis was represented, and the striking phylogenetic conservation of cell cycle regulatory mechanisms was readily evident. The meeting also served to demonstrate the impact of whole-genome analysis on basic cell biology, and these achievements will no doubt continue to drive rapid progress in this field. It was another landmark meeting for the cell cycle field, and the participants continue to look forward to equally exciting meetings in future years.

Funding for this meeting was provided by the National Cancer Institute.



J. Pomerening, D. Thron

O. Cohen-Fix, D. Morgan, S. Elledge

PROGRAM

Cell Cycle Control Mechanisms
Chairperson: J. Ferrell, *Stanford University School of Medicine, California*

Keynote Speaker
M. Kirschner, *Harvard Medical School*

G_1 Control I
Chairpersons: J.W. Harper, *Harvard Medical School, Boston, Massachusetts*; S. Van den Heuvel, *Utrecht University, The Netherlands*

G_1 Control II
Chairpersons: B. Edgar, *Fred Hutchinson Cancer Research Center, Seattle, Washington*; M. Tyers, *Samuel Lunenfeld Research Institute, Toronto, Canada*

S Phase
Chairpersons: S. Bell, *HHMI/Massachusetts Institute of Technology, Cambridge*; J. Walter, *Harvard Medical School, Boston, Massachusetts*

M Phase I: Mitotic Chromosomes and the Spindle
Chairpersons: D. Koshland, *HHMI/Carnegie Institute of Washington, Baltimore, Maryland*; C. Walczak, *Indiana University, Bloomington*

M Phase II: APC and the Spindle Checkpoint
Chairpersons: S. Biggins, *Fred Hutchinson Cancer Research Center, Seattle, Washington*; A. Musacchio, *European Institute of Oncology, Milan, Italy*

M Phase III: Anaphase and Cytokinesis
Chairpersons: M. Glotzer, *University of Chicago, Illinois*; F. Uhlmann, *Cancer Research UK London Research Institute*

DNA Damage Response
Chairpersons: K. Cimprich, *Stanford University, California*; S.J. Elledge, *Harvard Medical School, Boston, Massachusetts*

J. Lahti, K. Kitagawa

R. Scalfani, J. Hamlin

S. Stoynov, Y. Boyarchuk

Professor for a Day Program students S. Christedes, K. Bedkowski, J. Cohen, H. Lewkowitz-Shpuntoff, L. Libresco, M. Indaram

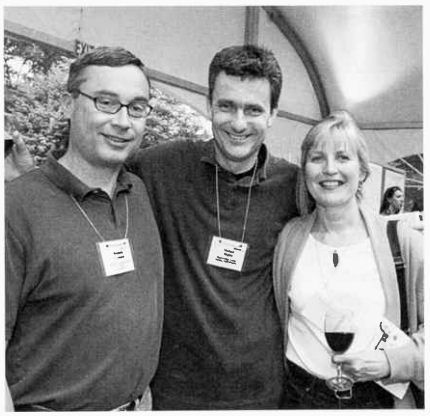
Retroviruses

May 23–28 459 participants

ARRANGED BY **Frederic Bushman,** University of Pennsylvania School of Medicine
Jaquelin Dudley, The University of Texas, Austin

This 31st annual meeting devoted to retroviruses was organized into two keynote addresses, ten sessions of short talks on specific topics, and three poster sessions.

Two distinguished scientists delivered the keynote addresses: Michael Malim described the discovery of the APOBEC antiretroelement system and the function of HIV Vif, and Marina Cavazzana-Calvo described results from the first unambiguously successful gene therapy trial in humans, as well as the first example of insertional activation of oncogenes in humans by a retrovirus vector. The choice of speakers allowed a wide array of retrovirus topics to be addressed in basic and applied retrovirology. Both talks were very well received.



F. Bushman, M. Malim, J. Dudley

Ten sessions were devoted to short talks on specific topics including virus entry, assembly, restriction factors, antiviral agents, integration, accessory genes, pathogenesis, reverse transcription, and RNA synthesis, processing, and export. Poster sessions were held on Wednesday evening, Thursday afternoon, and Friday evening and were organized alphabetically, so that poster presenters were able to see some of the posters in their fields. Evening poster sessions facilitated interactions by participants since there were no events immediately following. A multitude of significant new advances were reported, including new findings on pathogenesis, restriction factors, assembly, and integration. Because of the intense interest in retroviral restriction factors and assembly, there were two sessions on each of these subjects. The largest number of abstracts was presented on retroviral assembly, and this area was highlighted and summarized on the front cover of the abstract book by an excellent graphic kindly provided by Wes Sundquist.

In other matters, the meeting is increasingly user friendly. Strengths are the improved food, banquet, and A-V/computer support. Wireless Internet access was appreciated by many participants. Weaknesses are minor, but include off-site housing for some participants, proximity to Memorial Day, sporadic cell phone coverage, and small size of the bar. We very much appreciated the help and guidance of David Stewart, Mark Beavers, and the CSHL audiovisual staff, who greatly facilitated the meeting organization.

Funding for this meeting was provided by the CSHL Corporate Sponsor program.

A.M. Skalka, M. Katzman

PROGRAM

Pathogenesis
Chairpersons: J. Stoye, *National Institute for Medical Research, London, United Kingdom*; H. Fan, *University of California, Irvine*

Assembly I
Chairpersons: P. Cannon, *University of Southern California, Los Angeles*; H. Göttlinger, *University of Massachusetts Medical School, Worcester*

Restriction Factors I
Chairpersons: T.J. Hope, *Northwestern University, Chicago, Illinois*; V.N. KewalRamani, *National Cancer Institute, Frederick, Maryland*

Keynote Speaker: Two Decades of Vif Research—An Illustration of Viral Sophistication?
M. Malim, *King's College London*

Assembly II
Chairpersons: K. Musier-Forsyth, *University of Minnesota, Minneapolis*; E.O. Freed, *National Cancer Institute, Frederick, Maryland*

Entry
Chairpersons: L. Pedersen, *Aarhus University, Denmark*; M.J. Federspiel, *Mayo Clinic College of Medicine, Rochester, Minnesota*

Reverse Transcription/Antivirals
Chairpersons: A. Telesnitsky, *University of Michigan, Ann Arbor*; S.H. Hughes, *National Cancer Institute, Frederick, Maryland*

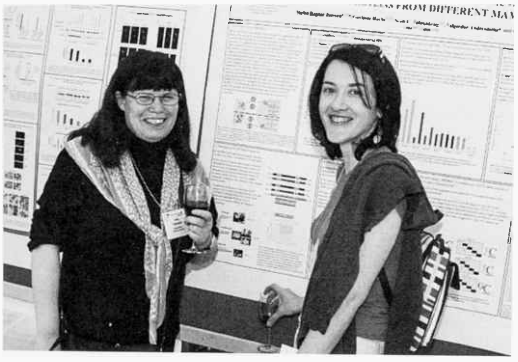
Integration
Chairpersons: P. Cherepanov, *Imperial College London, United Kingdom*; J. Grobler, *Merck Research Laboratories, West Point, Pennsylvania*

Keynote Speaker: Where Do We Stand Six Years after Gene Therapy?
M. Cavazzana-Calvo, *Hôpital Necker-Enfants Malades*

Transcription/Processing/Export/Translation/Packaging
Chairpersons: B. Berkout, *Academic Medical Center, University of Amsterdam, The Netherlands*; A.M. Lever, *University of Cambridge, United Kingdom*

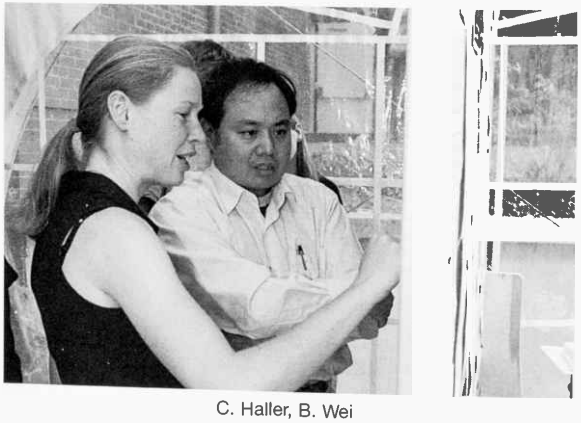
Restriction Factors II
Chairpersons: N.R. Landau, *Salk Institute, La Jolla, California*; K. Strebel, NIAID/*National Institutes of Health, Bethesda, Maryland*

Accessory Genes
Chairpersons: K. Boris-Lawrie, *Ohio State University, Columbus*; F. Kirchhoff, *University of Ulm, Germany*



G. Tachedjian, T. Hatziioannou

S. Baenziger, J. DeStefano



C. Haller, B. Wei

New York Structural Biology Summer Forum

July 19 250 participants

ARRANGED BY **Hao Wu,** Weill Medical College of Cornell University
David Stokes, Skirball Institute, New York University

This conference was the eighth in day-long meetings allowing structural biologists from all over the region to meet and discuss their latest results. It was open to structural biologists from different disciplines, including crystallographers, spectroscopists, computational biologists, and biochemists, with 250 participants from academia and industry from the tri-state area. The program featured 13 talks by faculty, postdocs and students, as well as a poster session. A beach barbecue concluded the meeting, allowing a wonderful opportunity for informal interactions. This meeting complements the bimonthly evening meetings of the group held at The Rockefeller University. No registration was required and participants were encouraged to set up posters.

Financial support was provided by Cell Press, FEI Corp., Gatan, GE Healthcare, Hampton Research, Imclone, JEOL USA, Merck, Rigaku, Roche Pharmaceuticals, and Varian.

Waiting in the auditorium for the meeting to begin

PROGRAM

Session I

Chairperson: D. Stokes, *Skirball Institute, New York University School of Medicine*; R. Ghose, *City College of New York*; Y. Huang, *Skirball Institute, New York University School of Medicine*; J. Nandakumar, *Memorial Sloan-Kettering Cancer Center*; S. Blanchard, *Weill Medical College of Cornell University*

Session II

Chairperson: H. Wu, *Weill Medical College of Cornell University*; F. Mancia, *Columbia University*; A. Stewart, *SUNY Stony Brook*; J. Fernandez, *Columbia University*

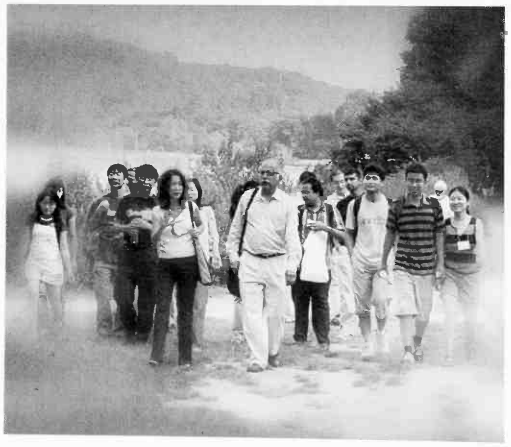
Session III

Chairperson: W. Hendrickson, *Columbia University College of Physicians & Surgeons, New York*; A. McDermott, *Columbia University*; M. Vettings, *Einstein College of Medicine*; G. Scapin, *Merck*

Session IV

Chairperson: L. Joshua-Tor, *Cold Spring Harbor Laboratory*; C. Simmerling, *SUNY Stony Brook*; E. Enemark, *Cold Spring Harbor Laboratory*; D. Patel, *Memorial Sloan-Kettering Cancer Center*

The picnic is always a treat



On the beach

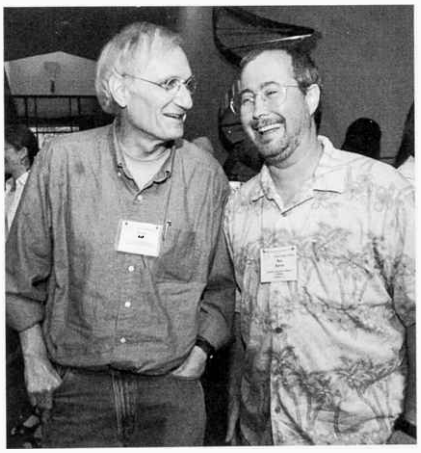
A leisurely lunch on the lawn

Glia in Health and Disease

July 20–24 211 participants

ARRANGED BY **Ben Barres,** Stanford University School of Medicine
Martin Raff, University College London

Glial cells constitute 90% of cells in the human nervous system, but their roles in the developing, adult, and diseased brain are still poorly understood. In this new meeting, students and scientists from across the world gathered to discuss their recent progress. In the first part of the meeting, sessions focused on understanding the normal roles of glia, including sessions on glial development, genetic analysis of glial function, myelination, axon-glial interactions, and astrocyte function at synapses and blood vessels. Next, recent progress on understanding the roles of glia in reactive gliosis, central nervous system regenerative failure, and disease processes including multiple sclerosis, AIDS, glioma, and neuropathic pain.



M. Raff, B. Barres

The meeting concluded with a session highlighting advances in understanding glia that have been made possibly by new techniques, including time-lapse imaging using two-photon microscopy, laser ablation, mosaic analysis with double markers in mice, and new transgenic mouse models. By the end of the meeting, there was a sense of excitement that the function of glia is finally at long last emerging from the shadows, with glia having central roles in normal brain development, function, and disease.

Funding for this meeting was provided by the CSHL Corporate Sponsor program.

M. Chheda, H. Higashimori

U. Gaul, M. Freeman

PROGRAM

Glial Development
Chairpersons: S. Temple, *Albany Medical College, New York*; C. Stiles, *Dana-Farber Cancer Institute, Boston, Massachusetts*

Genetic Analysis of Glial Function
Chairpersons: W. Talbot, *Stanford University School of Medicine, California*; S. Shaham, *The Rockefeller University, New York*

Myelinating Cells
Chairpersons: D. Colman, *McGill University, Montréal, Canada*; J. Griffin, *Johns Hopkins Hospital, Baltimore, Maryland*

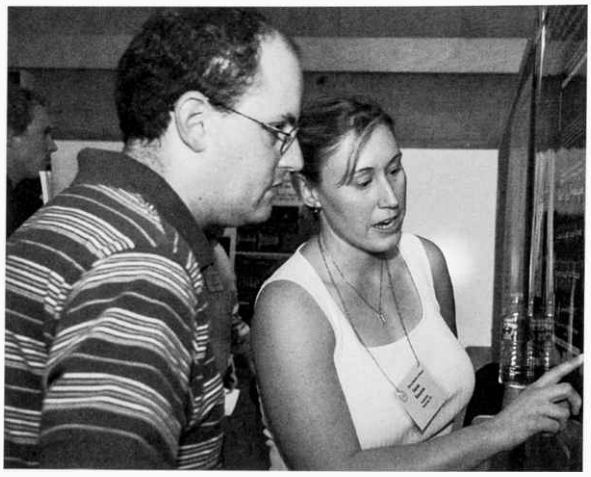
Astrocyte Function at Synapses and Blood Vessels
Chairpersons: U. Gaul, *The Rockefeller University, New York*; R. Robitaille, *University of Montréal, Canada*

Axon-Glial Interactions
Chairpersons: J. Salzer, *New York University Medical Center*; E. Peles, *Weizmann Institute of Science, Rehovot, Israel*; P. Magistretti, *University of Lausanne, Switzerland*

Reactive Gliosis and CNS Regenerative Failure
Chairpersons: Z. He, Children's Hospital, Boston, Massachusetts; M. Freeman, University of Massachusetts Medical School, Worcester

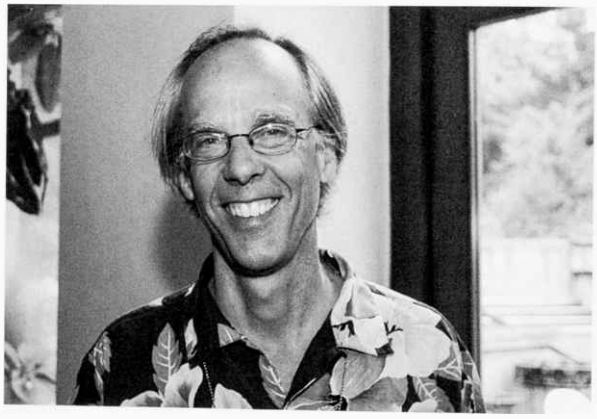
Glia and Disease
Chairpersons: L. Watkins, *University of Colorado, Boulder*; V.H. Perry, *University of Southampton, United Kingdom*

New Approaches to Understanding Glia
Chairpersons: W. Gan, *New York University Medical Center*; B. Tasic, *Stanford University, California*



M. Heiman, S. Kucenas

A. Mir, L. Dimou



D. Fields

New York Psychiatric Genetics Summer Forum

July 26 70 participants

ARRANGED BY **Anil Malhotra,** The Zucker Hillside Hospital, Glen Oaks, New York
Sydney Gary, Cold Spring Harbor Laboratory

This first meeting brought together neuroscientists, psychiatrists, and geneticists to meet and discuss their latest results. This inaugural meeting involved 70 participants from the tri-state area who welcomed this unique opportunity to interact with their local colleagues working to understand the genetic basis of psychiatric disorders. The program featured welcoming remarks from Jim Watson, 11 research talks, and a poster session. Presentations featured faculty, postdocs, and students. The day concluded with a barbecue on the shore of Cold Spring Harbor, allowing a wonderful opportunity for continued interactions in an informal setting.

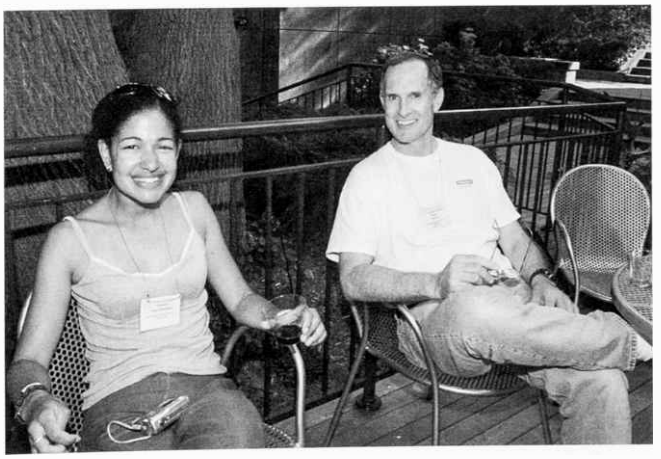
S. Gary, A. Malhotra

PROGRAM

Welcoming Remarks
James Watson

Session I
Chairpersons: A. Malhotra, *The Zucker Hillside Hospital, Glen Oaks, New York*; A. Fyer, *Columbia University, New York*; J. Gelernter, *Yale University, New Haven, Connecticut*; T. Lencz, *The Zucker Hillside Hospital, Glen Oaks, New York*; J. Gordon, *Columbia University, New York*

Session II
Chairpersons: P. Szezcko, The Zucker Hillside Hospital, Glen Oaks, New York; K. Burdick, *The Zucker Hillside Hospital, Glen Oaks, New York*; J. Chen, *Columbia University, New York*; J. Millonig, *UMDNJ Robert Wood Johnson Medical School, Piscataway, New Jersey*; L. Delisi, *New York University, Piermont*; J. Sebat, Cold Spring Harbor Laboratory



Y.S. Machado, B. Stevens

Lunch on the new Blackford deck

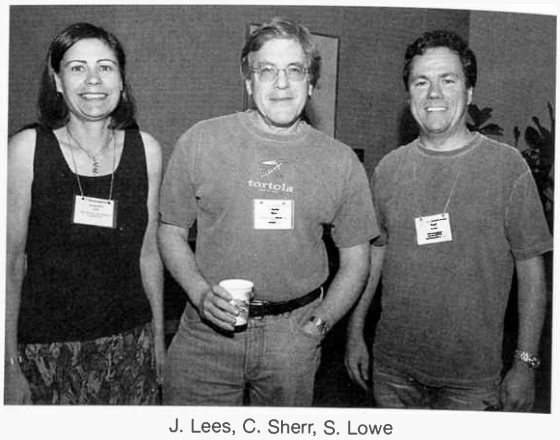
Meeting being shown on closed circuit TV in Grace Lobby

Mechanisms and Models of Cancer

August 16–20 459 participants

ARRANGED BY **Jacqueline Lees,** MIT Center for Cancer Research
Scott Lowe, Cold Spring Harbor Laboratory
Charles Sawyers, University of California, Los Angeles
Charles Sherr, HHMI/St. Jude's Children's Research Hospital

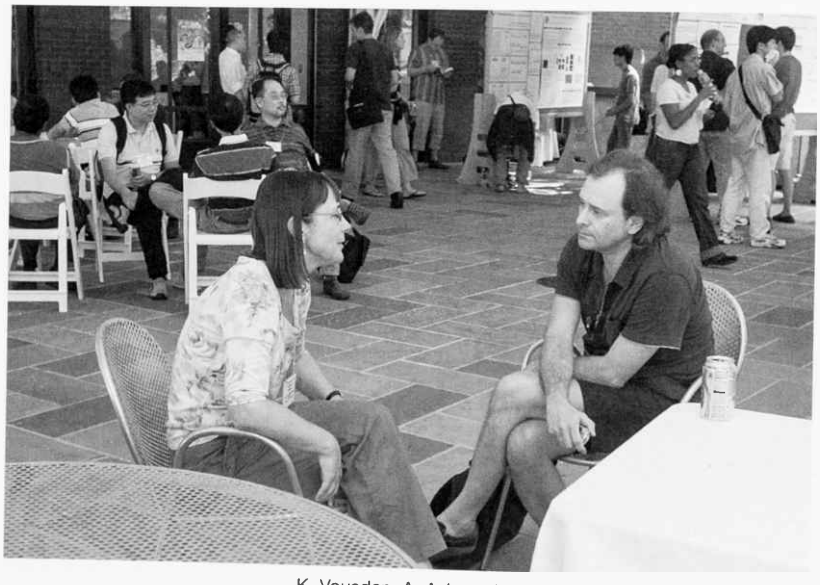
The first Mechanisms and Models of Cancer meeting held at the laboratory focused on a variety of topics related to cancer genetics, biology, and therapy. The meeting evolved from the former Cancer Genetics and Tumor Suppressor Genes meeting that occurred biannually since 1996 and reflected an increased emphasis on integrated approaches to studying signaling in normal and cancer cells, as well as the use of sophisticated in vivo mouse models to study various aspects of cancer biology.



J. Lees, C. Sherr, S. Lowe

More than 450 scientists, most of whom presented unpublished research through oral or poster presentations, attended the conference. The talks were highlighted by two keynote lectures: Michael Kastan discussed recent advances in DNA damage response pathways and Robert Eisenman discussed our increased understanding of the *myc* oncogene-signaling network. An international team of established investigators, whose expertise covered many of the topics highlighted during the meeting, chaired the individual sessions and facilitated discussion and interactions throughout the course of the meeting. Session topics are listed below under "Program." This meeting was enthusiastically supported, and the lectures and poster presentations led to extensive discussions and exchanges of information and ideas.

This conference was funded in part by the National Cancer Institute, a branch of the National Institutes of Health.


K. Vousden, A. Ashworth

PROGRAM

Checkpoints and Cell Cycle Regulation
Chairpersons: C.J. Sherr, *HHMI/St. Jude Children's Research Hospital, Memphis, Tennessee*; P.P. Pandolfi, *Memorial Sloan-Kettering Cancer Center, New York*

Keynote Speaker: DNA Damage Response Pathways and Human Disease
M.B. Kastan, *St. Jude Children's Research Hospital*

Cellular Senescence
Chairpersons: J. Campisi, *Lawrence Berkeley National Laboratory, California*; J. Lees, *MIT Center for Cancer Research, Cambridge, Massachusetts*

Genomes, Target Identification, and Therapeutics
Chairpersons: S. Elledge, *Harvard Medical School, Boston, Massachusetts*; P. Lamb, *Exelixis Inc., South San Francisco, California*

p53, ARF, MDM2, p53 Family
Chairpersons: L. Attardi, *Stanford University, California*; K. Vousden, *Beatson Institute for Cancer Research, Glasgow, United Kingdom*

Cancer Signaling Networks
Chairpersons: G. Evan, *University of California Comprehensive Cancer Center, San Francisco, California*; B. Neel, *Beth Israel Deaconess Medical Center, Boston, Massachusetts*

Keynote Speaker: Myc Goes Global
R. Eisenman, *Fred Hutchinson Cancer Research Center*

Signaling Pathways in Mouse Models
Chairpersons: A. Berns, *Netherlands Cancer Institute, Amsterdam;* L. Parada, *University of Texas Southwestern Medical Center, Dallas*

Tumor Microenvironment
Chairpersons: M. Bissell, *Lawrence Berkeley National Laboratory, California;* J. Allison, *Memorial Sloan-Kettering Cancer Center, New York*

DNA Damage and Repair
Chairpersons: A. Ashworth, *Breakthrough Breast Cancer Research Center, London, United Kingdom*; J. Wang, *University of California, San Diego*

H. Hermeking, M. Henriksson

S. Muthuswamy, M. Bissell

G. Caprara, P. Martinelli

L. Groth-Pedersen, L. Fang

Molecular Genetics of Bacteria and Phages

August 22–27 194 participants

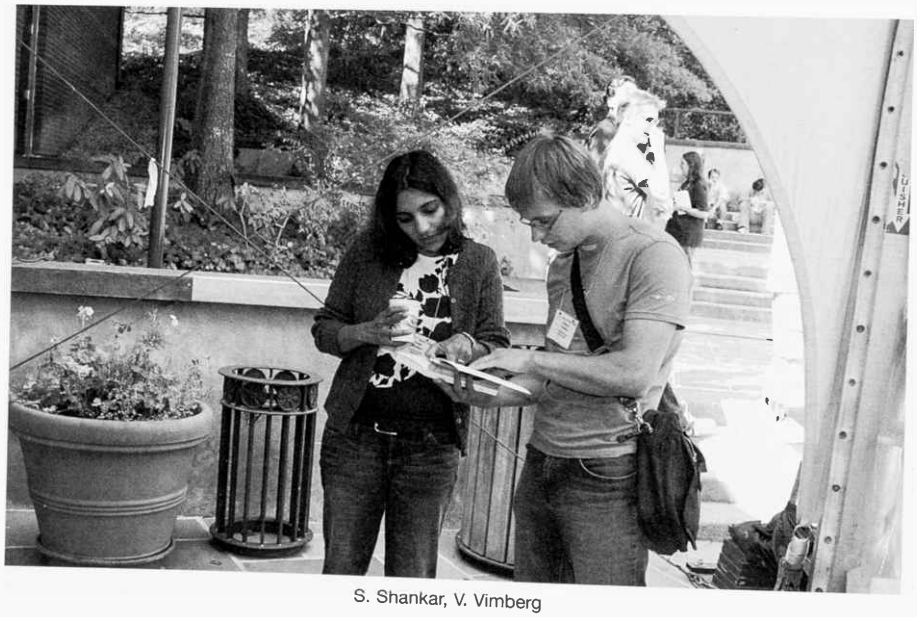
ARRANGED BY **Tina Henkin,** Ohio State University
Gary Dunny, University of Minnesota Medical School
Charles Turnbough, Jr., University of Alabama, Birmingham

This meeting featured 74 talks, arranged in eight oral presentation sessions, and two poster sessions. Bacteria and phages provide fantastic systems to study the chemistry of biology, and they grow ever more important in the study of disease, the understanding of our environment, and the development of new technologies. Topics discussed at the meeting are listed below under "Program."

Although many topics were discussed, a few prominent themes emerged, such as spatial and temporal regulation during cell growth and differentiation. Hubert Lam, the winner of the annual Nat Sternberg Award for the best thesis in prokaryotic molecular genetics, presented the first talk on this subject. Dr. Lam, who trained with Christine Jacobs-Wagner at Yale, described key proteins involved in establishing and perpetuating polarity in *Caulobacter crescentus*. Other talks described checkpoints, regulated proteolysis, and subcellular protein localization during sporulation of *Bacillus subtilis* and also the dynamics of chromosomal and plasmid partitioning.

Another theme was the application of genomics to study the inner workings of phage and bacteria. Genomic approaches were used by Roger Hendrix to characterize jumbo phages and by William Navarre to describe the role of H-NS in silencing horizontally acquired genes. Related approaches were used to examine the critical role of ppGpp during amino acid starvation, genome-wide binding profiles of *Escherichia coli* RNA polymerase, and the contribution of prophages to bacterial pathogenesis.

Gene expression and regulation were, as usual, major topics at the meeting. Richard Ebright described possible DNA scrunching during the initiation phase of transcription, Rick Gourse described a new recognition element in bacterial promoters, and Smita Shankar presented a model for Q-mediated antitermination. Another major theme was the connections between environmental signals, integrated signaling circuitries, and gene expression, and posttranscriptional regulation. Mechanisms for bacterial survival during stationary phase, heat shock, and other common stress conditions were described. Posttranscriptional



S. Shankar, V. Vimberg

regulation involving small RNAs was highlighted. For example, Gigi Storz described how the *SymE* toxin of *E. coli* is neutralized by the small RNA antitoxin SymR. A discussion of signaling would not be complete without talks on cell structure and surfaces, and there were many of these. Mixed in with these talks was Albert Siryaporn's use of a most colorful imaging assay to measure the extent of cross-talk between the many two-component signal transduction systems in live *E. coli* cells. The meeting concluded with a session of excellent talks on DNA recombination and repair.

Support for this meeting was provided by the National Science Foundation.

PROGRAM

Cell Division and Development
Chairperson: W. Burkholder, *Stanford University, California*

Bacteriophage and Genomics
Chairperson: R. Hendrix, *University of Pittsburgh, Pennsylvania*

Mechanism and Regulation of Transcription
Chairperson: R. Gourse, *University of Wisconsin, Madison*

Cellular Signaling and Stress Responses
Chairperson: E.P. Greenberg, *University of Washington, Seattle*

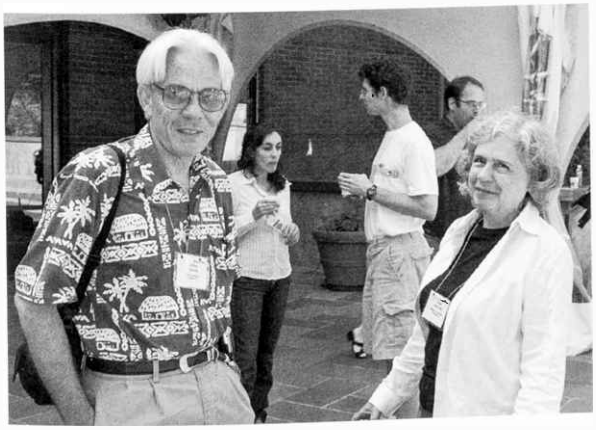
Bacterial Pathogenesis
Chairperson: S. Payne, *University of Texas, Austin*

Posttranscriptional Regulation
Chairperson: G. Storz, *NICHD/National Institutes of Health, Bethesda, Maryland*

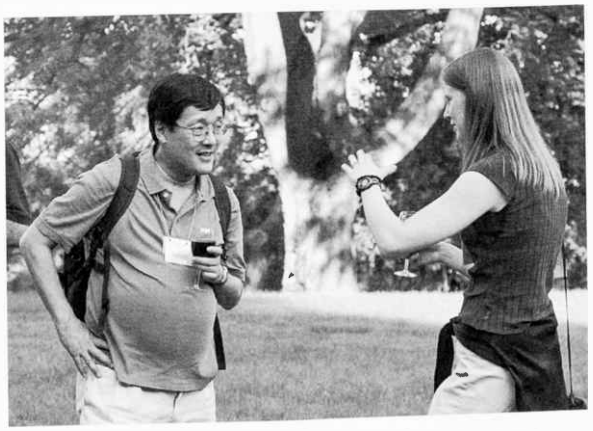
Prokaryotic Cytology and Surfaces
Chairperson: P. Christie, *University of Texas Health Science Center, Houston*

DNA Recombination and Repair
Chairperson: S. Lovett, *Brandeis University, Waltham, Massachusetts*

S. Jones, M. Laubacher



J. Roberts, S. Gottesman



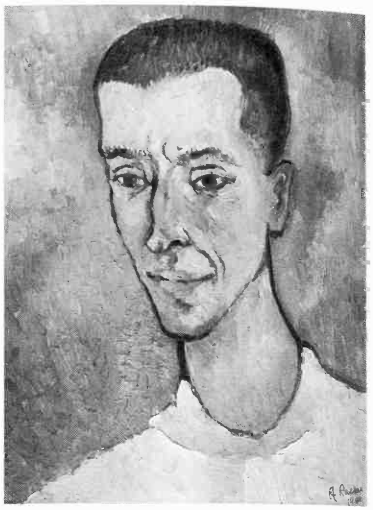
J. Hu, B. Burton

Max Delbrück Centennial

August 26–27

ARRANGED BY **David Stewart,** Cold Spring Harbor Laboratory

HONORARY COMMITTEE **Detlev Ganten,** Humboldt University Berlin/Charité
Peter Starlinger, Institute for Genetics, Koln, Germany
James D. Watson, Cold Spring Harbor Laboratory
John Wikswo, Vanderbilt University



The Max Delbrück Centennial to celebrate the 100th anniversary of his birth (September 4, 1906) was held immediately following the Molecular Genetics of Bacteria and Phages meeting, with which Delbrück was closely associated. The celebration was attended by all four of Max's children and their families, and other close friends and collaborators, and included reminiscences and thoughtful presentations by scientific colleagues and collaborators of Max, as well as current overviews of scientific questions that intrigued Delbrück throughout his life and career. A special exhibition celebrating Max Delbrück's life was assembled by the Laboratory's Library and Archives Director Mila Pollock and her staff, and it is now on tour to Vanderbilt University, the California Institute of Technology, and elsewhere. The celebration, attended by more than 100 people, also included a classical concert, a special dinner, and various other events. It concluded with a luncheon at Ballybung hosted by Jim and Liz Watson.

Beside the lobby display of Max Delbrück's story

Delbrück, Genes, and Phage

In 1906, Wilhelm II was Emperor of the German Empire and King of Prussia; Edward VII had succeeded his mother Queen Victoria and was in the fifth year of his reign as King of Great Britain, and Theodore Roosevelt was the 26th President of the United States of America. Max Delbrück was born that year in Berlin, into an academic family. His father was a professor of history and a noted political controversialist, and his mother's grandfather was the great chemist, Justus von Liebig. Delbrück early developed a passion for astronomy and entered the University of Tübingen as an astronomy student in 1924. Unpromisingly, German astronomy at this time consisted largely of measuring and cataloging the positions of stars, an enterprise devoid of intellectual rigor or excitement. Delbrück's interests changed to theoretical astrophysics, inspired in part by hearing a seminar given by Werner Heisenberg. In 1926, he moved to Göttingen, then a center of the new physics, with Max Born, Wolfgang Pauli and Heisenberg working together there. Delbrück moved intellectually, too, from astronomy to quantum physics, and completed his Ph.D. with Born in 1928.

Delbrück's interest in biology was awakened a few years later when he heard Niels Bohr give his influential lecture "Light and Life," in which he argued that life might not be reducible to atomic physics and that there was complementarity in biology as there was in physics. As Delbrück wrote later, he was inspired to pursue a research program in biology that would eventually "...lead to a paradoxical situation analogous to that into which classical physics ran in its attempts to analyze atomic phenomena."

Delbrück was intrigued that the "...stability of the gene and the algebra of genetics suggested something akin to quantum mechanics," and his first foray into biology was when he teamed up with the Russian geneticist Nikolai Timoféeff-Ressovsky and the radiobiologist Karl Zimmer to study the effects of radiation on fruit flies. In the third part of the "Three-man" or "Green Paper," published in 1935, Delbrück discusses the nature of the gene from the standpoint of atomic physics. This paper might have languished in obscurity had not Erwin Schrödinger made it the central argument in his book What is Life?"(1944). He drew on Delbrück's analysis to suggest that "a gene—or perhaps the whole chromosome fiber—to be an aperiodic solid." Although it is clear that much of it is wrong (and was wrong at the time he wrote it), the book had a significant impact, especially on some physicists, notably Gunther Stent, Seymour Benzer, and Francis Crick.

By 1937, Delbrück was committed to work on genetics. Stanley had recently crystallized tobacco mosaic virus, and Delbrück viewed viruses both as crystalline molecules and as living organisms. He viewed "...replication of viruses as a particular form of primitive replication of genes" and hoped that they might provide the key to an atomic level analysis of the gene. Delbrück received a Rockefeller Foundation Fellowship to work in T.H. Morgan's group at California Institute of Technology but, not surprisingly, found *Drosophila* genetics uninspiring. He was, however, introduced to bacteriophage by Emory Ellis, and together they published a classic paper, "The Growth of Bacteriophage," the description and analysis of one-step growth. Delbrück published three further papers on phage before his fellowship ended. In a brief reversion to physics, Delbrück coauthored a short note with Linus Pauling refuting the arguments put forward by Pascual Jordan that identical macromolecules had a special quantum mechanical attraction for one another.

Delbrück's second and third significant contribution as a biologist came from his association with Salvador Luria. They published a paper with Anderson on the electron microscopy of bacteriophage, but their most important collaborative research was their analysis of the appearance of bacteria resistant to phage infection (1943). The "fluctuation test" showed that mutations leading to resistance were present in the population of bacteria and were selected for following addition of phage, rather than resistance arising as an adaptive response to infection. Delbrück, Luria, and Alfred Hershey made up the core of the Phage Group that led to Delbrück's third contribution, and arguably the most influential: the Phage Course at Cold Spring Harbor. Delbrück and Luria first came together to Cold Spring Harbor in the summer of 1941 to do research. Delbrück was now at Vanderbilt University and during the winter of 1944–1945, he tentatively introduced a course on phage biology and genetics to be taught at Cold Spring Harbor. The first students, a grand total of six, took the first Phage Course in the summer of

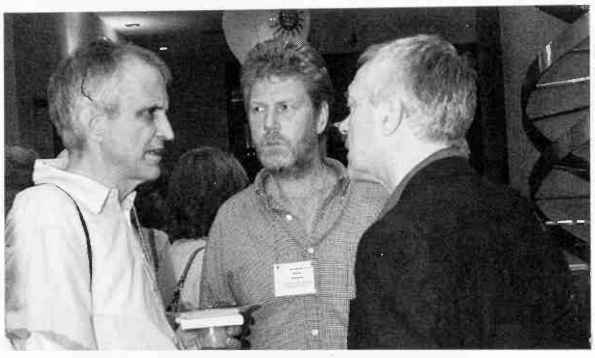
Benno Muller-Hill, Jeffrey Miller

Karin Moelling, John Inglis

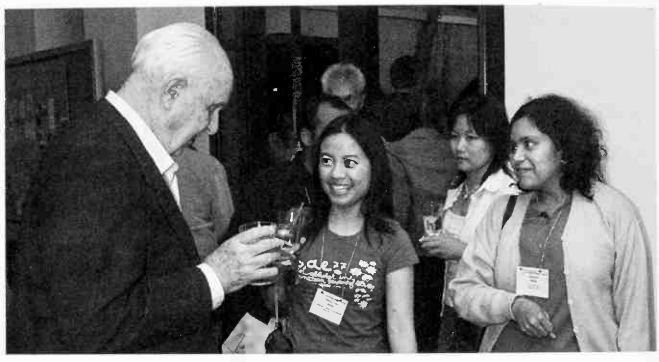
Daniel Wulff, Peter Starlinger

Bruce Stillman, Jonathan Delbrück

Jennifer Rohn, Harry Rubin

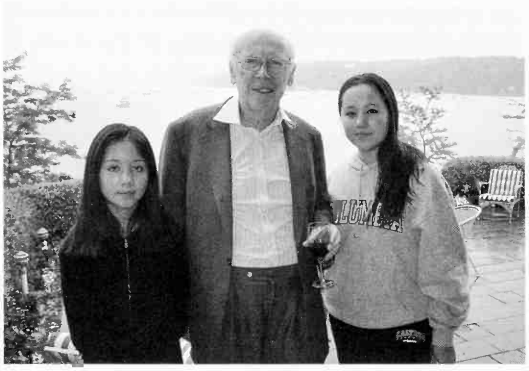


John Wikswo, David Stewart, Jan Witkowski



Waclaw Szbalski, Sheng-Tsien Hung, Simanti Datta

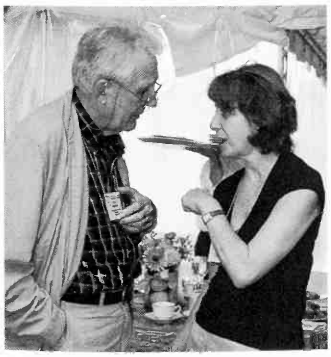
Julie Albers, cellist, in concert during the celebration



Elita Delbrück, James Watson, Lina Delbrück

Lina Delbrück, Yuri Delbrück, Kyuya Tamura

Tobi Delbrück, Christa Mayr Menzel



Klaus Rajewsky, Mila Pollock

Nicola Salmon, Liz Watson, Rick Salmon

Mathew Meselson, Alex Gann

Detler and Ursula Ganten

Beate Carriere, Giuseppe Bertani

1945. Delbrück taught the course for the first three summers and was succeeded by Mark Adams. The influence of the Phage Course is undeniable. Many of the luminaries of the phage and bacterial genetics underwent initiation in the Course not only into the biology of phage, but also into the Delbrück style of doing research: rigorous, quantitative, and no toleration of sloppy thinking.

Delbrück had less and less to do with phage in the early 1950s as his interest changed from genetics to studying sensory perception and transduction. He chose to study phototropism in *Phycomyces*, "...in the belief that in the field of transducer physiology, as in genetics, essential progress will require the use of a suitable microorganism."

Delbrück, Hershey, and Luria shared the 1969 Nobel Prize for Physiology or Medicine "...for their discoveries concerning the replication mechanism and the genetic structure of viruses." In his Nobel Lecture, Delbrück, returning perhaps to the philosophical challenges that 30 years earlier he and Bohr had seen in biology, challenged his audience to think about truth: "...even if we learn to speak about consciousness as an emergent property of nerve nets, even if we learn to understand the processes that lead to abstraction, reasoning and language, still any such development presupposes a notion of truth that is prior to all these efforts and that cannot be conceived as an emergent property of it, an emergent property of a biological evolution."

Jan Witkowski
Executive Director, Banbury Center

PROGRAM

OPENING REMARKS:
Bruce Stillman, *Cold Spring Harbor Laboratory*

REMINISCENCES
Chairperson: Mila Pollock, *Cold Spring Harbor Laboratory*

Jan Drake, *National Institute of Environmental Health Sciences*
Ernst Peter Fischer, *Universitat Konstanz*
Detlev Ganten, *Humboldt University Berlin/Charité*
Rashika Harshey,*University of Texas, Austin*
Makkuni Jayaram, *University of Texas, Austin*
Dale Kaiser, *Stanford University School of Medicine*
Peggy Lieb, *University of Southern California*
Carol Lipson, *Syracuse University*
Karl Maramorosch, *Rutgers University (Cook College)*
Matthew Meselson, *Harvard University*
Harry Rubin, *University of California, Berkeley*
Peter Starlinger, *University of Koln*
Gunther Stent, *University of California, Berkeley*
Waclaw Szybalski, *University of Wisconsin, Madison*
James D. Watson, *Cold Spring Harbor Laboratory*
Dan Wulff, *SUNY at Albany*

After Dinner Speakers
Jonathan Delbrück, *Atlas Pacific TC, Del Mar, California*
Lawrence M. Krauss, *Case Western Reserve University*

Current Science
Chairperson: John Wikswo, *Vanderbilt University*

Howard Berg, *Harvard University.* Inspired by conversations with Max Delbrück at CSHL, Howard began work on bacterial chemotaxis. The field has made substantial progress.
Molecular biology of behavior: Taming bacteria.

Enrique Cerdá-Olmedo, *University of Seville.* Postdoc and collaborator of Delbrück.
Phycomyces under the helm and in the wake of Max Delbrück.

Tobias Delbrück, *University of Zurich.* How Max started Neuromorphic Engineering, and what is that anyhow?

Karel Svoboda, *Cold Spring Harbor Laboratory/HHMI.* European physicist turned neurobiologist working at CSHL on experience-dependent plasticity in various sensory systems.
Imaging synaptic plasticity in vivo.

Closing Talk: Max Delbrück and Systems Biology
Lee Hood, *Institute for Systems Biology*

ATTENDEES

F. Carter Bancroft, Mount Sinai School of Medicine
Howard Berg, Harvard University
Anni Bergman, Northeastern University
Kostia Bergman, Northeastern University
Giuseppe Bertani, California Institute of Technology

F indicates family, family friends, etc.

Pat Burke (F)
Beate Carriere (F)
Enrique Cerdá-Olmedo, Universidad de Sevilla, Spain
Clare Clark, Cold Spring Harbor Laboratory
Robert Cohen, University of Florida

Luis Corrochano, Universidad de Sevilla, Spain
Jonathan Delbrück (F)
Tobi Delbrück (F)
Jan Drake, National Institute of Environmental Health Sci
Gary Dunny, University of Minnesota Medical School
Rada Dyson-Hudson (F)
Jan Eisenman, Cold Spring Harbor Laboratory
Ellen Fanning, Vanderbilt University
Ernst Peter Fischer, Universität Konstanz, Germany
Naomi Franklin, University of Utah
Paul Galland, University of Marburg, Germany
Rustem Gamow, University of Colorado (Retired)
Detlev Ganten, Humboldt University Berlin/Charité, Germany
Wally and Celia Gilbert (F)
Sue Harrison (F)
Rashika Harshey, University of Texas, Austin
Kiryn Haslinger, Cold Spring Harbor Laboratory
Tina Henkin, The Ohio State University
Leroy Hood, Institute for Systems Biology
John Inglis, Cold Spring Harbor Laboratory
Makkuni Jayaram, University of Texas, Austin
Dale Kaiser, Stanford University School of Medicine
Lawrence Krauss, Case Western Reserve University
Gordon Lark, University of Utah
Margaret Lieb, University of Southern California
Carol Lipson, Syracuse University
Edward Lipson, Syracuse University
Daniel Luria (F)
Zella Luria (F)
Karl Maramorosch, Rutgers University (Cook College)
Marisa Macari, Cold Spring Harbor Laboratory
Christa Menzel (F)
Matthew Meselson, Harvard University
Jeffrey Miller, University of California, Los Angeles
Karin Moelling, University of Zurich
Benno Muller-Hill, Universitat zu Koln, Germany
Mila Pollock, Cold Spring Harbor Laboratory
Klaus Rajewsky, CBR Institute for Biomedical Research
Jennifer Rohn, LabLit.com Magazine
Harry Rubin, University of California, Berkeley
Ludina Sallam (F)
Nicola Salmon (F)
Gorden Sato (F)
Walter and Audrey Shropshire (F)
Robert Sinsheimer, University of California, Santa Barbara
Frank Stahl, University of Oregon
Peter Starlinger, University of Koln, Germany
Gunther Stent, University of California, Berkeley
David Stewart, Cold Spring Harbor Laboratory
Bruce Stillman, Cold Spring Harbor Laboratory
Karel Svoboda, Cold Spring Harbor Laboratory
Waclaw Szybalski, University of Wisconsin, Madison
Kyuya Tamura (F)
Charles Turnbough, Jr., University of Alabama at Birmingham
Alexander Ulam (F)
James Watson, Cold Spring Harbor Laboratory
Simone Wenkel, University of Cologne
John Wikswo, Vanderbilt University
Evelyn Witkin, formerly Rutgers University
Jan Witkowski, Cold Spring Harbor Laboratory
Daniel Wulff, SUNY at Albany
Libby Zimmerman, Northeastern University

F indicates family, family friends, etc.

Mouse Molecular Genetics

August 30–September 4 243 participants

ARRANGED BY

François Guillemot, National Institute for Medical Research, London, United Kingdom
Janet Rossant, The Hospital for Sick Children, Toronto, Canada
Hiroyuki Sasaki, National Institute of Genetics, Japan
Anthony Wynshaw-Boris, University of California, San Diego

This meeting is held every other year and attracts researchers from across the globe. Scientists attending the meeting also come from very diverse areas of biology and are united by the common use of mouse mutants in their research. As a result, the meeting covers very diverse areas of biology in which the contribution of mouse genetics is essential to the progress of knowledge. The sustained interest of all participants throughout the meeting is ensured by the commonality of techniques, as well as by increasing evidence that basic mechanisms are conserved across diverse experimental systems.

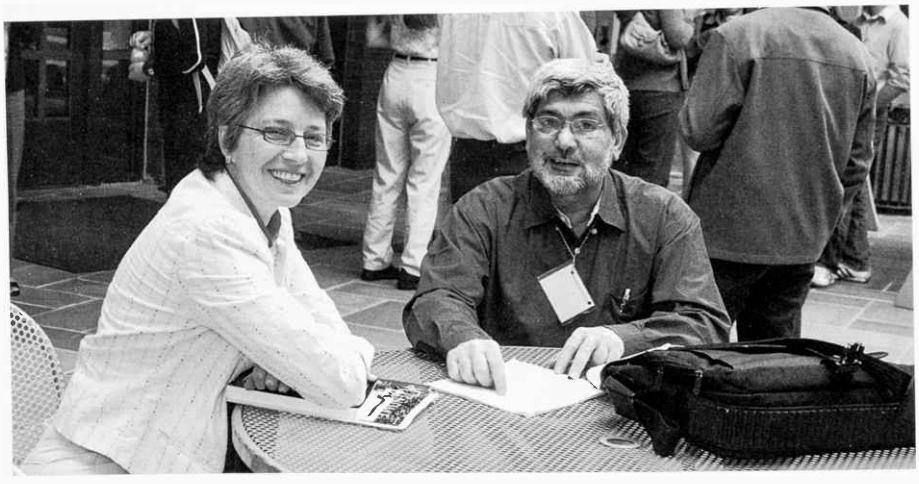
J. Rossant

The meeting was organized into 11 sessions, and included the Annual Rosa Beddington Lecture given by Virginia Papaioannou. Each session was arranged around two to three talks of general interests, given by both senior and more junior invited researchers. Between these more in-depth talks, five to six speakers per session were selected from the abstracts for more concise presentations, and included the presentation of several hundred posters in two afternoon sessions. The meeting ended with a farewell to François Guillmot and Janet Rossant, who stepped down after 4 years as organizers of the meeting.

A. Wynshaw-Boris

Some of the sessions have shifted in their emphasis the past 2 years. The growing emphasis on genetics/genomics and on human disease models, already noted in previous years, has also continued. Given the increasing importance of the mouse as a model to investigate disease mechanisms, and the sophistication with which diseases can now be modeled and analyzed in the mouse, the organizing committee has decided to emphasize the link between mouse and human genetics. This year, neurogenetic diseases were a focal point of the session featuring mouse models of human diseases. Besides a session on genetics/genomics, there was also a Mouse Genome Bionformatics workshop headed by Deanna Church of NCBI.

This conference was supported in part by funds provided by the National Cancer Institute, the National Institute of Child Health and Human Development, the National Institute of Diabetes & Digestive and Kidney Diseases, and the National Institute of Neurological Diseases and Stroke.



A. Naumova, A. Klar

PROGRAM

Epigenetics
Chairperson: T. Magnuson, *University of North Carolina, Chapel Hill*

Genetics and Genomics
Chairperson: D. Hilton, *Walter & Eliza Hall Institute of Medical Research, Parkville, Australia*

Stem Cells and Differentiation
Chairperson: E. Fuchs, *HHMI/The Rockefeller University, New York*

Neurobiology
Chairperson: L.-H. Tsai, *HHMI/Massachusetts Institute of Technology, Cambridge*

Mouse Genome Bioinformatics Workshop
D. Church, *National Center for Biotechnology Information, Bethesda, Maryland*

Rosa Beddington Lecture
V. Papaioannou, *Columbia University*

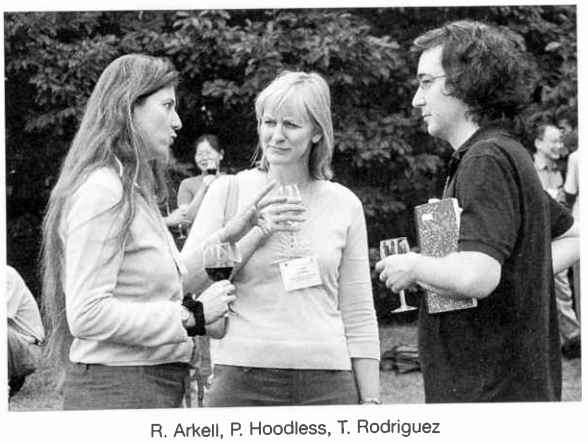
Signaling
Chairperson: S. Yamanaka, *Kyoto University, Japan*

Organogenesis
Chairperson: C. Wylie, *Children's Hospital Research Foundation, Cincinnati, Ohio*

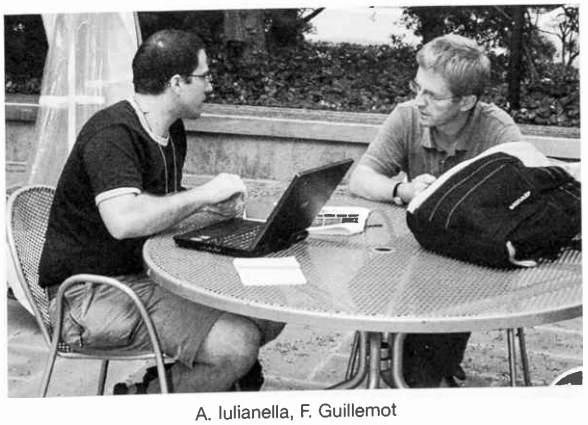
Human Disease Models: Neurogenetic Diseases
Chairperson: C. Walsh, *Beth Israel Deaconess Medical Center, Boston, Massachusetts*

Patterning
Chairperson: K. Anderson, *Memorial Sloan-Kettering Cancer Institute, New York*

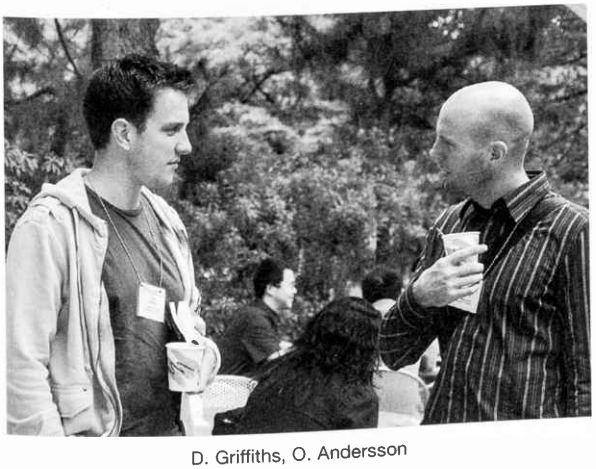
C. Ovitt, D. Barlow



R. Arkell, P. Hoodless, T. Rodriguez



A. Iulianella, F. Guillemot



D. Griffiths, O. Andersson

CSHL/Wellcome Trust Conferences

These conferences were held at the Hinxton Hall Conference Centre, located several miles south of Cambridge in the United Kingdom, which forms part of the Wellcome Trust Genome Campus, together with the Sanger Institute, and the European Bioinformatics Institute. The conferences are managed jointly by Cold Spring Harbor Laboratory and the Wellcome Trust and follow the Cold Spring Harbor style in that the majority of talks are chosen from openly submitted abstracts. The topics of the joint conference series emphasize genomics and bioinformatics, or topics of particular interest to science in the United Kingdom or Europe.

Interactome Networks

August 30–September 3 121 participants

ARRANGED BY

Ewan Birney, European Bioinformatics Institute
Anne-Claude Gavin, Cellzome Inc.
Marc Vidal, Dana-Farber Cancer Institute

This second meeting brought together senior and junior investigators, postdoctoral, and (post)graduate researchers in a range of disciplines to share existing research and experience. The conference addressed topics including ORFeome and other clone resources, Y2H and other binary assay maps; pull-down mass spectrometry approaches; finishing orthogonal binary assays; assembly/annotation: integration with phenotypic, transcriptome and localization clustering data; domain-domain networks; and interaction-defective genetics.

PROGRAM

ORFeome and Other Resources
Chairperson: D. Gerhard, *NCI/National Institutes of Health, Bethesda, Maryland*

DNA/Protein and Other Networks
Chairperson: G. Superti-Furga, *Austrian Academy of Sciences, Vienna*

Cocomplex Membership Maps
Chairperson: A.-C. Gavin, *EMBL, Heidelberg, Germany*

Binary Interaction Maps
Chairperson: J. Haas, *University of Munich, Germany*

Keynote Speaker: The Wall Of Genomics
R. Gibbs, *Human Genome Sequencing Center, Baylor College of Medicine*

Assembly, Annotation, Visualization
Chairperson: E. Birney, *EMBL-EBI, Hinxton, United Kingdom*

Data Integration, Interactome Modeling I
Chairperson: M. Vidal, *Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts*

Data Integration, Interactome Modeling II
Chairperson: D. Scholtens, *Northwestern University, Chicago, Illinois*

Domain-Domain Networks
Chairperson: R. Russell, *EMBL, Heidelberg, Germany*

Keynote Speaker: Mapping Regulatory Genomes and Their Evolution
B. Wold, *California Institute of Technology*

Francis Crick Auditorium

Genomic Perspectives to Host-pathogen Interactions

September 7–10 99 participants

ARRANGED BY **Matthew Berriman,** Wellcome Trust Sanger Institute
John Boothroyd, Stanford University
Julian Parkhill, Wellcome Trust Sanger Institute
George Weinstock, Baylor College of Medicine

This second meeting focused on how the rapid expansion in host, pathogen, and vector genome data and other large data sets is changing the way we think about host-pathogen-(vector) interactions. The conference brought together leading scientists in this growing field, and we strongly encourage researchers interested in a variety of different hosts, pathogens, and vectors to attend.

PROGRAM

Session 1
Chairpersons: D.M. Bird, *North Carolina State University, Raleigh*; D.F. Smith, *University of York, United Kingdom*

Session 2
Chairpersons: B. Wren, *London School of Hygiene and Tropical Medicine, United Kingdom*; S. Andersson, *Uppsala University, Sweden*

Session 3
Chairpersons: J.M. Musser, *The Methodist Hospital, Houston, Texas*; P. Keim, *Northern Arizona University, Flagstaff*

Session 4
Chairpersons: J. Berman, *University of Minnesota, Minneapolis*; T. Palzkill, *Baylor College of Medicine, Houston, Texas*

Session 5: Poster Session

Session 6
Chairpersons: A.A. James, *University of California, Irvine*; M.J. Lehane, *Liverpool School of Tropical Medicine, United Kingdom*

Session 7
Chairpersons: D. Roos, *University of Pennsylvania, Philadelphia*; M. Blaxter, *University of Edinburgh, United Kingdom*

Keynote Speaker: Phenotype and Genotype Evolution of Influenza A Virus
R.A.M. Fouchier, *Erasmus Medical Center*

Wellcome Trust Conference Center and Hinxton Hall

Genome Informatics

September 13–17 207 participants

ARRANGED BY **Tim Hubbard,** Wellcome Trust Sanger Institute
Suzanna Lewis, University of California, Berkeley
Jason Swedlow, University of Dundee

This sixth conference continued to highlight the latest developments in genome research and, once again, was a vital and exciting meeting. The explosion of biological data requires a concomitant increase in the scale and sophistication of information technology. This ranges from the storage of data and their associated data models, to the design of effective algorithms to uncover nonobvious aspects of these data sets, to ontologies to concisely describe biological information, and finally to software systems to support curation, visualization, and exploration. The conference brought together some of the leading scientists in this growing field, and researchers from other large-scale information handling disciplines were also invited to attend.

PROGRAM

Keynote Speaker
F. Kafatos, *Imperial College London*

Populations, Phenotypes, and Disease
Chairpersons: N. Hall, *The Institute for Genomic Research, Rockville, Maryland*; O. White, *The Institute for Genomic Research, Rockville, Maryland*

Assembly, Annotation, and Resources I
Chairpersons: R. Durbin, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*; A. Valencia, *Spanish National Cancer Research Centre, Madrid*

Comparative and Evolutionary Genomics I
Chairpersons: N. Goldman, *EMBL-EBI, Hinxton, United Kingdom*; J. Boore, *DOE Joint Genome Institute, Walnut Creek, California*

Comparative and Evolutionary Genomics II
Chairpersons: N. Goldman, *EMBL-EBI, Hinxton, United Kingdom*; J. Boore, *DOE Joint Genome Institute, Walnut Creek, California*

Epigenomics
Chairperson: S. Beck, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*

Images, Atlases, and Reconstruction
Chairpersons: M. Martone, *University of California, San Diego*; R. Baldock, *MRC Human Genetics Unit, Edinburgh, United Kingdom*

Assembly, Annotation, and Resources II
Chairpersons: R. Durbin, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*; A. Valencia, *Spanish National Cancer Research Centre, Madrid*

Regulation, Pathways, and Networks
Chairpersons: S. Teichmann, *MRC Laboratory of Molecular Biology, Cambridge, United Kingdom*; B.F.F. Ouellette, *University of British Columbia, Vancouver*

Grounds of the Wellcome Trust Genome Campus

Integrative Approaches to Brain Complexity

September 28–October 1 65 participants

ARRANGED BY

Seth Grant, Wellcome Trust Sanger Institute
Nathaniel Heintz, HHMI/The Rockefeller University
Jeffrey Noebels, Baylor College of Medicine

This second conference explored the structural and functional complexity of the vertebrate nervous system. Advances in genomics and proteomics are defining the molecular building blocks that underpin the structural complexity of the brain and it behavioral output. Combining genetic discoveries with anatomical, electrophysiological and behavioral findings, it is now feasible to integrate this knowledge to provide a more integrated understanding of brain function (and dysfunction).

PROGRAM

Human Genome and Genetics

Evolution of Brain Complexity

Keynote Lecture
S. Brenner, *Salk Institute of Biological Studies*

Brain Gene Expression: Cellular Approaches

Brain Gene Expression: Global Approaches and Atlases

Neuroscience Datamining Workshop

Electrophysiological Integration

Circuitry Mapping

Behavior and Brain Imaging

Consortium Presentations

Residential Court

Sanger Institute

Translational Control Meeting

September 6–10 449 participants

ARRANGED BY **Alan Hinnebusch,** NICHHD/National Institutes of Health
Nahum Sonenberg, McGill University, Canada
Gerhard Wagner, Harvard Medical School

This meeting attracted participants from around the world and included eight invited speakers, eight platform sessions, and three poster sessions that covered 346 abstracts. There were many novel findings presented concerning the mechanism of translation. It was reported that eIF4G contacts the e subunit of eIF3, implicated previously in tumor formation, and the binding site of one domain of eIF3j was mapped near the mRNA entry channel and A site of the 40S subunit. Distinct binding sites for eIF5 and eIF3c have been mapped on eIF1. There is new evidence that eIF1 dissociation from the 40S is a key step in AUG selection, and the amino and carboxyl termini of eIF1A oppositely regulate the transition from open to closed conformations of the scanning preinitiation complex (PIC). It now appears that eIF1A participates in subunit joining by stimulating GTP hydrolysis by eIF5B, and a contact point for eIF5B in 18S rRNA was uncovered by genetic suppressor analysis. Cryoelectron microscopy studies revealed the structure of human 80S ribosomes trying to melt a pseudoknot structure from a programmed frameshift site and suggested that bacterial RRF alters its position on terminating ribosomes to promote ribosome dissociation. The new bacterial elongation factor LepA, homologous to EF-G, was shown to mediate back-translocation, presumably to correct translocation errors at high ionic strength. An abortive termination event was described in which peptidyl tRNA is hydrolyzed after translation of a specific heptapeptide and translation resumes at the next Pro codon.

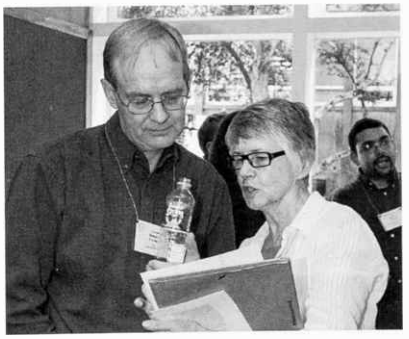
There were also important new insights into translational control mechanisms. Structural data reveal that the phosphorylation site in eIF2α (Ser51) is buried and that kinase PKR induces a conformational change that exposes the residue to the catalytic cleft. New data demonstrate the role of eIF2α phosphorylation in synaptic plasticity and memory in mammals. The structure of the domain in tumor suppressor protein PDCD4 that binds and sequesters RNA helicase eIF4A was found to resemble the eIF4A-binding domain in its natural partner eIF4G. A small molecule that inhibits eIF4E-eIF4G interaction has pro-apoptotic activity in Jurkat cells and inhibits proliferation in other cancer cell lines. *Drosophila* 4E-HP, which competes with eIF4E for mRNA caps but fails to bind eIF4G, was found to interact with the 3′ untranslated region (3′UTR)-binding translational repressors Bicoid and Nanos/Pum/Brat to prevent *caudal* and *hunchback* mRNAs from recruiting eIF4F. Cyfip1 emerged as a novel neuronal eIF4E-binding protein (4E-BP) that mediates repressive association between the fragile-X mental retardation protein (FMRP) and eIF4E. There is now evidence that 4E-BP promotes cap-independent translation of survival-promoting and angiogenesis factors in mammalian cells. It was demonstrated that the interferon-γ-activated inhibitor of translation (GAIT) complex binds the 3′UTR and silences translation of vascular endothelial growth factor (VEGF), ceruloplasmin, and probably other interferon-induced transcripts to coordi-

Enjoying the Wine and Cheese Party on Airslie Lawn

nately regulate inflammatory genes. The GAIT subunit ribosomal protein L13a, in a phosphorylation- and RNA-dependent way, binds eIF4G and blocks recruitment of the 43S PIC, possibly by impeding eIF3-eIF4G association. Translation of *ASH1* mRNA in yeast is regulated by phosphorylation-dependent binding of KHD1 to the E1 element in the open reading frame, and it appears that phospho-KHD1 binds eIF4G and prevents ribosome recruitment to the mRNA. It was shown that ZBP1 directs actin mRNA localization to dendrites of expanding neurons and also prevents translation of the mRNA during transport.

Other novel insights concerned meiotic maturation and neuronal differentiation via cytoplasmic polyadenylation. It was shown that the deadenylase PARN is active in *Xenopus* oocytes and opposes the GLD2 cytoplasmic polyadenylase to maintain short poly(A) tails before maturation. A sequential activation of the cytoplasmic polyadenylation element-binding protein (CPEB) and a protein (C3H-4) that induces ARE-mediated deadenylation was uncovered that temporally regulates target mRNAs with particular arrangements of CPE and ARE elements. CPEB and Musashi, a protein controlling cytoplasmic polyadenylation in maturing eggs, is also activated during neuronal differentiation elicited by NGF in PC12 cells. A novel role for CPEB was discovered in regulating cellular senescence in somatic cells. There is new evidence that translational repression by a *Drosophila* microRNA (miRNA) occurs at the level of PIC assembly, rather than elongation. P bodies store miRNA-repressed mRNAs, and a repressed message can reenter the translated pool in a manner regulated by ARE-binding protein HuR. mRNA trafficking between P bodies and ribosomes in yeast involves DEAD-box helicase UPF1, which functions in nonsense-mediated mRNA decay (NMD). Evidence was presented from yeast at odds with the idea that mRNAs are susceptible to NMD only in the "pioneer" round of translation, and it was shown that programmed frameshifting can reduce mRNA abundance by activating NMD. The structure of the exon junction complex, a *cis*-acting effector of NMD in mammals, was also described, and new results indicate that the ARE activates decay from both the 5′ and 3′ ends of the mRNA.

Support for this meeting was provided by the National Institute of Child Health and Human Development and Cell Signaling Technology, Inc.



D. Cavener, D. Gietzen

PROGRAM

Development, the CNS, and Signaling
Chairperson: N. Standart, *University of Cambridge, United Kingdom*

Viruses and Disease
Chairperson: I. Mohr, *New York University School of Medicine*

Initiation Factors and Mechanisms
Chairperson: O. Elroy-Stein, *Tel Aviv University, Israel*

MicroRNAs and Regulation of Factors
Chairperson: R. Kaempfer, *Hebrew University, Jerusalem, Israel*

mRNA Regulatory Elements
Chairperson: P. Fox, *Cleveland Clinic Foundation, Ohio*

Ribosome Structure and Function
Chairperson: C. Spahn, *Charité-Universitätsmedizin, Berlin, Germany*

mRNA Turnover
Chairperson: D. Schoenberg, *Ohio State University, Columbus*

Elongation and Termination
Chairperson: K. Nierhaus, *Max-Planck Institut für Molekulare Genetik, Berlin, Germany*

J. Richter, J. Keene

V. Camerini, I. Napoli

Axon Guidance, Synaptogenesis, and Neural Plasticity

September 13–17 392 participants

ARRANGED BY **Anirvan Ghosh,** University of California, San Diego
Christine Holt, University of Cambridge
Mu-Ming Poo, University of California, San Diego

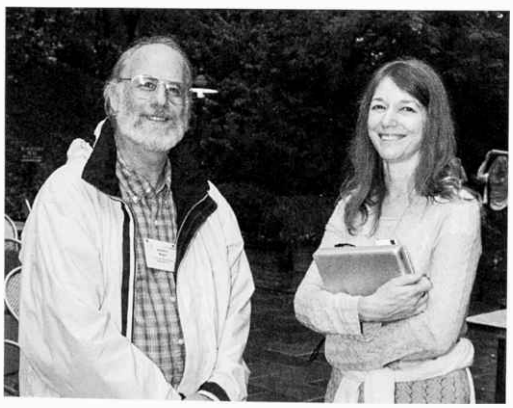
The human brain has billions of nerve cells (neurons) and each neuron is typically connected to hundreds of other neurons via synapses in a highly precise fashion. This complex neural wiring underlies the ability of humans, and other animals, to interact with the outside world, to learn and to perform complex behaviors. The complexity of the brain, in fact, surpasses that of a computer, and one of the major challenges for the field of neuroscience is to understand how nerve connections are made accurately and reliably.

Connections between different regions of the brain are first established during embryogenesis and result in the formation of a stereotyped pattern of axon pathways or fiber tracts. Projection neurons are often a long distance away from their synaptic targets, and the first step in establishing connectivity is to extend an axon tipped with a growth cone. The growth cone is the sensory/motile apparatus responsible for steering an accurate course through the early neuroepithelium of the embryonic brain. What directs the navigation of growth cones along the correct pathway? Once the growth cone reaches its target area, it must then find the correct region to terminate and begin to make synapses. How do axons select the correct cells with which to make synapses? Finally, after synapses are initially made, they can be modified by activity and experience leading to "plastic" changes that are important in important processes such as learning and memory. What determines whether a synapse is maintained or lost? How are these plastic changes controlled? This meeting focused on these key issues in axon guidance, synaptogenesis, and plasticity.

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. Progress has been assisted by the finding that these mechanisms are highly conserved across evolution, so that both biochemical approaches in vertebrates and genetic approaches in invertebrates (and increasingly, in vertebrates as well) have led to mutually reinforcing discoveries that have helped fuel further advances.

As the pace of discovery has quickened, the field has grown enormously, making it more difficult for scientists to keep abreast of new developments. To help facilitate communication in the field, a biennial CSH conference series on "Axon Guidance and Developmental Plasticity of the Nervous System" was initiated in 1998. This year, the fifth of these meetings involved sessions devoted to particular problems in the assembly of the nervous system, with speakers chosen from among the participants submitting abstracts by Session chairs who are leaders in the field. Other abstracts were presented as posters.

J. Sanes, C. Mason



J. Raper, C. Bargmann

Like the previous four meetings, the response of the field to the 2006 conference was one of overwhelming enthusiasm. Of the 392 participants, 254 submitted abstracts, and 63 abstracts were selected for talks, in eight sessions. Senior researchers, starting assistant professors, postdoctoral fellows, and graduate students were well represented as speakers and participants. 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 two keynote addresses: Dr. Corey Goodman reviewed the history of the field of axon guidance and included some of the key contributions made by his own group, and Dr. Joel Richter focused on the control of polyadendylation-mediated translation and its role in synaptic plasticity. The 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. 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.

Funding for this meeting was provided by the National Institute of Neurological Diseases and Stroke.

PROGRAM

Axon Guidance I: Guidance Molecules
Chairpersons: A. Kolodkin, *HHMI/Johns Hopkins University School of Medicine, Baltimore, Maryland*; L. Zipursky, *HHMI/University of California, Los Angeles*

Synapse Formation I
Chairpersons: C. Bargmann, *HHMI/The Rockefeller University, New York*; P. Scheiffele, *Columbia University, New York*

Special Lecture: The Molecular Biology of Axon Guidance Revisited
C. Goodman, *HHMI/University of California, Berkeley*

Neural Circuits and Plasticity I
Chairpersons: D. Feldman, *University of California, San Diego*; C. Shatz, *Harvard Medical School, Boston, Massachusetts*

Axon Guidance II: Guidance Mechanisms
Chairpersons: R. Klein, *Max-Planck Institute for Neurobiology, Martinsried, Germany*; P. Bovolenta, *Cajal Institute, Madrid, Spain*

Neural Circuits and Plasticity II: Developmental Plasticity in Sensory Cortex
Chairpersons: D. Feldman, *University of California, San Diego*; C. Shatz, *Harvard Medical School, Boston, Massachusetts*

Axon Guidance III: Topographic Maps
Chairpersons: J. Flanagan, *Harvard Medical School, Boston, Massachusetts*; C. Mason, *Columbia University, New York*

Synapse Formation II: Targeting
Chairpersons: J. Sanes, *Harvard University, Cambridge, Massachusetts*; M. Bate, *University of Cambridge, United Kingdom*

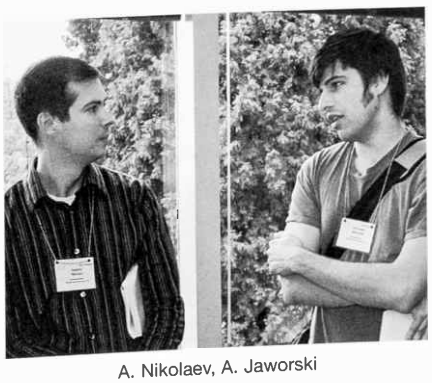
Special Lecture: Polyadenylation-mediated Translation in the CNS
J. Richter, *University of Massachusetts Medical School*

Axon Guidance IV: Novel Mechanisms and Regeneration
Chairpersons: C.-B. Chien, *University of Utah, Salt Lake City*; L. Luo, *Stanford University, California*

NIDA Funding Opportunities in Developmental Biology
Chairperson: D.-Y. Wu, *NIDA/National Institutes of Health, Bethesda, Maryland*

Neuronal Polarity and Dendritic Patterning
Chairpersons: H. Cline, *Cold Spring Harbor Laboratory*; Y.-N. Jan, *HHMI/University of California, San Francisco*


A. Debant, A. Briaçon-Marjollet


A. Nikolaev, A. Jaworski


S. Margolis, D. Conón-Ramos

Dynamic Organization of Nuclear Function

September 27–October 1 310 participants

ARRANGED BY

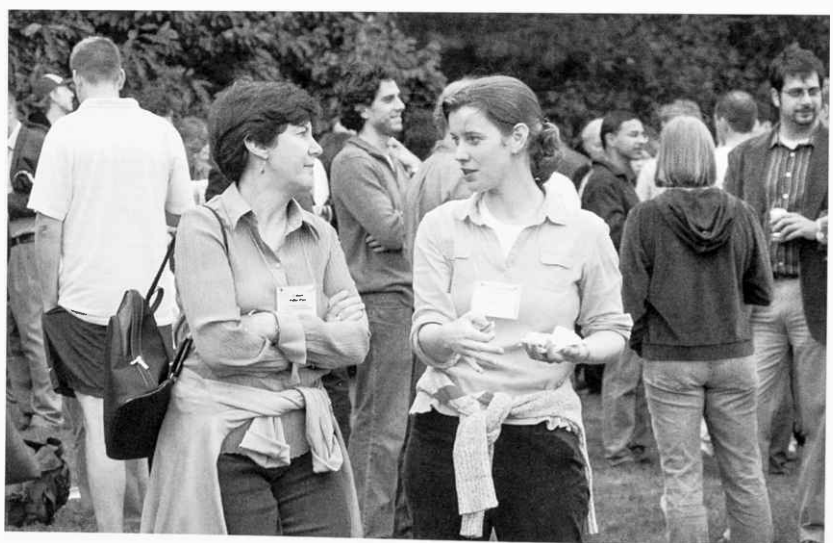
Genevieve Almouzni, Institut Curie, France
David Spector, Cold Spring Harbor Laboratory
Susan Wente, Vanderbilt University Medical Center

This meeting focused on the relationships between nuclear structure and function. The opening session highlighted current studies on Chromosome Organization and DNA Replication, the session chair (David Gilbert) describing his studies using a cell-free replication to dissect components that regulate replication timing. Although the nucleus is highly dynamic, the genome is nonrandomly organized with the mobility of chromatin constrained by its proximity to specific anchorage sites whose positions define the functional organization of the nucleus. This session tackled questions of replication and nuclear organization, coordinating chromatin assembly with replication, as well as how genomes are organized in the nucleus for transcription.

D. Spector, G. Almouzni, S. Wente

The following morning's session on Nuclear Bodies chaired by Greg Matera illustrated the ongoing and lively debate about the function of these entities as bona fide sites of macromolecular assembly or merely representing aggregate byproducts of other nuclear processes. After a lively poster session and wine and cheese party, the session on RNA Processing and Export that evening was chaired by Javier Caceres, and served to emphasize the high degree of connection that exists among different RNA processing events, and many specific examples were discussed that provided significant insights for experienced observers and newcomers alike.

The Nuclear Structure and Disease session gave multiple direct examples of how the machinery involved in nuclear organization and function is linked to the underpinnings of several pathophysiologic states. Robert Goldman gave a broad and lucid introduction of general lamin structure and function, summarized recent discoveries of roles in spindle assembly, and provided clear evidence for defective processing of lamin A being a causative agent of the premature aging disease, progeria. In the Chromosomes and Cell Cycle session, session chair William Earnshaw described the regulation of mitotic chromosome struc-



A.-M. Jacobs, F. Fuller-Pace

ture by condensin and his group's identification of a novel activity provisionally termed RCA (regulator of mitotic chromosome architecture). He postulated that that RCA normally drives mitotic chromosome condensation, with the role of condensin being to stabilize the condensed chromosomes. The Technologies to Access Nuclear Organization session was chaired by Jan Ellenberg and provided an exciting overview of state-of-the-art and emerging technologies to access nuclear organization at different scales of biological organization, from whole living cells all the way to single mRNA molecules and nucleosomes.

Although several of the prior sessions pointed to connections to nuclear pore complex proteins (nucleoporins/Nups) and the nuclear envelope, the Nuclear Periphery session allowed an in-depth update on these topics, ably chaired by Douglass Forbes who presented provocative work on the role of the Nup107-160 nucleoporin complex in mitotic spindle assembly. The final session on Transcription and Genome Function was chaired by Maria Carmo-Fonseca who introduced a very fundamental question: How does intranuclear positioning relate to gene function? In particular, she highlighted connections between pre-mRNA splicing, Pol II transcription and mRNA release. Several laboratories then presented their most recent progress on mapping multiple genes and regulatory sequences in the nucleus, identifying the molecules that mediate genomic confinement relative to different landmarks, and determining whether positioning is a cause or consequence of gene activity.

The enthusiasm of the meeting participants was overwhelming, and the meeting will be held again in the fall of 2008.

PROGRAM

Chromosome Organization and DNA Replication
Chairperson: D. Gilbert, *Florida State University, Tallahassee*

Nuclear Bodies
Chairperson: G. Matera, *Case Western Reserve University, Cleveland, Ohio*

RNA Processing and Export
Chairperson: J. Caceres, *MRC Human Genetics Unit, Edinburgh, United Kingdom*

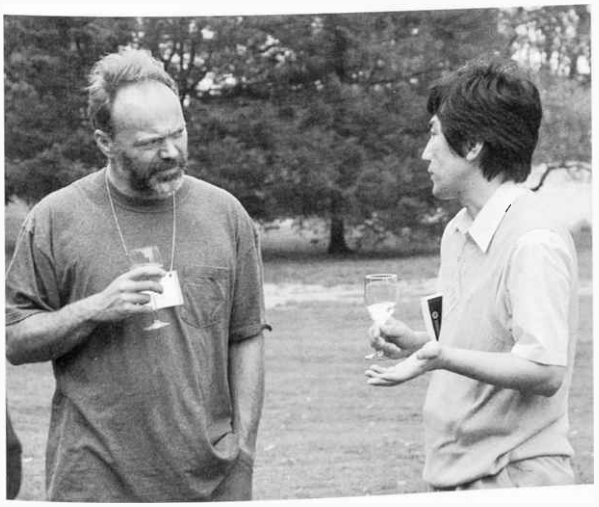
Nuclear Structure and Disease
Chairperson: R. Goldman, *Northwestern University Medical School, Chicago, Illinois*

Chromosomes and the Cell Cycle
Chairperson: W. Earnshaw, *University of Edinburgh, United Kingdom*

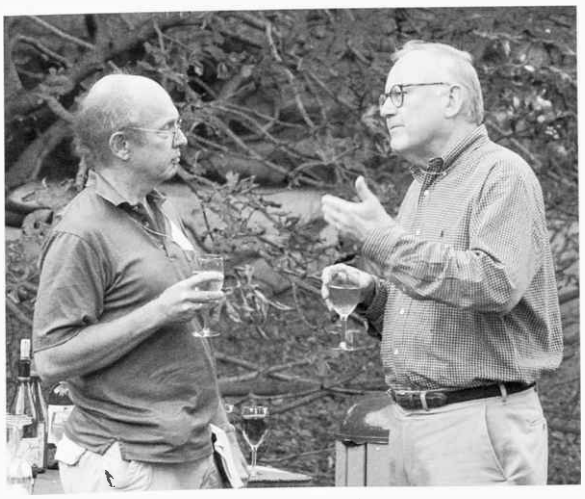
Technologies to Access Nuclear Organization
Chairperson: J. Ellenberg, *European Molecular Biology Laboratory, Heidelbeg, Germany*

The Nuclear Periphery
Chairperson: D. Forbes, *University of California, San Diego*

Transcription and Genome Function
Chairperson: M. Carmo-Fonseca, *University of Lisbon, Portugal*



J. Campbell, T. Yoshihisa



M. Engelharolt, T. Pederson

Molecular Genetics of Aging

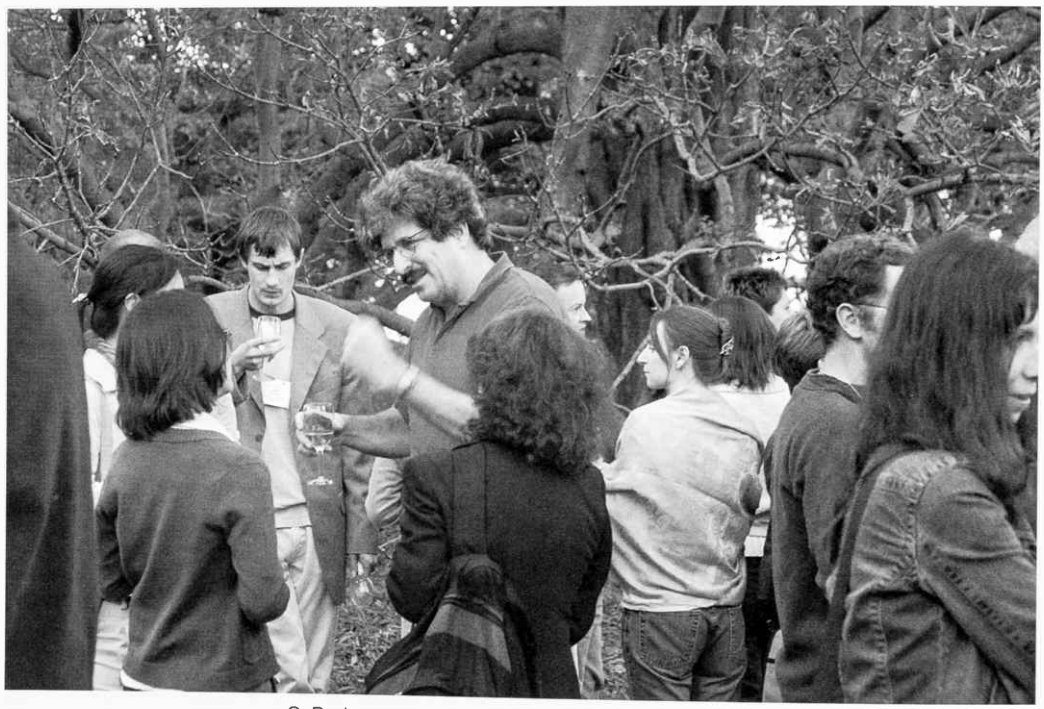
October 4–8 338 participants

ARRANGED BY **Judith Campisi,** Lawrence Berkeley National Laboratory
Lenny Guarente, Massachusetts Institute of Technology
Gary Ruvkun, Massachusetts General Hospital

Aging is controlled by genetics and the environment, and leads to biological imbalances that increase the probability of death. Remarkable advances have been made in recent years in understanding how genes and genetic processes control and influence aging. This meeting focused on these advances and stimulated lively discussions on the relative contributions of various genetic pathways to the aging process in simple model organisms and complex organisms such as mice and humans.

Two sessions focused specifically on genetics, highlighting the growing list of mutations that extend the life span of simple model organisms such as nematodes and fruit flies. A separate session focused on genetic manipulations that modulate aging and life span in mice. Sessions were also devoted to the roles that mitochondria and nutrient metabolism play in the aging process. One session concentrated on the brain and genomic approaches to understand the genes that regulate aging, another explored the role of stress and cellular processes such as apoptosis and senescence in the aging of tissues and organisms, and finally, another session focused on the roles of telomeres and genomic instability in both aging and the development of late-life cancer. The meeting revealed the rapid progress that is being made in understanding the molecular bases for aging, the many important questions that have yet to be answered, and the rising hopes that interventions into fundamental aging processes may be possible.

Funding for this meeting was provided by the National Institute on Aging.



G. Ruvkun expounding his thoughts to a rapt audience

PROGRAM

Genetics I
Chairpersons: D. Sinclair, *Harvard Medical School, Boston, Massachusetts*; M. Driscoll, *Rutgers University, Piscataway, New Jersey*

Metabolism
Chairpersons: B. Kahn, *Beth Israel Deaconess Medical Center, Boston, Massachusetts*; G. Shulman, *HHMI/Yale University School of Medicine, New Haven, Connecticut*

Genomics/The Brain
Chairpersons: T. Prolla, *University of Wisconsin, Madison*; B. Yankner, *Harvard Medical School, Boston, Massachusetts*

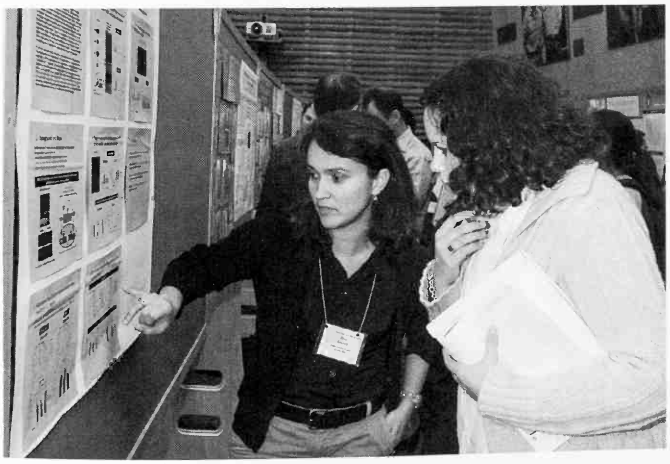
Mice
Chairpersons: Y.-i. Nabeshima, *Kyoto University, Japan*; H. Scrable, *University of Virginia, Charlottesville*

Senescence/Stress
Chairpersons: D. Bredesen, *Buck Institute for Age Research, Novato, California*; W. Wright, *University of Texas Southwestern Medical Center, Dallas*

Genetics II
Chairpersons: L. Partridge, *University College London, United Kingdom*; C. Kenyon, *University of California, San Francisco*

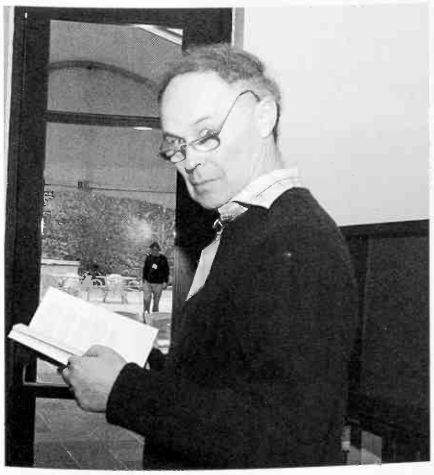
Mitochondria
Chairpersons: D. Wallace, *University of California, Irvine*; S. Hekimi, *McGill University, Montreal, Canada*

Telomeres/Cancer
Chairperson: M. Blasco, *Spanish National Cancer Center, Madrid*

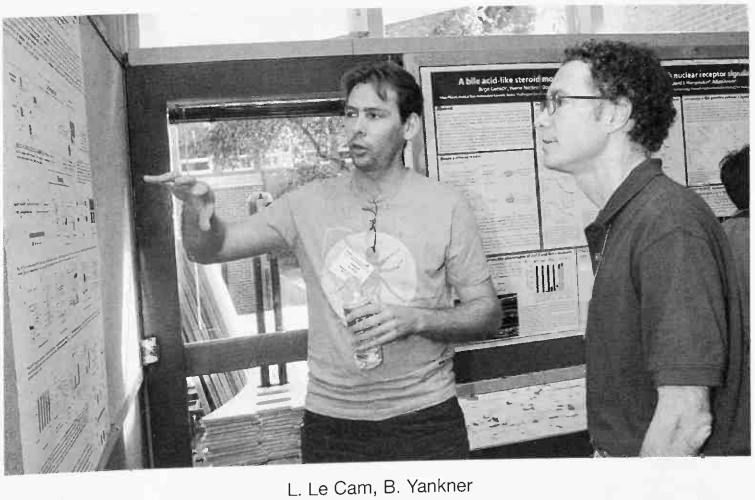


R. Garvin, M. Grabowski

L. Partridge, S. Pletcher



N. Wade



L. Le Cam, B. Yankner

Germ Cells

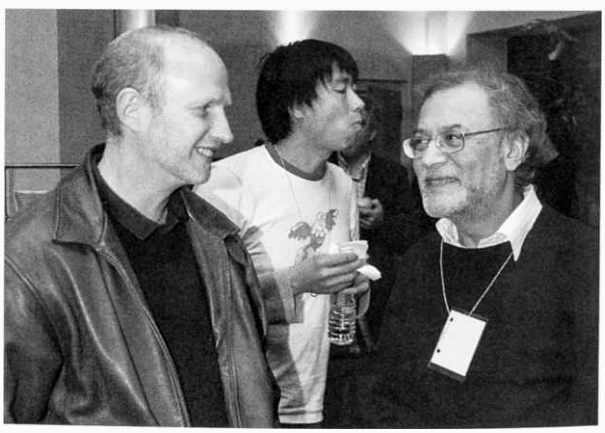
October 11–15 229 participants

ARRANGED BY **Susan Strome,** Indiana University
Azim Surani, Wellcome Trust/CRC Institute

One major advance reported at this meeting was on the mechanism of imprinting in the mouse germ line and in the *Arabidopsis* gametophyte. Tim Bestor together with C. David Allis reported the crystallographic structure of Dnmt3L, which is detected only in germ cells. The structural dimension of Dnmt3L can accommodate a nucleosome. The Dnmt3L structure also contains Dnmt3A and Dnmt3B, and this core structure can "read" histone modification marks and induce DNA methylation of appropriate regions, such as the control elements of imprinted genes in developing mouse oocytes. Expression of the maternally imprinted SET domain gene, *MEDEA*, in the *Arabidopsis* gametophyte is, however, regulated by a demethylase, *DEMETER*, a protein with a glycosylase domain, which functions to excise 5-methylcytosine. At the same time, Christof Niehrs has identified a putative DNA demethylase from *Xenopus* that may have a role in the reprogramming of somatic nuclei in the oocyte. This apparently promotes DNA repair to erase DNA methylation marks. Further studies on the reprogramming of somatic nuclei transferred into mammalian oocytes was described by Rudolf Jaenisch, who also described the role of transcriptional factors such as Oct4/Sox2 and epigenetic modifiers such as the polycomb group proteins in regulating pluripotent stem cells. Germ cells also show expression of pluripotency markers such as Nanog, and Ian Chambers showed that this gene is essential for the maintenance of the germ-cell lineage in mice. Mitinori Saitou showed how germ-cell specification in mice is critically dependent on Blimp1, a transcriptional repressor that represses the somatic program that is adopted by neighboring somatic cells. From further analysis, he described that Prdm14 is also critical for formation of the founder population of primordial germ cells in mice.

Two prominent themes in several presentations on germ-line development in invertebrate model systems were epigenetic regulation and regulation at the level of RNA. A feature shared by the primordial germ cells of worms and flies is transcriptional repression. Bill Kelly, Akira Nakamura, and Ruth Lehmann described several levels at which this repression is mediated in worms and flies. Key regulators include PIE-1 in worms, PGC in flies, Nanos, and histone modifiers. Gene expression in the adult germ line in worms seems to be regulated largely at posttranscriptional levels. Judith Kimble described a network of translational regulators that operate in different regions of the gonad, and Chris Merritt demonstrated the importance of genes' coding and/or 3′UTR regions (but not promoters) for achieving proper protein distributions. Craig Mello discussed his and Andrew Fire's Nobel-winning studies of RNAi and Argonaute proteins, after which Greg Hannon, Rene Ketting, and Haifan Lin presented advances on the Piwi subclass of Argonautes, including their association with small (~30 nucleotides) RNAs and

S. Strome, K. Blackwell



R. Martiennsen, A. Surani

with chromatin regulators. Several presentations focused on stem cell biology, including a presentation by Margaret Fuller demonstrating that during the stem cell divisions in fly testes, the stem cell daughters retain the mother "Eve" centrosome.

The next Germ Cells meeting will be held in the fall of 2008 and will be organized by Anne Ephrussi, Renee Reijo Pera, and David Zarkower.

This meeting was supported in part by the March of Dimes Birth Defects Foundation Grant No. 4-FY05-1255, the National Institute of Child Health and Human Development, and the Lalor Foundation.

PROGRAM

Themes in Germ-cell Biology
Chairpersons: S. Strome, *Indiana University, Bloomington*; A. Ephrussi, *European Molecular Biology Laboratory, Heidelberg, Germany*

Epigenetic Regulation
Chairpersons: M. Fuller, *Stanford University School of Medicine, California*; T. Bestor, *Columbia University College of Physicians & Surgeons, New York*

RNA Regulation
Chairpersons: R. Ketting, *Hubrecht Laboratory, Utrecht, The Netherlands*; C. Mello, *University of Massachusetts Medical School, Worcester*

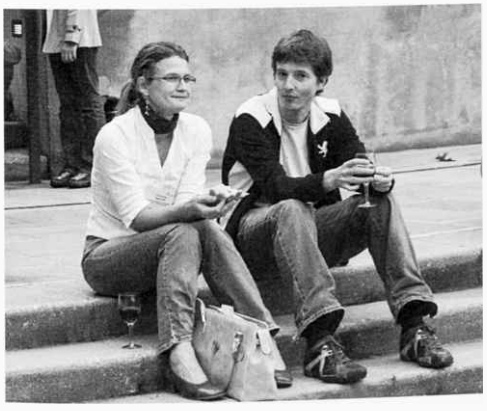
Germ Line and Reprogramming
Chairpersons: R. Jaenisch, *Whitehead Institute, Massachusetts Institute of Technology, Cambridge*; C. Niehrs, *Deutsches Krebsforschungzentrum, Heidelberg, Germany*

Germ-line Stem Cells
Chairpersons: J. Kimble, *HHMI/University of Wisconsin, Madison*; N. Geijsen, *Massachusetts General Hospital, Charlestown*

Germ-cell Patterning and Specification
Chairpersons: R. Lehmann, *HHMI/New York University Medical Center*; G. Seydoux, *Johns Hopkins University School of Medicine, Baltimore, Maryland*

Germ-line Programs
Chairpersons: T. Orr-Weaver, *Whitehead Institute, Massachusetts Institute of Technology, Cambridge*; A. Villeneuve, *Stanford University School of Medicine, California*

Gametogenesis and Fertilization
Chairpersons: A. Surani, *University of Cambridge, United Kingdom*; D. Page, *Whitehead Institute, Massachusetts Institute of Technology, Cambridge*



M. Segerstahl, R. Hobbs

M. McGovern, K. Walstrom

J. Kimble, H. Lin

C. Mello, L. Gann

Nuclear Receptors: Bench to Bedside

November 1–5 164 participants

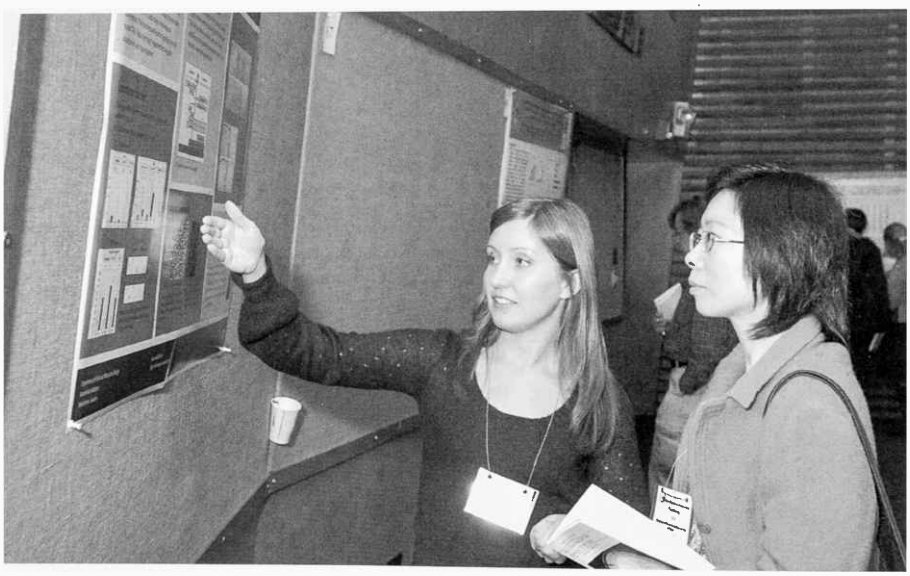
ARRANGED BY **Ronald Evans,** The Salk Institute
Sohaib Khan, University of Cincinnati
Keith Yamamoto, University of California, San Francisco

R. Evans, S. Khan, K. Yamamoto

The discovery of the estrogen receptor 50 years ago spawned the nuclear receptor superfamily field, now comprising 48 or 49 receptors in mammals that control diverse developmental and physiological functions in the body and are implicated in a wide variety of diseases, including cancer and metabolic and neural disorders. At least half of the nuclear receptor superfamily products are therapeutic targets. The intent of this meeting was to bring together investigators whose research expertise in nuclear receptors ranged from basic to clinical, for a detailed appraisal of the current challenges and opportunities across this field.

Nuclear receptors, which function as transcription factors, modulate target gene transcription in cooperation with comodulators, overseeing complex transcriptional networks that produce global physiological effects. Deciphering the molecular mechanisms of nuclear receptor action has enhanced our basic understanding of gene expression and illuminated promising avenues for development of therapeutic agents. The diverse biological functions of the nuclear receptors and their ligands were discussed, with an emphasis on their involvement in normal physiology and disease, and their implications for cancer, metabolic and insulin disorders, heart and muscle disease, inflammation, and neurological disorders. Discussions also focused on the prospects for rational drug design based on nuclear receptor crystal structure. The next Nuclear Receptor meeting is planned for the fall of 2008.

Partial funding for this meeting was provided by Wyeth.



K. Wallis, Y. Shi

PROGRAM

Keynote Address: Etiology and Treatment of Insulin Resistance
G. Shulman, *HHMI/Yale University School of Medicine*

Metabolic and Insulin Resistance Disorders
Chairperson: J. Auwerx, *IGBMC, Illkirch, France*

Nuclear Receptors in Cancer
Chairperson: J.A. Manson, *Brigham & Women's Hospital, Boston, Massachusetts*

Nuclear Receptors in Heart and Muscle Disease
Chairperson: R. Lifton, *Yale University School of Medicine, New Haven, Connecticut*

Round Table Discussion

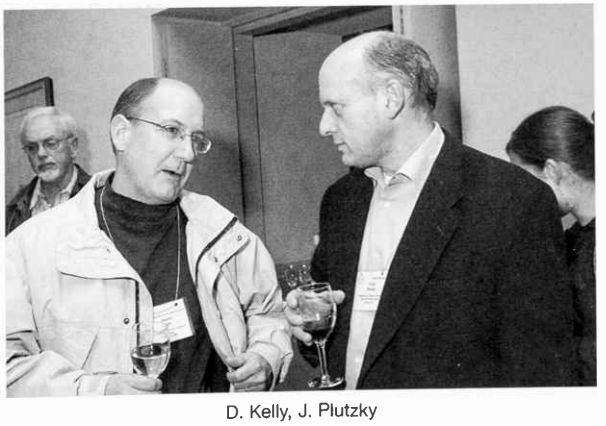
Nuclear Receptors in Inflammation
Chairperson: D. Mangelsdorf, *University of Texas Southwestern Medical Center, Dallas*

Nuclear Receptor Architecture and Designer Drugs
Chairperson: W. Chin, *Eli Lilly & Co., Indianapolis, Indiana*

Where Are We with Estrogen?
Chairperson: E. Jensen, *University of Cincinnati College of Medicine, Ohio*

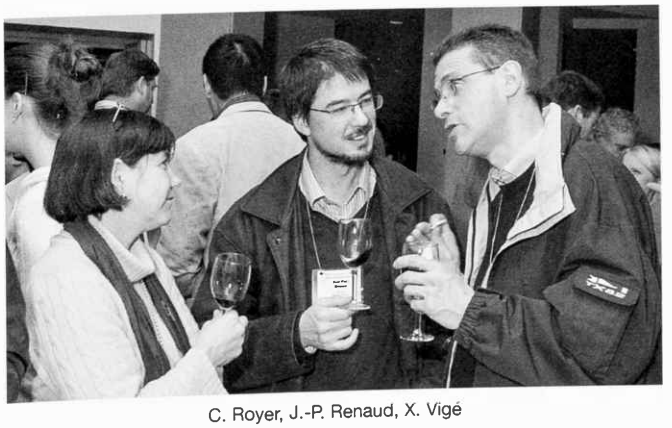
Nuclear Receptors in the Brain
Chairperson: B. Vennström, *Karolinska Institute, Stockholm, Sweden*

S.-M. Choi, H. Park



D. Kelly, J. Plutzky

P. Ornella, K. Bramlett



C. Royer, J.-P. Renaud, X. Vigé

Pharmacogenomics

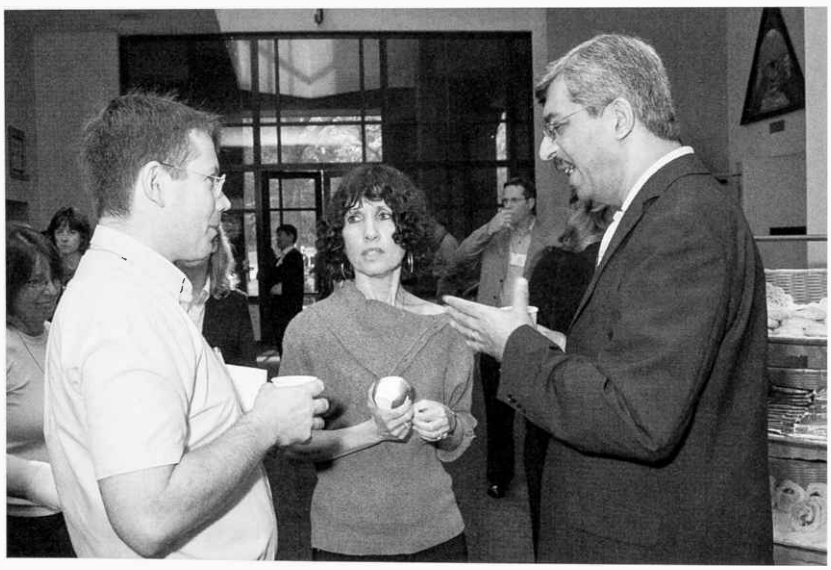
November 15–18 215 participants

ARRANGED BY **Alan Guttmacher,** National Human Genome Research Institute
J. Steven Leeder, Children's Mercy Hospital, Kansas City
Deborah Nickerson, University of Washington
Munir Pirmohamed, University of Liverpool
Richard Weinshilboum, Mayo Medical School, Minnesota
C. Roland Wolf, University of Dundee

This fourth annual meeting was a joint project of Cold Spring Harbor Laboratory and the Wellcome Trust and funded in part by the National Institute of General Medical Sciences, a branch of the National Institutes of Health. The primary objective of this series of meetings, held in alternating years on the Cold Spring Harbor Laboratory and Sanger Center campuses, continues to be the stimulation of interest from a broadly based constituency representing basic scientists and clinical investigators from both academic and corporate environments. This goal is accomplished by focusing on diverse issues related to the application of genomic approaches to investigations of variability in drug response in humans.

The meeting opened with a session highlighting examples of current research areas within the NIGMS-led Pharmacogenetics Research Network by Kathy Giacomini, a description of the "Biobank Japan" project at RIKEN in Japan by Yusuke Nakamura, and an overview by Francis Collins of current genomic initiatives at NHGRI relevant to Pharmacogenomics. The second day of the meeting was devoted to an update of global pharmacogenomic and database initiatives with five continents being represented in the program. Major subject areas for the remainder of the meeting included functional genomics and model systems, application of genome-wide approaches to pharmacogenomic studies, and two sessions devoted to "translational pharmacogenomics." The latter two sessions covered several disease-related and therapeutic areas with a primary emphasis on cancer and cardiovascular pharmacogenomics. A series of presentations specifically highlighted recent developments in the United States and Germany uncovering the relationship between *CYP2D6* genotype and the response to tamoxifen therapy in breast cancer.

The meeting was attended by 215 registrants from 23 countries, the broadest international representation to date; 67% of the attendees were from academia and 23% from industry. Women represented 37% of attendees and Ph.D. students and postdoctoral fellows constituted 23%. This year's meeting



I. MacPhee, K. Giacomini, M. Pirmohamed

was the most extensive integration of the genomics and pharmacogenomics communities to date, and future efforts will be directed toward further increasing representation at the meeting from the genetics and genomics community and providing more opportunities for trainees and junior faculty members to present their work in oral platform sessions. The Pharmacogenomics meeting is the premier meeting of its type and continues to provide an outstanding forum to explore the impact of genomics on the treatment of human disease and to describe the science underlying pharmacogenomics.

Support for this meeting was provided by the National Institute of General Medical Sciences, a part of the National Institutes of Health.

PROGRAM

Opening Session
Chairpersons: R.M. Weinshilboum, *Mayo Clinic College of Medicine, Rochester, Minnesota*; J.S. Leeder, *Children's Mercy Hospital, Kansas City, Missouri*

Global Pharmacogenomics Research and Database Initiatives
Chairpersons: K. Giacomini, *University of of California, San Francisco*; H.Z. Ring, *University of California, San Francisco*

Recent Advances in Global Pharmacogenomics Research
Chairpersons: K. Giacomini, *University of of California, San Francisco*; H.Z. Ring, *University of California, San Francisco*

Functional Genomics and Model Systems
Chairpersons: H. Jacob, *Medical College of Wisconsin, Milwaukee*; C.R. Wolf, *University of Dundee, United Kingdom*

Translational Pharmacogenomics I
Chairpersons: A. Hattersley, *Peninsula Medical School, Exeter, United Kingdom*; M. Pirmohamed, *University of Liverpool, United Kingdom*

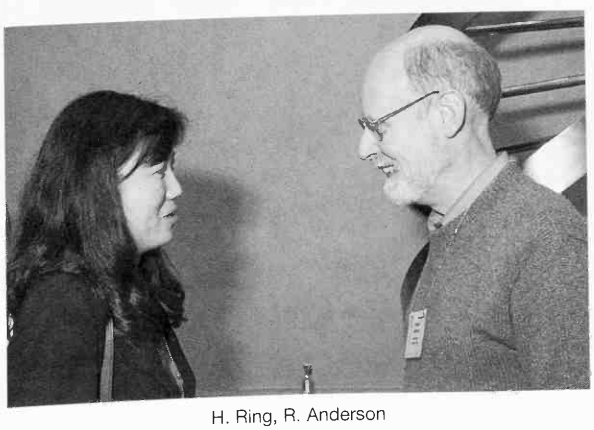
Genome-wide Approaches
Chairpersons: D. Nickerson, *University of Washington, Seattle*; J. Hirschhorn, *Children's Hospital, Harvard Medical School, Boston, Massachusetts*

Translational Pharmacogenomics II
Chairpersons: D. Flockhart, *Indiana University School of Medicine, Indianapolis*; M. Eichelbaum, *Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, Germany*

S. Weiss, R. Weinshilboum

F. Arena, E.A. Ortega, G. Suarez-Kurtz

D.S. Leeder, L. Kaminsky, R. Tyndale



H. Ring, R. Anderson

Winter Biotechnology Conference: Neurodegenerative Diseases: Biology and Therapeutics

November 30–December 3 128 participants

ARRANGED BY

Sam Gandy, Thomas Jefferson University
Virginia Lee, University of Pennsylvania School of Medicine
Marcy MacDonald, Massachusetts General Hospital
Peter Snyder, University of Connecticut

As many as one half of those aged 85 years or older will develop debilitating degenerative disease of the central nervous system, usually characterized by a decade or more of dependent living, accompanied by progressive failure of cognitive function and/or coordinated movement. Although these illnesses appear most commonly in the absence of obvious heredibility or identifiable genetic mutations, it has been possible during the last 20 years to discover risk-modifying DNA changes in some examples and predictable causative changes in others. From these findings, transgenic technology has rapidly led to the development of mouse, fruit fly, and nematode model systems that partially recapitulate the clinical abnormalities of the human diseases, as well as some of the hallmark molecular and morphological pathology of the conditions.

Rational biochemical and cell-based screens have generated lead compounds that show promise in living animal models. Most importantly, animal models have enabled the discovery of entirely unanticipated therapeutic strategies (such as amyloid-β immunotherapy). Some of these rationally discovered compounds and unexpected immunotherapies are now in Phase II and III clinical trials. The progress of compounds and rational strategies from the animal model to the human clinical trial; the design, results, and conclusions of the trials; and the return to the animal model with questions raised during human trials; are the areas of particular emphasis for these biannual Winter Biotechnology Conferences instituted in December 2000 with the explicit goal of facilitating the translation of "breakthrough" science into effective medicines.

For this meeting, seven 3- or 4-hour 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. Although the discussion of new, unpublished data was emphasized, the group was tolerant of a wide range of intellectual property conventions. Poster pre-

P. Snyder, M. MacDonald, S. Gandy, V. Lee

sentations were also encouraged. Posters were displayed for an extended period during the meeting, and poster viewing was especially encouraged during the cocktail hours before the evening meals.

Topics discussed at this meeting are listed below under "Program." Diseases such as Alzheimer's, Huntington's, spinocerebellar atrophies, enzymopathies such as Gaucher's disease, ALS, spinal muscular atrophy, prion diseases, Parkinson's, tauopathies, and synucleinopathies, as well as the newly described TDP-43-related diseases, were also considered.

PROGRAM

Federal and Foundation Perspectives on Neurodegenerative Disease Drug Discovery

Presymptomatic Evaluation: Imaging Methodology Development

Amyloid Imaging in Humans and its Applications to Mouse Models

Pathogenesis of Sporadic Alzheimer's: Synthesizing Evidence from Imaging, Cell Biology, and Genetics

Presymptomatic Evaluation with Nonimaging Biomarkers: Genetic, Biochemical, and Physiological Markers

Innovative Trial Design and an Update on Alzheimer's Trials

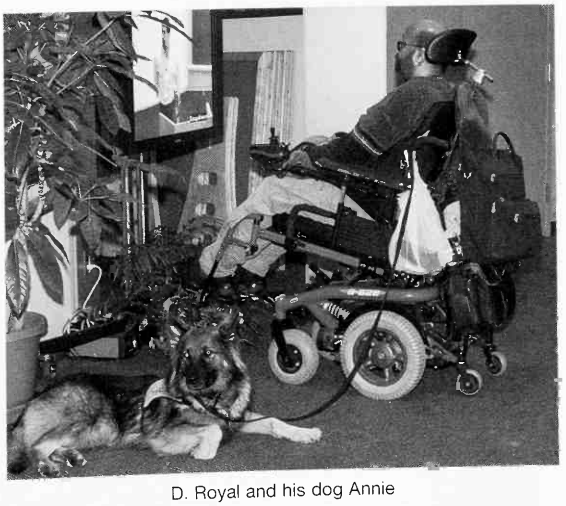
Drug Discovery Strategies in Academic and Pharma Sectors

Modulation of Disease by Aging, Metabolism, Excitotoxicity, and Extracellular Matrix

Protein Conformational Considerations: Prion, Aβ and Poly Q Oligomers

Motor Neuron–Frontotemporal Dementia–Parkinson's Spectrum of Disease(s)

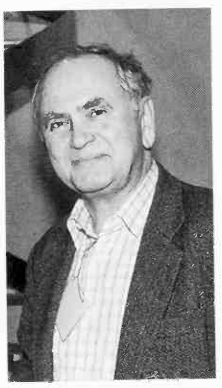
S. Hata, T. Suzuki



D. Royal and his dog Annie

R. Tanzi, J. Talon, J. Rothstein

T. Veldman, M. Agostino



P. Seck

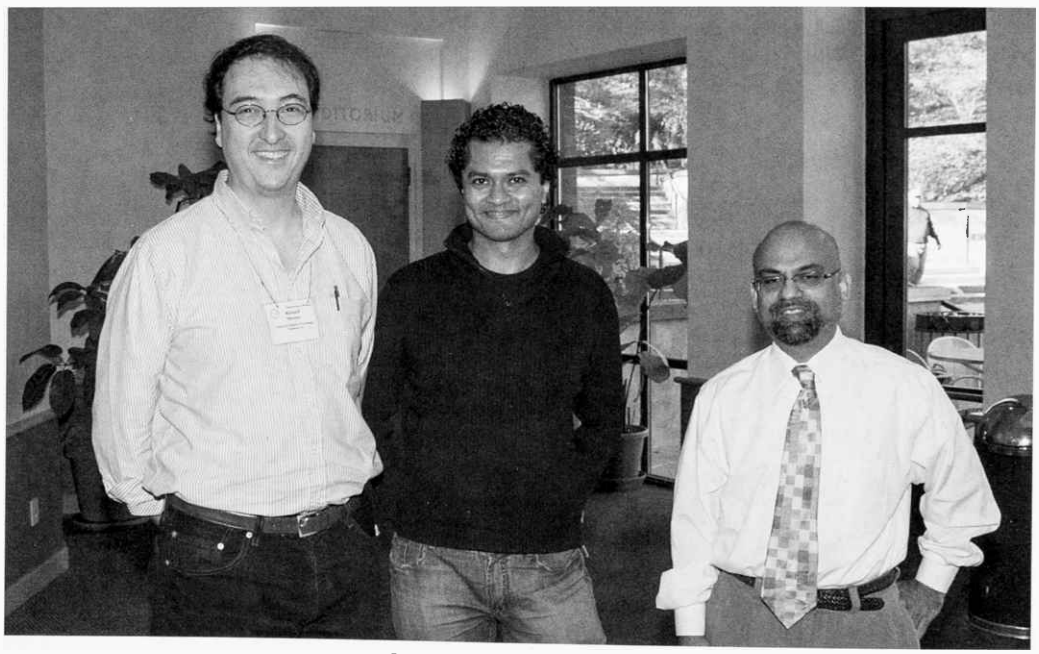
Winter Biotechnology Conference: Engineering Principles in Biological Systems

December 3–6 101 participants

ARRANGED BY **Bud Mishra,** New York University School of Medicine
Partha Mitra, Cold Spring Harbor Laboratory
Richard Murray, California Institute of Technology

This year saw the addition of a new meeting to the CSHL repertoire. The first meeting on Engineering Principles in Biological Systems was held in early December, following on an earlier planning workshop at the Banbury conference center in May of the same year. There were two key ideas behind this meeting: that theoretical principles which have been developed in the context of human engineered systems are useful in understanding biological function and that these principles apply across scales, from the cellular to the organism level. In keeping with these ideas, we hoped to, and succeeded in, attracting researchers in a broad range of fields ranging from bacterial systems biology to neural systems, with shared interest in engineering principles.

Sessions were broken up according to broad areas of engineering, and there was also a session on evolution. The introductory session set the tone of the meeting: introductory remarks (by Partha Mitra) about the need of a theoretical canon in biology, was followed on by an experimental talk about engineering biochemical circuits in cells, design principles in metabolic and gene regulation circuits, a talk about insect locomotion, and a plenary talk by John Doyle. An innovative aspect of the meeting much appreciated by the attendees was tutorial talks on control theory by Richard Murray and on machine learning by Partha Niyogi. Attendees also expressed appreciation of the broad spectrum of the meeting, and it was felt that the meeting was quite unique in its reach and scope. On the basis of this enthusiasm, the plans are to repeat the meeting in December 2008.



R. Murray, P. Mitra, B. Mishra

PROGRAM

INTRODUCTION
P. Mitra, *Cold Spring Harbor Laboratory*

Feedback, Dynamics, and Control I
Chairperson: B. Mishra, *New York University School of Medicine*

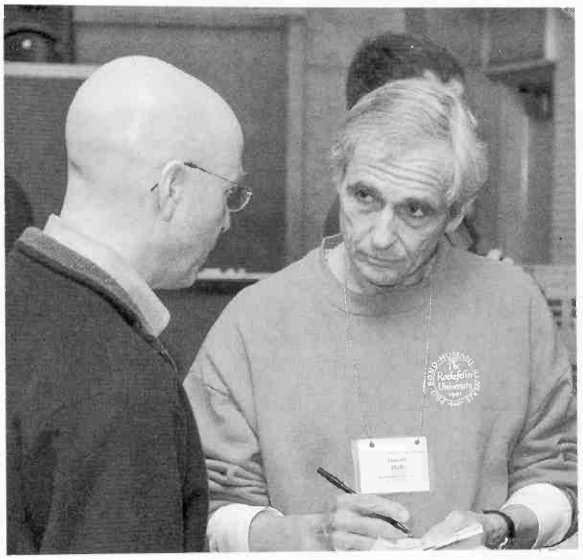
Synthetic Biology, Biomedical Applications
Chairperson: H. El-Samad, *University of California, San Francisco*

Feedback, Dynamics, and Control II
Chairperson: H. Breiter, *Massachusetts General Hospital, Boston*

Learning, System Identification, Perception
Chairperson: R. Murray, *California Institute of Technology, Pasadena*

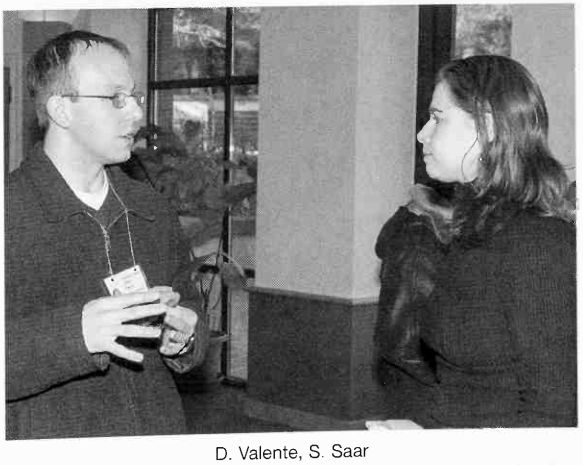
Evolution, Evolvability
Chairperson: L. Carporale, *Columbia University, New York*

Signaling and Communications
Chairperson: P. Niyogi, *University of Chicago, Illinois*

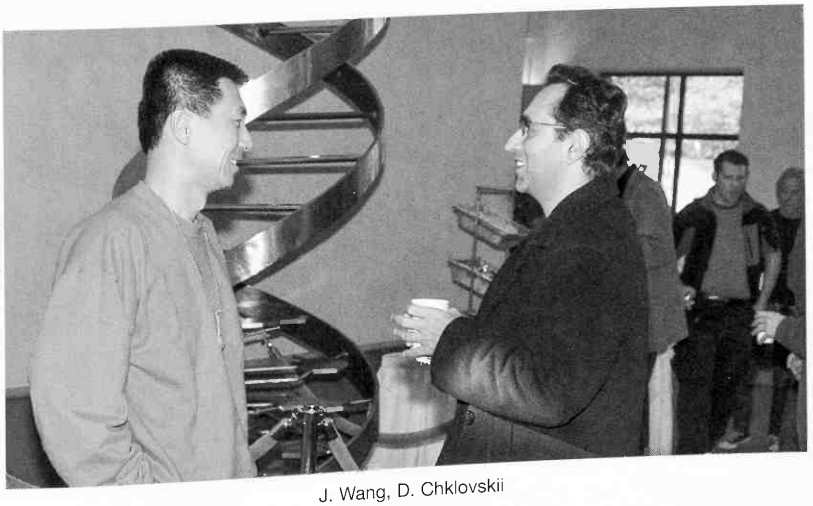


S. Schiff, D. Pfaff

H. El-SaMad, C. Smolke, T. Chouard



D. Valente, S. Saar



J. Wang, D. Chklovskii

The GABAergic System

December 6–10 135 participants

ARRANGED BY **Gyorgy Buzsáki,** Rutgers University
Joshua Huang, Cold Spring Harbor Laboratory
Henry Markram, Brain & Mind Institute
Roger Nicoll, University of California

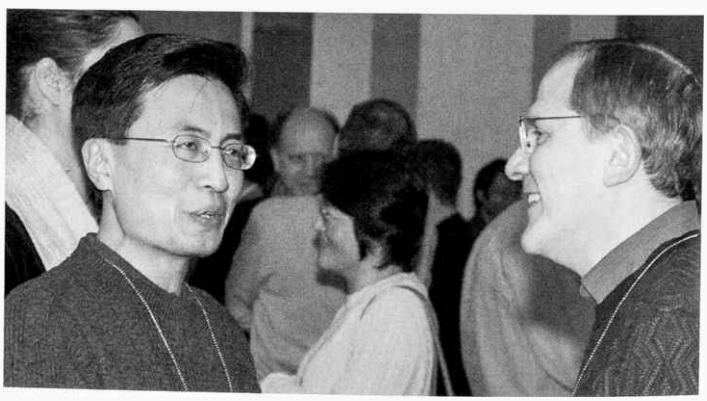
GABA is one of the major neurotransmitters and the only known mediator of inhibition in the brain. In addition to synaptic transmission, homeostatic feedback, oscillations, shaping network patterns, and assisting in the formation of cell assemblies, this simple molecule has been implicated in a number of other aspects of neuronal functions, including, e.g., trophic effects, morphogenesis, cell proliferation, and control of local blood flow. Importantly, GABA dysfunction has been implicated in a range of neurological, neurodevelopmental, and psychiatric disorders. Progress in understanding the genetic design, construction, and mode of operation of the GABAergic system will significantly advance our knowledge of brain development and function. Paradoxically, no regular, high-quality scientific conference has been devoted to the discussion of GABAergic system that would stimulate the interactions between investigators working in different disciplines but with a common interest in GABA function. To facilitate the interdisciplinary approach to the GABA system, this first meeting brought together scientists using molecular and genomic approaches, developmental neurobiologists, physiologists, system neuroscientists, and clinicians, with the explicit goal of fostering future work in this critical domain of research.

The program included a keynote address by Professor Thomas M. Jessell, who has been studying the assembly of neural circuit in spinal cord. His work has profound implication on the genetic logic underlying the construction and function of neural circuits in general.

The oral sessions are listed below under "Program." The speakers included mixtures of established investigators presenting their recent results as well as graduate students and postdoctoral fellows presenting their new findings. These balanced presentations, in the tradition of CSH meetings, are considered to be major motivation for many participants. They reflect not only some acknowledgment, but also a wider forum for young researchers. The talks and poster sessions generated lively, interactive discussions. Several presentations described new methods to solve complex problems in GABA signaling and function. Some of the discussions have already fostered fruitful scientific collaborations.

Describing the specific sessions is not relevant to this short report. Nevertheless, it should be mentioned that it became clear for all participants that the field a GABAergic signaling has expanded over the past decade beyond the niche of specialized areas. In fact, the various subfields, represented at this meeting emerged semi-independently from the various larger disciplines, and the interactions of the newly emerged subdivisions allowed for creating a novel forum of discussion under the common

G. Buzsáki, H. Lester



J. Huang, A. De Blas

umbrella of this simple molecule (GABA). The importance of this convergence is clearly reflected by the ever-increasing number of papers and citations beyond the mean average of neuroscience papers.

PROGRAM

From Genomics to Cell Types
Chairpersons: S. Nelson, *Brandeis University, Waltham, Massachusetts*; R. Yuste, *Columbia University, New York*

Panel Discussion on Cell Types
Moderator: R. Yuste, *HHMI/Columbia University*
S. Nelson, *Brandeis University*
G. Buzsáki, *Rutgers University*
G. Fishell, *New York University*
S. Anderson, *Cornell University*

Development
Chairpersons: J. Rubenstein, *University of California, San Francisco*; Y. Ben-Ari, *INMED, INSERM, Marseille, France*

GABA Transporters
Chairpersons: K. Kaila, *University of Helsinki, Finland*; H. Lester, *California Institute of Technology, Pasadena*

GABA Receptors
Chairpersons: S. Moss, *University of Pennsylvania, Philadelphia*; I. Mody, *University of California, Los Angeles*

Keynote Address
T. Jessell, *HHMI/Columbia University College of Physicians & Surgeons*

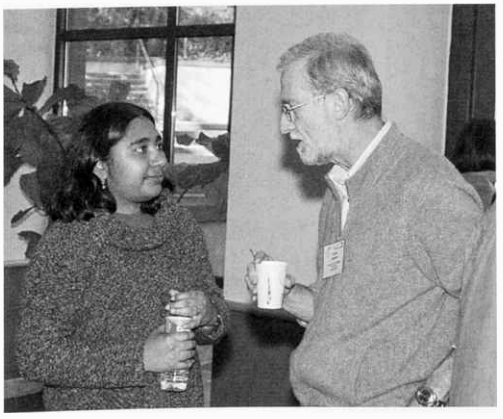
Cellular Physiology
Chairpersons: A. Thomson, *University of London School of Pharmacy, United Kingdom*; P. Jonas, *University of Freiburg, Germany*

Plasticity
Chairpersons: D. Kullman, *University College London, United Kingdom*; C. McBain, *NICHD/National Institutes of Health, Bethesda, Maryland*

Network, System, Behavior
Chairpersons: T.F. Freund, *Hungarian Academy of Sciences, Budapest*; H. Monyer, *University of Heidelberg, Germany*

C. Wu, A. Thomson

H. Gache, M. Vithlani



B. Chattopadhyaya, C. Avendaño

POSTGRADUATE COURSES

The Postgraduate Courses program at Cold Spring Harbor Laboratory is aimed at meeting the special need for training in interdisciplinary subjects that are either so new or so specialized that universities do not adequately treat them. Our aim is to provide intensive study in the most recent developments and techniques in these subjects and to prepare students to enter directly into research in a particular area. To ensure up-to-date coverage of current research work, we bring together course faculty from many laboratories around the world and supplement this staff with a series of seminar speakers.

Protein Purification and Characterization

March 29–April 11

INSTRUCTORS

K. Adelman, National Institutes of Health/NIEHS, Research Triangle Park, North Carolina
R. Burgess, University of Wisconsin, Madison
A. Courey, University of California, Los Angeles
S.-H. Lin, M.D. Anderson Cancer Center/University of Texas, Houston

ASSISTANTS

S. Duellman, University of Wisconsin, Madison
N. Fuda, Cornell University, Ithaca, New York
B. Glaser, University of Wisconsin, Madison
H. Hess, Massachusetts Institute of Technology, Cambridge
Y.-C. Lee, M.D. Anderson Cancer Center/University of Texas, Houston
S. Nechaev, University of California, San Diego, La Jolla
M. Nie, University of California, Los Angeles
M. Marr, University of California, Berkeley
C. Winkler, University of California, Los Angeles

This course was for scientists who were not familiar with techniques of protein isolation and characterization. It was a rigorous program that included laboratory work all day and a lecture with discussion session every evening. Each student became familiar with each of the major techniques in protein purification by actually performing four separate isolations including (1) a regulatory protein from muscle tissue, (2) a sequence-specific, DNA-binding protein, (3) a recombinant protein overexpressed in *Escherichia coli*, and (4) a membrane-bound receptor. A variety of bulk fractionation, electrophoretic, and chromatographic techniques included precipitation by salts, pH, and ionic polymers; ion-exchange, gel-filtration, hydrophobic interaction, and reverse-phase chromatography; lectin affinity, ligand affinity, oligonucleotide affinity, and immunoaffinity chromatography; polyacrylamide gel electrophoresis and electroblotting; and high-performance liquid chromatography.

Procedures were presented for solubilizing proteins from inclusion bodies and refolding them into active monomeric forms. Methods of protein characterization were utilized to include immunological and biochemical assays, peptide mapping, amino acid analysis, protein sequencing, and mass spectrometry. Emphasis was placed on strategies of protein purification and characterization. Guest lecturers discussed protein structure, modification of proteins, methodologies for protein purification and characterization, and applications of protein biochemistry to cell molecular and cancer biology.

Speakers included Gerald Hart, Michael Myers, William Tansey, Lemoor Joshua-Tor, Michael Marr, Richard Burgess, Al Courey, Sue-Hwa Lin, and Andrew Link.

PARTICIPANTS

Capotosti, F., M.S., Universite de Lausanne, Switzerland
Gao, H., Ph.D., Howard Hughes Medical Institute at Wadsworth Center, Albany, New York
Heusner, C., Ph.D., University of California, Los Angeles
Huang, S., Ph.D., Baylor College of Medicine, Houston, Texas
Laloraya, S., Ph.D., Indian Institute of Science, Bangalore, India
Li, C., B.S., University of California, Los Angeles
Li, Z., Ph.D., New York University School of Medicine, New York
Link, A., Ph.D., Vanderbilt University School of Medicine, Nashville, Tennessee
Luke, B., Ph.D., ISREC, EPFL, Lausanne, Epalinges, Switzerland
O'Donoghue, P., Ph.D., University of Illinois at Urbana-Champaign
Oldrini, B., M.D., FIRC Institute of Molecular Oncology, Milan, Italy
Paden, E., B.S., University of North Carolina, Chapel Hill
Payne, A., M.S., Georgia Institute of Technology, Atlanta
Payne, C., Ph.D., Harvard University, Cambridge, Massachusetts
Zamudio, J., B.S., University of California, Los Angeles

SEMINARS

Burgess, R., University of Wisconsin, Madison: Introduction to protein purification, immunoaffinity chromatography. RNA polymerase-sigma factor interactions and use of LRET-based assays for drug discovery and biochemistry.
Courey, A., University of California, Los Angeles: Spreading the silence: Groucho, Yan, and polycomb-mediated repression.
Hart, G., Johns Hopkins, Baltimore, Maryland: Protein glycosylation: The most ubiquitous posttranslational event in eukaryotes.
Joshua-Tor, L., Cold Spring Harbor Laboratory: Protein crystallography introduction and demonstration. The crystal structure of Agronaute: The secret life of slicer.
Lin, S.-H., M.D. Anderson Cancer Center, Houston, Texas: Osteoblasts and prostate cancer metastasis.
Link, A., Vanderbilt University, Nashville, Tennessee: Proteomic analysis of the yeast translational machinery.
Marr, M., HHMI, University of California, Berkeley: Characterization of protein-protein and protein-DNA interactions in vivo.
Myers, M., Cold Spring Harbor Laboratory: Introduction to mass spectroscopy for analyzying biological samples.
Tansey, W., Cold Spring Harbor Laboratory: Transcriptional control, cancer, and the ubiquitin proteosome system.

Cell and Developmental Biology of *Xenopus*

April 1–11

INSTRUCTORS **J. Heasman,** Children's Hospital Medical Center, Cincinnati, Ohio
C. Wylie, Children's Hospital Research Foundation, Cincinnati, Ohio

ASSISTANTS **M. Kofron,** Children's Hospital Medical Center, Cincinnati, Ohio
A. Mir, Children's Hospital Medical Center, Cincinnati, Ohio
L. Zacchigna, University of Padua, Italy

Xenopus is the leading vertebrate model for the study of gene function in development. The combination of lineage analysis, gene-knockout strategies, experimental manipulation of the embryo, and genomic/bioinformatic techniques makes it ideal for studies on the molecular control of embryo pattern-

ing, morphogenesis, and organogenesis. The course combined intensive laboratory training with daily lectures from recognized experts in the field. Students learned both emerging technologies and classical techniques to study gene function in *Xenopus* development. An important element was the informal interaction between students and course faculty.

Technologies covered included oocyte and embryo culture, lineage analysis and experimental manipulation of embryos, gain- and loss-of-function analyses using mRNAs and antisense oligos, whole-mount in situ hybridization, immunocytochemistry, RT-PCR, genomic/bioinformatic techniques, preparation of transgenic embryos, and use of *Xenopus tropicalis* for genetic analyses. This course was designed for those new to the *Xenopus* field, as well as for those wanting a refresher course in the emerging technologies. The course was open to investigators from all countries.

Additional lecturers included Timothy Grammer, Ray Keller, Mustafa Khokha, Carole LaBonne, Stefano Piccolo, Jonathan Slack, Janet Weber, and Aaron Zorn.

PARTICIPANTS

Belak, Z., M.S., University of Saskatchewan, Saskatoon, Canada
Bordalo, P., Ph.D., Harvard Medical School, Boston, Massachusetts
Delgado, I., M.Sc., Universidad Andres Bello, Santiago, Chile
Gallop, J., M.bioc., Harvard Medical School, Boston, Massachusetts
Jia, Z., Ph.D., NIH/NCI-Frederick, Maryland
Lacoste, N., Ph.D., Curie Institute, Paris, France
Nerlick, S., B.A., University of Miami, Florida
Pabbidi, M., BVSc., Southern Illinois University, Springfield
Perez, O., Licen., Pontificia Universidad Catolics del Ecuador, Quito, Ecuador
Porter, K., Ph.D., U.S. Army Center for Environmental Health Research, Fort Detrick, Maryland
Sanchez, V.A., B.A., Centro de Investigacion Principe Felipe, Valencia, Spain
Snedden, D., B.S., University of Notre Dame, Indiana
Taelman, V., Ph.D., Universite Libre de Bruxelles, Gosselies, Beligum
Wu, M., B.A., Cancer Research U.K., London
Young, J., M.S., Van Andel Research Institute, Grand Rapids, Michigan

SEMINARS

Grammer, T., University of Berkeley, California: *Xenopus tropicalis* genetics.
Heasman, J., Children's Hospital Medical Center, Cincinnati, Ohio: Maternal control of development.
Keller, R., University of Charlottesville, Virginia: The early morphogenesis of the *Xenopus* embryo.
Khoka, M., University of California, San Francisco: *Xenopus tropicalis* bioinformatics and genomics.
LaBonne, C., University of Northwestern, Chicago, Illinois: Neural crest.
Piccolo, S., University of Padua, Italy: Establishment of the primary germ layers.
Slack, J., University of Bath, United Kingdom: Tail regeneration. Tail Development.
Weber, J., National Institutes of Health, Bethesda, Maryland: NCBI tools and resources.
Wylie, C., Children's Hospital Research Foundation, Cincinnati, Ohio: Classical experiments in *Xenopus*.
Zorn, A., Children's Hospital Medical Center, Cincinnati, Ohio: Organogenesis of the endoderm.

Genetics of Complex Human Diseases

June 7–13

INSTRUCTORS **A. Al-Chalabi,** FRCP, Institute of Psychiatry, 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 o complex disorders such as diabetes, cardiovascular disease, cancer, and autism. We discussed genetic-epidemiologic study designs, including family, parent-child trio, 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 the appropriate contexts for their use. Study design and methods for analysis of quantitative risk factors related to complex diseases was covered as well as the latest ideas in data reduction such as haplotype mapping and SNP tagging. Copy-number variation as an influence on complex disease risk and Bayesian coalescent models for the fine mapping of disease loci were also discussed. Illustrations were provided through discussion of results from ongoing studies of a variety complex diseases and related risk factors.

Speakers included Paul De Bakker (Massachusetts General Hospital), John Gallacher (Cardiff University, UK), Michael Hauser (Duke University), Toby Johnson (University of Edinburgh, UK), Eden Martin (Duke University), Cathryn Lewis (Kings College, London), Shaun Purcell (Kings College, London), Jonathan Sebat (Cold Spring Harbor Laboratory), Pak Sham (University of Hong Kong, China), and Janet Sinsheimer (University of California, Los Angeles).

PARTICIPANTS

Agam, A., B.S., University of Oxford, United Kingdom
Burdick, K., Ph.D., Zucker Hillside Hospital-NSLIJHS, Hillside, New York
Deo, A., B.S., New York University, American Museum of Natural History, New York
Gallacher, J., Ph.D., Cardiff University, United Kingdom
Gotay, N., Ph.D., National Institute of Mental Health, Bethesda, Maryland
Hildebrandt, J., Ph.D., Medical University of South Carolina, Charleston
Houlihan, L., M.S., Indian Institute of Science, Bangalore
Jun, J., M.S., University of Connecticut, Storrs
Kasperaviciute, D., Ph.D., University College, London
Kennerson, M., Ph.D., University of Sydney, Concord, Australia
Lazaridis, K., Ph.D., Mayo Clinic & Foundation/NHGRI-NIH, Bethesda, Maryland
Malhotra, D., M.S., Cold Spring Harbor Laboratory
Martina, G., Ph.D., University of Parma, Italy
Mihailov, E., B.S., University of Tartu, Estonia
Pasaniuc, B., B.S., University of Connecticut, Storrs
Rana, N., Ph.D., Cambridge University, United Kingdom
Rocheleau, G., Ph.D., McGill University, Montreal, Canada
Shah, P., B.S., MRC Institute of Neurology, London
Simpson, C., Ph.D., Institute of Psychiatry, London
Sookoian, S., Ph.D., Instituto de Investigaciones Medicas, Buenos Aires, Argentina
Su, H., Ph.D., NIH/NIAID, Bethesda, Maryland
Tinkle, S., Ph.D., National Institutes of Environmental Health Sciences, Research Triangle, North Carolina
Zia, A., Ph.D., Columbia University, New York
Zipprich, J., B.S., Columbia University, New York

SEMINARS

Al-Chalabi, A., Kings College, London: Linkage introduction.
Almasy, L., Southwest Foundation for Biomedical Research, San Antonio, Texas: Welcome and introduction to course. Methods for analysis of quantitative risk factors.
Almasy, L. and Al-Chalabi, A., Southwest Foundation for Biomedical Research, San Antonio, Texas: Genetics programs practical.
De Bakker, P., Massachusetts General Hospital, Boston: SNP tagging, data integration LD and haplotype blocks, genome-wide association.
Gallacher, J., Cardiff University, United Kingdom: Genetic epidemiology from the epidemiologist's perspective.
Hauser, M., Duke University, Durham, North Carolina: From locus to gene: Complement factor H in AMD.
Johnson, T., University of Edinburgh, United Kingdom: Evolution and population genetics.
Lewis, C., Kings College, London, United Kingdom: Association studies: An introduction.
Martin, E., Duke University Medical Center, Durham, North Carolina: Linkage disequilibrium and association studies.
Purcell, S., Kings College, London: Large-scale association analysis.
Sebat, J., Cold Spring Harbor Laboratory: Analysis of genome copy-number variation in human disease.
Sham, P., University of Hong Kong, China: Power and multiple testing issues in association studies.
Sinsheimer, J., David Geffen School of Medicine, University of California, Los Angeles: Statistics 101.

Advanced Bacterial Genetics

June 7–27

INSTRUCTORS
J. Kirby, Georgia Institute of Technology, Atlanta
S. Lovett, Brandeis University, Waltham, Massachusetts
A. Segall, San Diego State University, California

ASSISTANTS
J. Foti, Brandeis University, Waltham, Massachusetts
S. Orchard, University of Wisconsin, Madison
H. Vlamakis, Harvard Medical School, Boston, Massachusetts

The course presented logic and methods used in the genetic dissection of complex biological processes in eubacteria. Laboratory methods used included classical mutagenesis using transposons, mutator strains, and chemical and physical mutagens; the mapping of mutations using genetic and physical techniques; modern approaches to the generation and analysis of targeted gene disruptions and fusions using PCR and cloning methods; epitope insertion mutagenesis; and site-directed mutagenesis. Key components of the course were the use of sophisticated genetic methods in the analysis of model eubacteria and the use of the wealth of new genomic sequence information to motivate these methods. Invited lecturers presented various genetic approaches to study eubacterial mechanisms of metabolism, development, and pathogenesis.

Speakers included Abigail Salyers (University of Illinois at Urbana-Champaign), Ann Hochschild (Harvard Medical School), Christine Jacobs-Wagner (Yale University), Igor Jouline (University of

Tennessee), John Roth (University of California), Robert Kolter (Harvard Medical School), Stanley Maloy (San Diego State University), Susan Golden (Texas A&M University), William Jacobs (Albert Einstein College of Medicine), and Forest Rohwer (San Diego State University).

PARTICIPANTS

Aldea, M., M.S., Texas A&M University, College Station
Allen, R., Ph.D., University of Edinburgh, United Kingdom
Carr, J., Ph.D., Massachusetts Institute of Technology, Cambridge
Cohen, D., B.S., University of Minnesota, Minneapolis
Fero, M., Ph.D., Stanford University School of Medicine, California
Flaherty, P., Ph.D., Massachusetts Institute of Technology, Cambridge
Forbes, S., B.S., University Albany and Wadsworth Center, Albany, New York
Guzman-Verri, C., Ph.D., Universidad Nacional, Heredia, Costa Rica
Long, T., B.S., Princeton University, New Jersey
Pereira, C., M.S., Instituto Gulbenkian de Ciencia, Oeiras, Portugal
Pichler, F., Ph.D., The University of Auckland, New Zealand
Roper, M.C., B.S., University of California, Davis
Samoilov, M., Ph.D., HHMI/University of California, Berkeley
Tobin, C., B.Sc., Uppsala University, Sweden
Wagner, S., M.S., Stockholm University, Sweden
Wilson, L., Ph.D., University of British Colombia, Vancouver, Canada

SEMINARS

Golden, S., Texas A&M University, College Station: Winding up the cyanobacterial circadian clock.
Hochschild, A., Harvard Medical School, Boston, Massachusetts: Using a bacterial two-hybird assay to study dynamics at the sigma/core interface of bacterial RNA polymerase.
Jacobs, W., Albert Einstein College of Medicine/HHMI, Bronx, New York: Fulfilling Koch's coroliar for mycobacterium tuberculosis. The five unassailable truths of bacterial genetics: Elucidating the mechanism of action of isoniazid on mycobacterium tuberculosis.
Jacobs-Wagner, C., Yale University, New Haven, Connecticut: Cell polarity, cell shape, and the cytoskeleton in *Caulobacter crescentus*.
Jouline (Zhulin), I., The University of Tennessee, Oakridge: Comparative genomics of signal transduction.
Kolter, R., Harvard Medical School, Boston, Massachusetts: *B. subtilis* multicellular development.
Maloy, S., San Diego State University, California: Deranged chromosomes in bacteria.
Rohwer, F., San Diego State University, California: The awesome power of metagenomics.
Roth, J., University of California, Davis: Natural (and unnatural) selection and their different effects on genetic adaptation.
Salyers, A., University of Illinois at Urbana-Champaign: Horizontal gene transfer in the human intestine.

Ion Channel Physiology

June 7–27

INSTRUCTORS
M. Farrant, University College London, United Kingdom
M. Hausser, University College London, United Kingdom
N. Spruston, Northwestern University, Evanston, Illinois

COINSTRUCTORS
B. Clark, University College London, United Kingdom
J. Diamond, National Institutes of Health, Bethesda, Maryland
I. Soltesz, University of California, Irvine

ASSISTANTS
G. Dugue, Ecole Normale Suprieure, Paris, France
T. Jarsky, Northwestern University, Chicago, Illinois
S. Moore, Northwestern University, Evanston, Illinois
A. Scimemi, National Institutes of Health, Bethesda, Maryland

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, or (4) are developmentally required and regulated. The research interests of guest lecturers reflect these areas of emphasis.

The laboratory component of the course introduced students to electrophysiological approaches for the study of ion channels in their native environments. Hands-on exercises included patch-clamp

recording of ion-channel activity in neurons in brain slice preparations. Different recording configurations were used (e.g., whole-cell, cell-free, and nucleated patches) to examine macroscopic or single-channel activity. The advantages and disadvantages of each method, preparation, and recording techniques were considered with respect to the specific scientific questions being asked. Admissions priority was given to students and postdocs with a demonstrated interest, specific plans, and a supportive environment to apply these techniques to a defined problem.

Guest speakers Bruce Bean (Harvard Medical School), Jeffrey Diamond (National Institutes of Health), Florian Engert (Harvard University), Daniel Johnston (University of Texas at Austin), Jeffrey Magee (LSUHSC), Indira Raman (Northwestern University), Massimo Scanziani (University of California, San Diego), Steve Sigelbaum (Columbia University), Ivan Soltesz (University of California), and Tomoyuki Takahashi (University of Tokyo Graduate School of Medicine).

PARTICIPANTS

Ahmed, O., B.Sc., Brown University, Providence, Rhode Island
Domingos, A., Ph.D., The Rockefeller University, New York
Gomez-Ospina, N., B.A., Stanford University, California
Komiyama, T., B.S., Stanford University, California
Kress, G., B.S., Washington University in St. Louis, Missouri
Kwan, K., Ph.D., Harvard Medical School/HHMI, Boston, Massachusetts
Ma, G., Ph.D., The Rockefeller University, New York
Schafer, J., B.S., Johns Hopkins University, Baltimore, Maryland
Stanley, S., Ph.D., The Rockefeller University, New York
Uchida, N., Ph.D., Cold Spring Harbor Laboratory
Vervaeke, K., M.Sc., University of Oslo, Norway
Witt, R., B.S., Harvard Medical School, Boston, Massachusetts

SEMINARS

Bean, B., Harvard Medical School, Boston, Massachusetts: Pacemaking in central neurons.
Diamond, J., National Institutes of Health, Bethesda, Maryland: Excitatory synaptic transmission: Glutamate diffusion, uptake, and receptor activation.
Engert, F., Harvard University, Cambridge, Massachusetts: Spike-timing-dependent plasticity of synaptic connections: An overview.
Farrant, M., University College London, United Kingdom: GABA receptors and synaptic inhibition.
Hausser, M., University College London, United Kingdom: Synaptic integration.
Johnston, D., University of Texas, Austin: Voltage-dependent potassium channels.
Magee, J., LSUHSC, New Orleans, Louisianna: Dendritic integration of synaptic input.
Raman, I., Northwestern University, Evanston, Illinois: Resurgent sodium current and action potential firing in cerebellar Purkinje neurons.
Scanziani, M., University of California, San Diego, La Jolla: Short-term synaptic plasticity.
Sigelbaum, S., Columbia University, New York: HCN1 hyperpolarization-activated channels: A gate of learning and memory.
Soltesz, I., University of California, Irvine: Plasticity of endocannabinoid regulation of GABA release.
Takahashi, T., University of Tokyo Graduate School of Medicine, Japan: Voltage-dependent calcium channels.

Molecular Embryology of the Mouse

June 7–27

INSTRUCTORS **B. Capel,** Duke University Medical Center, Durham, North Carolina
M. Shen, UMDNJ–Robert Wood Johnson Medical School, Piscataway, New Jersey

COINSTRUCTORS **D. Threadgill,** University of North Carolina, Chapel Hill
P. Trainor, Stowers Institute for Medical Research, Kansas City, Missouri

ASSISTANTS **D. Escalante-Alcalde,** Institute of Cellular Physiology-UNAM, Mexico
J. Rivera, University of North Carolina, Chapel Hill
K. Yuna, Duke University Medical Center, Durham, North Carolina

This intensive laboratory and lecture course was designed for biologists interested in applying their expertise to the study of mouse development. Lectures provided the conceptual basis for contemporary research in mouse embryogenesis and organogenesis, whereas laboratory practicals provided extensive hands-on introduction to mouse embryo analysis. Experimental techniques covered included in vitro culture and manipulation of pre- and postimplantation embryos, embryo transfer, culture and genetic manipulation of embryonic stem cells, production of chimeras by embryo aggregation and by ES cell injection, and transgenesis by pronuclear microinjection. In addition, this year's practicals featured increased emphasis on phenotypic analysis of mutants, including techniques of histology, in situ hybridization, immunohistochemistry, skeletal preparation, organ culture, and tissue recombination.

Confirmed speakers for this year included Kathryn Anderson, Richard Behringer, Allan Bradley, Denis Duboule, Kevin Eggan, Kat Hadjantonakis, Greg Hannon, Gerard Karsenty, Robin Lovell-Badge, Terry Magnuson, Ann McLaren, Andy McMahon, Andras Nagy,Luis Parada, Janet Rossant, Davor Solter, Oliver Smithies, Colin Stewart, David Threadgill, Patrick Tam, and Paul Trainor.

PARTICIPANTS

Benito-Gutierrez, E., B.Sc., Universitat de Barcelona, Spain
Chaivorapol, C., B.S., University of California, San Francisco
Chen, F., M.D., Boston University School of Medicine, Massachusetts
Dlugosz, M., B.S., Stony Brook University, Stony Brook, New York
Eriksson, M., M.S., Karolinska Institutet, Stockholm, Sweden
Girard, A., B.S., Cold Spring Harbor Laboratory
Hofmann, C., Ph.D., Fox Chase Cancer Center, Philadelphia, Pennsylvania
Liu, Z., Ph.D., Washington University in St. Louis Medical School, Missouri
Luo, Y., Ph.D., Princeton University, New Jersey
Mekel-Bobrov, N., B.A., University of Chicago, Illinois
Scott, D., Ph.D., Baylor College of Medicine, Houston, Texas
Stafford, D., Ph.D., University of California, Berkeley
Thakar, R., Ph.D., Cold Spring Harbor Laboratory
Vigneau, S., M.S., Pasteur Institute, Paris, France

SEMINARS

Anderson, K., Memorial Sloan-Kettering Institute, New York: Forward genetic screens.
Behringer, R., The University of Texas/M.D. Anderson Cancer Center, Houston: Transgenics and insertional mutants.
Bradley, A., Wellcome Trust Sanger Institute, Hinxton, United Kingdom: Large-scale screens.
Capel, B., Duke University Medical Center, Durham, North Carolina: Immediate mesoderm.
Duboule, D., University of Geneva, Switzerland: *Hox* genes/ limb patterning.
Eggan, K., Stowers Medical Institute/Harvard University, Cambridge, Massachusetts: Nuclear reprogramming.
Escalante, D., Institute of Cellular Physiology-UNAM, Mexico: Lipid phosphate phosphatase-3 (LPP3) deficiency affects embryo development due to alterations in several signaling pathways.
Hadjantonakis, K., Columbia University, New York: Optical imaging.
Hannon, G., Cold Spring Harbor Laboratory: RNA interference: Biology and applications.
Karsenty, G., Baylor College of Medicine, Houston, Texas: Skeletal biology.
Lovell-Badge, R., MRC National Institute for Medical Research, The Ridgeway, United Kingdom: *Sox* genes, neural development, and stem cells in the CNS.
Magnuson, T., University of North Carolina, Chapel Hill: Epigenetics.
McLaren, A., University of Cambridge, United Kingdom: Germ line.
McMahon, A., Harvard University, Cambridge, Massachusetts: Kidney organogenesis.
Nagy, A., Samuel Lunenfeld Research Institute, Mt. Sinai Hospital, Toronto, Canada: Gene targeting.
Parada, L., University of Texas Southwestern Medical Center, Dallas: Cancer models.
Rivera-Perez, J., University of North Carolina, Chapel Hill: Axial development: A viscera C (endoderm) view.
Rossant, J., The Hospital for Sick Children, Toronto, Canada: Chimera analysis.
Shen, M., UMDNJ–Robert Wood Johnson Medical School, Piscataway, New Jersey: Nodal signaling and embryo patterning.
Smithies, O., University of North Carolina, Chapel Hill: Physiology.
Solter, D., Max-Planck Institut für Immunbiology, Freiburg, Germany: Preimplantation development.
Stewart, C., National Cancer Institute, Frederick, Maryland: ES cells.
Tam, P., Children's Medical Research Institute, Sydney, Australia: Introduction to mouse development. Gastrulation and formation of the body plan.
Threadgill, D., University of North Carolina, Chapel Hill: Quantitative traits.
Trainor, P., Stowers Institute for Medical Research, Kansas City, Missouri: Craniofacial development.

Applications in Next-generation Sequencing

June 8–15

INSTRUCTORS
G. Hannon, Cold Spring Harbor Laboratory
G. Irzyk, 454 Life Sciences, Branford, Connecticut
E. Mardis, Washington University School of Medicine, St. Louis, Missouri
R. McCombie, Cold Spring Harbor Laboratory
J. McPherson, Baylor College of Medicine, Houston, Texas

ASSISTANTS
V. Balija, Cold Spring Harbor Laboratory
M. Kramer, Cold Spring Harbor Laboratory
V. Magrini, Washington University School of Medicine, St. Louis, Missouri
L. Nascimento, Cold Spring Harbor Laboratory
L. Spiegel, Cold Spring Harbor Laboratory

TECHNICAL FACULTY
J. Lanza, 454 Life Sciences, Branford, Connecticut
C. Perbost, 454 Life Sciences, Branford, Connecticut

During the past decade, large-scale DNA sequencing has markedly changed the landscape of modern biology. With the introduction of new technologies, time and expenses have been reduced by orders of magnitude, facilitating investigators to propose and undertake more complex and elaborate experiments and analyses. A combination of the results of de novo sequencing and the vast data already generated by the genome projects can now be applied to a very diverse set of scientific questions in prokaryotic and eukaryotic biology. New technologies that facilitate massively parallel sequencing are available that offer the opportunity to further probe function in genomic data.

This intensive 8-day course explored applications of next-generation sequencing technology, with a particular focus on currently available methods based on scalable emulsion-based pyrosequencing. Successful applicants were instructed in the details and pitfalls of (1) emulsion-based sample preparation, (2) pyrosequencing, (3) base-calling, (4) quality control, (5) shotgun assembly, and (6) general data handling and analysis. However, the major emphasis of the course was on the experimental design and intepretation of the resulting data sets in the context of existing sequence information. A diverse range of biological questions were explored including de novo DNA sequencing of bacterial genomes, DNA (re-)sequencing of genomic arrangements in cancer, and the use of these technologies in studying small RNAs.

We encouraged applicants from a diversity of scientific backgrounds including molecular evolution, development, neuroscience, cancer, plant biology, and microbiology.

PARTICIPANTS

Bayes, M., Ph.D., Centre de Regulacio Genomica, Barcelona, Spain
Briggs, A., B.A., Max-Planck Institute for Evolutionary Anthropology, Leipzig, Germany
Bhandari, A., B.S., Cold Spring Harbor Laboratory
Domingo, A., B.S., U.S. Department of Homeland Security, Frederick, Maryland
Fritz, B., Ph.D., Fred Hutchinson Cancer Research Center, Seattle, Washington
Hnath, J., M.S., U.S. Department of Homeland Security, Frederick, Maryland
Huisinga, K., Ph.D., Washington University, St. Louis, Missouri
Li, C., M.S., University of Massachusetts Medical School, Worcester
Liu, W., Ph.D., Novartis Institute for BioMedical Research, Inc., Cambridge, Massachusetts
Persson, H., M.S., Lund University, Sweden
Quinlan, A., B.S., Boston College, Chestnut Hill, Massachusetts
Rosa Amaral, P., B.A., University of Sao Paulo, Brazil
Schroeder, B., Ph.D., Applied Biosystems, Foster City, California
Terracciano, A., Ph.D., Catholic University, Rome, Italy
Trinh, H., B.S., University of Arizona, Tucson
Verratti, K., B.A., U.S. Department of Homeland Security, Frederick, Maryland
Wing, R., Ph.D., University of Arizona, Tucson
Young, A., B.A., NISC/NHGRI/NIH, Bethesda, Maryland
Yu, Y., Ph.D., Arizona Genomics Institute, Tucson

Bentley, D., Solexa Ltd., Cambridge, United Kingdom: The solexa IGb sequencer.

Chen, F., Joint Genome Institute, Walnut Creek, California: Sequencing bacterial genomes using the 454 GS-20.

Hannon, G., Cold Spring Harbor Laboratory: microRNA discovery using the 454 GS-20 sequencer.

Knight, J., 454 Life Sciences, Branford, Connecticut: Signal processing pipeline and bioinformatics-based analyses using 454 pyrosequencing data.

Mardis, E., Washington University School of Medicine, St. Louis, Missouri: Sequencing cDNA libraries with the 454 GS-20.

McPherson, J., Baylor College of Medicine, Houston, Texas: Population genetics using amplicon sequencing on the 454 GS-20.

Methe, B., The Institute of Genome Research, Rockville, Maryland: Integration of 454 life sciences sequencing technology in de novo assembly of prokaryotic genomes.

Rohwer, F., San Diego State University, California: Metagenomics.

Watson, J., Cold Spring Harbor Laboratory: Ethics and the large-scale sequencing of human DNA.

Integrated Data Analysis for High-throughput Biology

June 14–27

INSTRUCTORS **H. Bussemaker,** Columbia University, New York
V. Carey, Harvard University, Boston, Massachusetts
P. Mitra, Cold Spring Harbor Laboratory
M. Reimers, National Cancer Institute, Bethesda, Maryland
A. Sengupta, Rutgers University, Piscataway, New Jersey

ASSISTANTS **J. Mar**, Harvard University, Boston, Massachusetts
J. Zhang, Dana-Farber Cancer Institute, Boston, Massachusetts
J. Zhang, Cornell Medical College, New York

High-throughput biology, epitomized by the ubiquitous DNA microarray, is rapidly generating enormous observation sets. Biologists seeking to make sense of this growing body of data needed to have a firm grasp of statistical methodology. This course was designed to build competence in quantitative methods for the analysis of high-throughput molecular biology data, from which meaningful inferences about biological processes were drawn.

- Review of multivariate statistics.
- R minitutorial.
- Expression and other microarrays: Experimental design, scanning and image analysis, quality control, normalization and probe-level analysis for spotted arrays or prefabricated chips, exploratory analysis, tests of significance, and multiple testing, using R and Bioconductor.

- Discrimination and classification of samples.
- Identifying general regulation themes (e.g., gene ontology categories) in gene lists by statistical means.
- Protein identification and quantification using mass spectrometry.
- Promoter analysis in yeast using CHIP and expression data.
- Identifying regulatory polymorphism using SNP and expression data.
- Characterizing the effect of DNA amplifications and deletions on gene expression in cancer using CGH and expression data on the same samples.

The first week of the course concentrated on analysis of specific types of microarray data (expression, Affymetrix, CGH, CHIP-chip, and SNP arrays) and proteomics. The second week explored biological problems involving the integration of several types of high-throughput data. Data sets were drawn from yeast, human polymorphisms, and cancer biology.

Confirmed speakers included Rick Young (MIT: yeast and CHIP), Audrey Gasch (Wisconsin: yeast microarrays), Bruce Futcher (SUNY: microarray techniques), Terry Speed (Berkeley: proteomics), Rafa Irizarry (Johns Hopkins: Affymetrix arrays), and Keith Baggerly (Texas; proteomics).

PARTICIPANTS

Chen, S., B.S., University of Chicago, Illinois
Colak, D., Ph.D., King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia
Cvek, U., Sc.D., Louisiana State University and LSU Health Sciences Center, Shreveport
de Ridder, J., M.S., Delft University of Technology, The Netherlands
Engelmann, J., M.S., University of Wurtzburg, Germany
Figueroa, M., M.D., Albert Einstein College of Medicine, Bronx, New York
Hassane, D., Ph.D., University of Rochester Medical Center, Rochester, New York
Jackson, S., B.S., U.S. FDA, Laurel, Maryland
Kavak, E., B.S., Bogazici University, Istanbul, Turkey
Kurkin, S., Ph.D., Philips Research, Eindhoven, The Netherlands
Large, T., B.S., Michigan State University, E. Lansing
Lim, L., B.S., Genome Institute of Singapore
Lyou, Y., B.S., SUNY Upstate Medical University, Syracuse, New York
Malone, J., M.S., University of Texas, Arlington
Marchand, J., Ph.D., Tufts University School of Medicine, Boston, Massachusetts
Mathe, E., Ph.D., National Cancer Institute, Bethesda, Maryland
Pagie, L., Ph.D., Netherlands Cancer Institute, Amsterdam, The Netherlands
Puniyani, A., Ph.D., Cold Spring Harbor Laboratory
Rabadan, R., Ph.D., Institute for Advanced Study, Princeton, New Jersey
Riley, T., M.S., Rutgers University, Piscataway, New Jersey
Trutschl, M., Sc.D., Louisiana State University and LSU Health Sciences Center, Shreveport
Xu, L., Ph.D., University of New Orleans-Research Institute for Children, New Orleans, Louisiana

SEMINARS

Baggerly, K., M.D. Anderson Medical Center, Houston, Texas: Quantitative proteomics I: More mass spec and lysate. Quantitative proteomics II: More mass spec and lysate.
Cheung, V., University of Pennsylvania, Philadelphia: Genetics of gene expression.
Futcher, B., SUNY at Stony Brook, New York: The yeast cell cycle, in shades of green and red.
Gasch, A., University of Wisconsin, Madison: Deciphering regulatory networks from stress-induced genomic expression programs in yeast.
Irizzaray, R., Johns Hopkins University, Baltimore, Maryland: Oligoarrays. Cross-platform.
Iyer, V., University of Texas, Austin: Spotted DNA microarrays: Array and experimental design and quantitation. Analyzing DNA-protein interactions with chromatin immunopreciptation (ChIP) and microarrays.
Melnick, A., Albert Einstein College of Medicine, Bronx, New York: Epigenomics.
Speed, T., University of California, Berkeley: SNP arrays. Peptide identification in MS.
Spielman, R., University of Pennsylvania, Philadelphia: Genetics of variation in gene expression (jointly with V. Cheung)
Weinstein, J., National Cancer Institute, Bethesda, Maryland: Integromic analysis of the NCI-60 cancer cell panel: Biostatistics and bioinformatics.
Wigler, M., Cold Spring Harbor Laboratory: CGH methods.
Young, R., Whitehead Institute/Massachusetts Institute of Technology, Cambridge: Regulatory/transcription systems.
Zeitlinger, J., Massachusetts Institute of Technology, Cambridge: ChIP technicalities.

Computational Neuroscience: Vision

June 16–29

INSTRUCTORS **J. Demb,** University of Michigan, Ann Arbor
E. Simoncelli, New York University, New York
S. Treue, German Primate Center, Goettingen, Germany

ASSISTANTS **G. Field,** Salk Institute, La Jolla, California
N. Rust, 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 in a computer laboratory, this intensive course examined color vision, spatial pattern analysis, motion analysis, oculomotor function, attention, and decision-making.

Lecturers in this year's course included Larry Abbott, David Brainard, Matteo Carandini, Marisa Carrasco, Eduardo Chichilnisky, Yang Dan, Jack Gallant, Wilson Geisler, David Heeger, Nancy Kainwisher, Michael Lewicki, J. Anthony Movshon, Michael Platt, Pamela Reinagel, Dario Ringach, and Jonathan Victor.

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 the tennis court, pool, and private beach.

PARTICIPANTS

Anderson, E., B.S., Salk Institute, La Jolla, California
Benucci, A., Ph.D., Smith Kettelwell Eye Research Institute, San Francisco, California
Busse, L., B.S., German Primate Center, Goettingen, Germany
Clark, A., B.S., University of Chicago, Illinois
Heckman, G., B.S., University of California, Santa Monica
Hohl, S., B.S., University of California, San Francisco
Ishikawa, A., B.S., Osaka University, Toyonaka, Japan
Kalmar, R., B.S., Stanford University, Menlo Park, California
Kiani, R., B.S., University of Washington, Seattle
Majaj, N., B.S., New York University, New York
Marre, O., B.S., CNRS, Gif sur Yvette, France
Masse, N., B.S., McGill University, Montreal, Canada
Merriam, E., Ph.D., New York University, New York
Nauhaus, I., B.S., University of California, Los Angeles
Ng, M., B.S., Salk Institute, La Jolla, California
Pilz, K., B.S., Max Plank Institute, Tubingen, Germany
Rathbun, D., B.S., University of California, Davis
Rothkopf, C., B.S., University of Rochester, New York
Sher, A., Ph.D., University of California, Santa Cruz
Stocker, A., Ph.D., New York University, New York
Wark, B., B.S., University of Washington, Seattle
Witthoft, N., B.S., Massachusetts Institute of Technology, Boston
Xio, B., B.S., University of Pennsylvania, Philadelphia
Yeh, C.-I., B.S., SUNY State College of Optometry, New York

SEMINARS

Abbott, L., Columbia University, New York: Population coding/decoding.
Brainard, D., University of Pennsylvania, Philadelphia: Contrast adaptation: Psychophysics. Linear algebra/trichromacy. Color: Constancy/adaptation.
Carandini, M., Smith-Kettelwell Eye Research Institute, San Francisco, California: Predicting the responses of the early visual system to complex stimuli.
Carrasco, M., New York University, New York: Psychophysics of attention.
Chichilnisky, E.J., The Salk Institute, La Jolla, California: Linear-nonlinear model. Retina: Luminance adaptation. Retina: Population coding.
Demb, J., University of Michigan, Ann Arbor: Retina: Contrast adaptation. Retina: Pathways/linearity. Retina: Nonlinearities/mechanisms.
Dan, Y., University of California, Berkeley: V1: Adaptation/plasticity.
Field, G., The Salk Institute, La Jolla, California: Transduction.
Gallant, J., University of California at Berkeley: Cortex: Ventral stream, form vision.
Geisler, W., University of Texas at Austin: Pattern vision and natural scenes.
Heeger, D., New York University, New York: Probing human vision with fMRI.
Kanwisher, N., Massachusetts Institute of Technology, Cambridge: fMRI investigations of visual recognition in humans.
Lewicki, M., Carnegie Mellon University, Pittsburgh, Pennsylvania: Image statistics and neural representation.
Movshon, J.A., New York University, New York: MT: Physiology.
Platt, M., Duke University, Durham, North Carolina: Neural basis of decision-making.
Reinagel, P., University of California at San Diego, La Jolla: Efficient coding hypothesis: LGN physiology.
Ringach, D., University of California, Los Angeles: V1 electrophysiology.
Rust, N., Massachusetts Institute of Technology, Cambridge: Fourier transforms. Spike-triggered covariance analysis in V1.
Simoncelli, E., New York University, New York: Visual motion modeling. Linear systems. Estimation/decision/bayes. Welcome and introduction.
Treue, S., German Primate Center, Goettingen, Germany: The role of attention in visual information processing.
Victor, J., Weill Medical College of Cornell, New York: Spikes and the neural code.

Proteomics

June 30–July 13

INSTRUCTORS
P. Andrews, University of Michigan Medical School, Ann Arbor
J. LaBaer, Harvard Institute of Proteomics, Cambridge, Massachusetts
A. Link, Vanderbilt University School of Medicine, Nashville, Tennessee

ASSISTANTS
R. Bish, Cold Spring Harbor Laboratory
E. Hainsworth, Harvard Medical School, Boston, Massachusetts
J. Jennings, Vanderbilt University School of Medicine, Nashville, Tennessee
W. Montor, Harvard Institute of Proteomics, Cambridge, Massachusetts
N. Ramachandran, Harvard Institute of Proteomics, Cambridge, Massachusetts
E. Simon, University of Michigan Medical School, Ann Arbor
S. Volk, University of Michigan, Blissfield

This intensive laboratory and lecture course focused on two major themes in proteomics: protein profiling and functional proteomics. In the profiling section of the course, students learned about cutting-edge protein separation methods, including hands-on experience with two-dimensional gel electrophoresis, multidimensional liquid chromatography, and affinity purification of protein complexes. The course covered both MALDI and ESI high-sensitivity mass spectrometry, including peptide mass mapping and tandem mass spectrometry, quantification, and phosphoproteomics. Students learned to use several informatics tools available for analyzing the data. In the functional proteomics section of the course, students learned about recombinational cloning, high-throughput protein isolation, and protein

microarrays. Students used robots to execute high-throughput methods including expression, purification, and characterization of proteins. They also printed and analyzed their own self-assembling protein microarrays, which was used for protein-protein interaction studies. The overall aim of the course was to provide each student with a fundamental knowledge and hands-on experience necessary to perform and analyze proteomics experiments and to learn to identify new opportunities in applying proteomics approaches to his/her own research.

Speakers included Brian Chait (The Rockefeller University), Pierre Chaurand (Vanderbilt University School of Medicine), Karl Clauser (Broad Institute), Ileana Critea (The Rockefeller University), Neil Kelleher (University of Illinois), Akhilesh Pandey (Johns Hopkins University), Michael Snyder (Yale University), and Forest White (Massachusetts Institute of Technology).

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Borrego, M.-J., Ph.D., Instituto Nacional de Sade, Lisboa, Portugal
Carruthers, N., B.S., Wayne State University, Detroit, Michigan
Darling-Reed, S., Ph.D., College of Pharmacy & Pharmaceutical Sciences, Tallahassee, Florida
Ericson, J., M.S., Goteborg University, Gothenburg, Sweden
Felicio, A., M.S., Universidade Estadual Paulista, Araraquara, Brazil
Fusetti, F., Ph.D., University of Gronigen, The Netherlands
Guha, U., Ph.D., Memorial Sloan-Kettering Cancer Center, New York
La Cour, J., M.S., University of Copenhagen, Denmark
Lim, J., Ph.D., University of Auckland, Grafton, New Zealand
Marionneau, C., Ph.D., Washington University, St. Louis, Missouri
Novo, M.T., Ph.D., Universidade Federal de Sao Carlos, Brazil
Oh, S.-H., Ph.D., University of California, Santa Barbara
Sarek, G., M.S., University of Helsinki, Finland
Singh, S., Ph.D., Sandia National Laboratories, Albuquerque, New Mexico
Sundberg, E., Ph.D., Boston Biomedical Research Institute, Watertown, Massachusetts
Yang, L., Ph.D., National Center Institute, Rockville, Maryland

SEMINARS

Chait, B., The Rockefeller University, New York: Purification of proteins complexes using a magnetic IP.
Chaurand, P., Vanderbilt University School of Medicine, Nashville, Tennessee: Profiling tissues by mass spectrometry.
Clauser, K., Broad Institute, Cambridge, Massachusetts: De novo interpretation of tandem mass spectra.
Kelleher, N., University of Illinois, Urbana: Top-down mass spectrometry analysis of whole proteins.
Pandey, A., Johns Hopkins University, Baltimore, Maryland: Proteomics databases.
Pappin, D., Applied Biosystems, Framingham, Massachusetts: Quantitative mass spectrometry.
Snyder, M., Yale University, New Haven, Connecticut: Application of protein microarrays.
White, F., Massachusetts Institute of Technology, Cambridge: Phosphoproteomics.

Molecular Approaches to Plant Science

June 30–July 20

INSTRUCTORS
J. Bender, Johns Hopkins University School of Public Health, Baltimore, Maryland
L. Hobbie, Adelphi University, Garden City, New York
H. Ma, Pennsylvania State University, University Park
S. McCormick, Plant Gene Expression Center, Albany, California

ASSISTANTS
M. Alandete-Saez, Plant Gene Expression Center, Albany, California
L. Boavida, University of California, Berkeley
L. Quan, Pennsylvania State University, University Park

This course provided an intensive overview of topics in plant physiology and development, focusing on molecular genetic approaches to understanding plant biology. It emphasized recent results from *Arabidopsis thaliana* and other plants and provided an introduction to current methods used in plant molecular biology. It was designed for scientists with some experience in molecular techniques or in plant biology who wish to work with *Arabidopsis* and other plants using the latest technologies in genetics and molecular biology. The course consisted of a vigorous lecture series, a hands-on laboratory, and informal discussions. Discussions of important topics in plant research were presented by the instructors and by invited speakers. These seminars included plant morphology and anatomy; plant development (such as development of flowers, leaves, male and female gametophytes, and embryos); perception of light and photomorphogenesis; and synthesis, function, and perception of hormones.

Lectures describing bioinformatics tools available to the plant community and the resources provided by plant genome projects were also included. Speakers provided overviews of their fields, followed by in-depth discussions of their own work. The laboratory sessions provided an introduction to important techniques currently used in plant research. These included studies of plant development, mutant analysis, histochemical staining, transient gene expression, applications of green fluorescent protein fusions, protein interaction and detection, proteomics approaches, transcription profiling, and techniques commonly used in genetic and physical mapping. The course also provided several short workshops on important themes in genetics.

Invited speakers included Jody Banks (Purdue University), Justin Borevitz (University of Chicago), John Celenza (Boston University), Savithramm Dinesh-Kumar (Yale University), Ueli Grossniklaus (University of Zurich), Roger Hangarter (Indiana University), Paul Herzmark (University of California, San Francisco), Vivian Irish (Yale University), Thomas Jack (Dartmouth College), David Jackson (Cold Spring Harbor Laboratory), Cris Kuhleimeier (Universitat Bern), Wolfgang Lukowitz (Cold Spring Harbor Laboratory), June Nasrallah (Cornell University), Jennifer Normanly (University of Massachusetts), Thomas Nuhse (The Sainsbury Laboratory), Eric Richards (Washington University), John Schiefelbein (University of Michigan), David Sommers (Ohio State University), Tai-ping Sun (Duke University Medical Center), and Marja Timmermans (Cold Spring Harbor Laboratory).

PARTICIPANTS

Brandenburg, A., M.S., University of Bern, Switzerland
Crawford, S., B.A., University of York, United Kingdom
Dozier, U., M.Ed., Howard University, Washington, D.C.
Goff, K., B.S., The University of Alabama, Tuscaloosa
Hertel, S., Diplom., Humboldt-University of Berlin, Germany
Jarve, K., M.S., Tallinn University of Technology, Estonia
Johnson, R., B.S., Texas Woman's University, Denton
Jones, K., Ph.D., Massachusetts Institute of Technology, Cambridge
Koniger, M., Ph.D., Wellesley College, Wellesley, Massachusetts
Matveeva, E., M.S., ETH Zentrum, Zurich, Switzerland
Miller, T., B.A., Marquette University, Milwaukee, Wisconsin
Mondragon-Palomino, M., Ph.D., University of Jena, Germany
Muralidharan, M., B.S., Arizona State University, Tempe
Nour-Eldin, H., B.S., The Royal Veterinary and Agricultural University, Copenhagen, Denmark
Sauer, M.-L., Ph.D., South Dakota State University, Brookings
Veerappan, V., M.S., Texas Tech University, Lubbock

SEMINARS

Banks, J., Purdue University, W. Lafayette, Indiana: Gametophytes and evolution.
Bender, J., Johns Hopkins University School of Public Health, Baltimore, Maryland: Epigenetics II.
Borevitz, J., University of Chicago, Illinois: Adaptation to light.
Celenza, C., Boston University, Cambridge, Massachusetts: Genetics of secondary metabolism.
Grossniklaus, U., University of Zurich, Switzerland: Female gametophytes.
Hangarter, R., Indiana University, Bloomington: Light responses.
Herzmark, P., University of California, San Francisco: Microscopy lecture and lab.
Hobbie, L., Adelphi University, Garden City, New York: Auxin signaling.
Irish, V., Yale University, New Haven, Connecticut: Evolution of flower development.
Jack, T., Dartmouth College, Hanover, New Hampshire: Flower development.
Kumar-Dinesh, S., Yale University, New Haven, Connecticut: Plant-pathogen interactions.
Lukowitz, W., Cold Spring Harbor Laboratory: Embryo development. Genetics II: Double mutants.
Ma, H., Pennsylvania State University, University Park: Genetics I: Mutagenesis. Genetics II: Double mutants. Plant Anatomy. Microsporogenesis.
McCormick, S., Plant Gene Expression Center, Albany, California: Pollen development. Bioinformatics. Scientific writing workshop.
Nasrallah, J., Cornell University, Ithaca, New York: Self-incompatibility.
Nelson, T., Yale University, New Haven, Connecticut: Vascular development.
Normanly, J., University of Massachusetts, Amherst: Metabolomics and auxin homeostasis.
Nuhse, T., John Innes Centre, Norwick, United Kingdom: Proteomics.
Richards, E., Washington University, St. Louis, Missouri: Epigenetics I.
Schaller, G.E., Dartmouth College, Hanover, New Hampshire: Ethylene.
Schiefelbein, J., University of Michigan, Ann Arbor: Root hair development.
Sommers, D.,, Ohio State University: The circadian clock.
Sun, T.-p., Duke University Medical School, Durham, North Carolina: GA signaling.
Timmermans, M., Cold Spring Harbor Laboratory: Maize shoot patterning.
Wildermuth, M., University of California, Berkeley: Metabolomics and SA function.

Neurobiology of *Drosophila*

June 30–July 20

INSTRUCTORS
G. Bashaw, University of Pennsylvania, Philadelphia
S. Waddell, University of Massachusetts Medical School, Worcester
B. Zhang, University of Texas, Austin

ASSISTANTS
S. DasGupta, University of Massachusetts Medical School, Worcester
D. Garbe, University of Pennsylvania, Philadelphia
J.P. Labrardor, University of Pennsylvania School of Medicine, Philadelphia
P. Perrat, University of Massachusetts Medical School, Worcester
C. Spaeth, University of Texas, Austin

This laboratory/lecture course was intended for researchers at all levels from beginning graduate students through established primary investigators who want to use *Drosophila* as an experimental system for nervous system investigation. The 3-week course was divided into the study of development, physiology/function, and behavior. Daily seminars introduced students to a variety of research topics and developed those topics by including recent experimental contributions and outstanding questions in the field. Guest lecturers brought original preparations for viewing and discussion and direct laboratory exercises and experiments in their area of interest. The course provided students with hands-

on experience using a variety of experimental preparations that are used in the investigation of current neurobiological questions.

The lectures and laboratories focused on both the development of the nervous system and its role in controlling larval and adult behaviors. In addition to an exposure to the molecular genetic approaches available in *Drosophila*, students learned a variety of techniques including embryo in situ hybridization, labeling of identified neurons, electrophysiological recording from nerves and muscles, and the analysis of larval and adult behavior. Collectively, the course provided a comprehensive and practical introduction to modern experimental methods for studying the *Drosophila* nervous system.

This year's lecturers included), Marcus Allen (University of Kent), Justin Blau (New York University), Heather Broihier (Case Western Reserve University), Vivian Budnik (University of Massachusetts), Sarah Certel (Harvard University), Yick-Bun Chan (Harvard Medical School), Josh Dubnau (Cold Spring Harbor Laboratory), Daniel Eberl (University of Iowa), David Featherstone (University of Illinois at Chicago), Marc Freeman (University of Massachusetts Medical School), Donald Gailey (California State University, Hayward), Fen Biao Gao (The Gladstone Institutes at UCSF), Tanja Godenschwege (University of Massachusetts), Stephen Goodwin (University of Glasgow), Gregory Jefferis (University of Cambridge), Edward Kravitz (Harvard Medical School), Mary Logan (University of Massachusetts Medical School), Greg Macleod (University of Arizona), Ben Parrott (University of Georgia), Peng Shen (University of Georgia), Bryan Stewart (University of Toronto), Glenn Turner (California Institute of Technology), and Bruno van Swinderen (Neurosciences Institute).

PARTICIPANTS

Cevik, M., Ph.D., Abant Izzet Baysal Universite, Bolu, Turkey
Cho, J.Y., Ph.D., The Johns Hopkins University School of Medicine, Baltimore, Maryland
Colon-Cesario, W., Ph.D., Stowers Institute for Medical Research, Kansas City, Missouri
Hill, J., B.A., University of Massachusetts, Amherst
Hoare, D., B.Sc., The University of Manchester, United Kingdom
McKellar, C., Ph.D., Harvard Medical School, Boston, Massachusetts
Nagaraja, K., Ph.D., University of Michigan, Ann Arbor
Pollarolo, G., M.S., VIB and University of Leuven School of Medicine, Leuven, Belgium
Repnikova, E., M.S., Texas A&M University, College Station
Smith, R., B.S., University of Alabama, Birmingham
Tabone, C., B.S., University of Nevada, Las Vegas
Weaver, C., Ph.D., University of California, San Diego
Wu, P.-S., M.D., Columbia University, New York

SEMINARS

Allen, M., University of Kent, United Kingdom, and Godenschwege, T., University of Massachusetts, Amherst: The giant fiber system.
Bashaw, G., University of Pennsylvania, Philadelphia: Axon guidance.
Blau, J., New York University, New York: Circadian rhythm and larvae.
Brohier, H., Case Western Reserve, Cleveland, Ohio: Neuronal identity.
Budnik, V., University of Masschusetts, Worcester: NMJ Development. Discussion on the mystery mutant results. Student activity: Mystery mutants.
Dubnau, J., Cold Spring Harbor Laboratory: Long-term memory.
Eberl, D., University of Iowa, Iowa City, and Turner, G., California Institute of Technology, Pasadena, California: Hearing and smell.
Featherstone, D., University of Illinois at Chicago: Glutamate receptors and synaptic plasticity.
Freeman, M., University of Massachusetts, Worcester: Glial development and Fn.
Gao, F.B., University of California, San Francisco: Dendrite morphogenesis.
Jefferis, G., Cambridge University, United Kingdom: Mosaic genetic techniques. Olfactory system development.
Kravitz, E. and Certel, S., Harvard Medical School, Boston, Massachusetts: Aggression.
Macleod, G., University of Arizona, Tucson: Imaging neural activity at the fly NMJ.
Shen, P., University of Georgia, Athens: Feeding behavior.
Stewart, B., University of Toronto, Canada: Electrophysiology 101. Synaptic release. Student activity: NMJ recording.
van Swinderen, B., Neuroscience Institute, San Diego, California: Visual learning.
Waddell, S., University of Massachusetts Medical School, Worcester: Introduction. Olfactory learning and memory.
Zhang, B., University of Texas, Austin: Synaptic vesicle recycling.

Mechanisms of Neural Differentiation and Brain Tumors

July 6–12

INSTRUCTORS **A. Guha,** University of Toronto, Canada
S. Majumuder, M.D. Anderson Cancer Center, Houston, Texas

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 interacted with senior investigators on a one-to-one basis in an informal environment.

Topics included Clinical Overview of Brain Tumors, Neuro-oncology/Surgery/Radiology/Pathology, Nervous System Pre-Disposition Syndromes, Genetic Alterations in Sporadic Brain Tumors, Chromatin Remodeling, DNA Replication, Extracellular Matrix and Migration, Stem Cells and Cancer Stem Cells, Neuronal, Glial, and Oligodendrocyte Differentiation, Genetically Engineered Mouse System, Detection Methods including Nanotechnology, Mechanism-based Therapeutics, Epidemiology, and NCI strategies and funding mechanisms.

Speakers included Oliver Bogler (M.D. Anderson Cancer Center), Melissa Bondy (M.D. Anderson Cancer Center), Melvin DePamphilis (National Institutes of Health), Charles Eberhart (Johns Hopkins University), Gregory Fuller (M.D. Anderson Cancer Center), Candece Gladson (University of Alabama, Birmingham), Steve Goldman (University of Rochester), David Gutmann (Washington University School of Medicine), Paul Knoepfler (Fred Hutchinson Cancer Research Center), Victor Levin (M.D. Anderson Cancer Center), Guillermina Lozano (M.D. Anderson Cancer Center), Tak Mak (AMDI OCI University Health Network), Gail Mandel (HHMI/SUNY Stony Brook), Christina Meyers (M.D. Anderson Cancer Center), Judy Mietz (National Cancer Institute), Andras Nagy (Samuel Lunenfeld Research Institute), Roeland Nusse (HHMI, Stanford University), Luis Parada (University of Texas Southwestern Medical Center), Mahendra Rao (Invitrogen), Ray Sawaya (M.D. Anderson Cancer Center), Evan Snyder (Burnham Institute), Charles Stiles (Dana Farber Cancer Institute), Al Yung (M.D. Anderson Cancer Center), and Miqin Zhang (Washington University)

This course was supported with generous funding provided by the American Brain Tumor Association.

PARTICIPANTS

Aboody, K., Ph.D., Beckman Research Institute, Duarte, California
Agathocleous, M., B.S., University of Cambridge, United Kingdom
Barish, M., Ph.D., Beckman Research Institute, Duarte, California
Benitez, J., B.S., CINVESTAV, Mexico City, Mexico
deCarvalho, A., Ph.D., Henry Ford Hospital, Detroit, Michigan
Evans, S., Ph.D., University of Pennsylvania School of Medicine, Philadelphia
Feng, C., Ph.D., University of Nevada, Reno
Havrda, M., B.S., Dartmouth Medical School, Lebanon, New Hampshire
Kagalwala, M., B.S., M.D. Anderson Cancer Center, Houston, Texas
Kamnasaran, D., Ph.D., Hospital for Sick Children, Toronto, Canada
Kang, N., Ph.D., University of Texas, Houston
Lam, P., Ph.D., National Cancer Center, Singapore
Ma, J., B.S., University of California, Davis
Maria, B., Ph.D., Medical University of South Carolina, Charleston
Mazumdar, T., B.S., Kent State University, Cleveland, Ohio
Nakashima, H., Ph.D., Ohio State University Medical Center, Columbus
Narita, Y., Ph.D., National Cancer Center Hospital, Tokyo, Japan
Onorati, M., Ph.D., University of Pisa, Ghezzano, Italy
Phillips, J., B.S., University of California, San Francisco
Rich, J., Ph.D., Duke University Medical Center, Durham, North Carolina
Singh, S., Ph.D., M.D. Anderson Cancer Center, Houston, Texas
Skalli, O., Ph.D., Louisiana State University, Shreveport
Soroceanu, L., Ph.D., CPMC Research Institute, San Francisco, California
Uziel, T., Ph.D., St. Jude Children's Research Hospital, Memphis, Tennessee

Bogler, O., University of Texas, M.D. Anderson Cancer Center, Houston: Tyrosine kinases in glioma. Proteomics and novel cytotoxins in glioma.

DePamphilis, M., National Institutes of Health, Bethesda, Maryland: DNA replication.

Fuller, G., M.D. Anderson Cancer Center, Houston, Texas: The role of neuropathology in clinical and investigative neuro-oncology.

Gladson, C., Univerity of Alabama, Birmingham: Extracellular matrix, migration, angiogenesis.

Guha, A., Hospital for Sick Children, Toronto, Canada: Genetic mechanisms.

Gutmann, D., Washington University School of Medicine, St. Louis, Missouri: Using mouse models to dissect the molecular and cellular pathogenesis of brain tumors.

Levin, V., M.D. Anderson Cancer Center, Houston, Texas: Understanding treatment strategies for gliomas.

Lozano, G., University of Texas M.D. Anderson Cancer Center, Houston: Genetic mechanisms.

Mak, T., Princess Margaret Hospital, Toronto, Canada: PTEN pathways.

Mandel G., Howard Hughes Medical Institute, SUNY Stony Brook, New York: The rise and fall of REST: Creating the nervous system.

Meyers, C., M.D. Anderson Cancer Center, Houston, Texas: Neuropsychology.

Parada, L., University of Texas Southwestern Medical Center, Dallas: Genetic Mechanisms.

Sawaya, R., M.D. Anderson Cancer Center, Houston, Texas: New techniques in surgery for brain tumors.

Stiles, C., Dana Farber Cancer Institute, Boston, Massachusetts: Transcription factors and glial development.

Yung, A., University of Texas M.D. Anderson Cancer Center, Houston: Mechanism-based therapeutics.

Advanced Techniques in Molecular Neuroscience

July 6–20

INSTRUCTORS
J. Eberwine, University of Pennsylvania Medical School, Philadelphia
T. Hughes, Montana State University, Bozeman
C. Lai, Scripps Research Institute, La Jolla, California

COINSTRUCTORS
K. Becker, National Institutes on Aging/NIH, Baltimore, Maryland
K. Haas, University of British Columbia, Vancouver, Canada
R. Lansford, California Institute of Technology, Pasadena

ASSISTANTS
H. Butler, Montana State University, Bozeman
R. Mealer, Montana State University, Bozeman
T. Peritz, University of Pennsylvania Medical Center, Philadelphia
G. Poynter, California Institute of Technology, Pasadena
J. Tan, Scripps Research Institute, La Jolla, California
W. Wood, III, National Institutes on Aging/NIH, Baltimore, Maryland
K. Wu, University of Pennsylvania Medical Center, Philadelphia

This newly revised laboratory and lecture course was designed to provide neuroscientists at all levels with a conceptual and practical understanding of several of the most advanced techniques in molecular neuroscience. The course curriculum was divided into three sections: an extensive and up-to-date set of laboratory exercises, daily lectures covering the theoretical and practical aspects of the various methods used in the laboratory, and a series of evening research seminars. The informal and interactive evening lectures were given by leading molecular neuroscientists and served to illustrate the ways in which the various experimental approaches have been used to advance specific areas of neurobiology.

In this year's course, the laboratory portion included topics such as an introduction to the design and use of animal virus vectors in neurobiology; the use of small interfering RNAs (siRNA) for regulating the expression of specific genes in neurons; practical exercises in gene-delivery systems including mammalian cell transfection protocols and single-cell electroporation techniques for targeted gene transfer in vivo; an introduction to overall strategies, use, and design of BAC transgenic vectors; 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 were available to molecular neuroscientists.

Speakers included Robert Darnell (HHMI/The Rockefeller University), Philip Haydon (University of Pennsylvania), Donald Lo (Duke University Medical Center), Ardem Patapoutian (Scripps Research Institute), Marie Anne Quinn (Montana Molecular LLC), Angeles Riberia (UCDHSC at Fitzsimons), and Lino Tessarollo (National Cancer Institute).

PARTICIPANTS

Black, M., Ph.D., Georgia State, Atlanta
Chung, K.Y., D.Phil., Columbia University, New York
Delalle, I., Ph.D., Boston University Medical School, Massachusetts
Gardner, T., Ph.D., Massachusetts Institute of Technology, Cambridge
Gkogkas, C., M.Sc., University of Edinburgh, United Kingdom
Griveau, A., M.S., Universite Paris VI, Paris, France
Kuhn, C., Ph.D., Gothenburg University, Goteborg, Sweden
Ma, C., D.Phil., Harvard Medical School, Charlestown, Massachusetts

McIver, S., B.A., Washington University, St. Louis, Missouri
Myers, K., Ph.D., Emory University, Atlanta, Georgia
Olveczky, B., Ph.D., Massachusetts Institute of Technology, Cambridge
Petrinovic, M., M.S., University of Zurich, Switzerland
Pierce, A., M.S., University of Memphis, Tennessee
Russell, S., M.D., New York University School of Medicine, New York
Siegert, S., Dipl., Friedrich Miescher Institute, Basel, Switzerland
Thayer, D., B.S., Scripps Research Institute, La Jolla, California

SEMINARS

Darnell, R., The Rockefeller University, New York: Applying molecular biology to study the role of RNA-binding proteins in neurologic disease.
Eberwine, J., University of Pennsylvania, Philadelphia: Molecular biology of the dendrite.
Haydon, P., University of Pennsylvania, Philadelphia: Glia: Listening and talking to the synapse.
Patapoutian, A., Scripps Research Institute, La Jolla, California: How do you feel? Molecular basis of temperature sensation.
Ribera, A., Universtity of Colorado Health and Sciences Center at Fitzsimons, Denver: Molecular neurobiology of the embryonic zebra fish nervous system.
Tessarollo, L., National Cancer Institute, Frederick, Maryland: Dissecting neurotrophin functions in vivo: Lessons from engineered mouse models.

Biology of Social Cognition

July 14–20

INSTRUCTORS **R. Adolphs,** California Institute of Technology, Pasadena
D. Skuse, University College London, United Kingdom

ASSISTANT **D. Newmann,** California Institute of Technology, Pasadena

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 provided a comprehensive overview of these topics. Although the emphasis was on social cognition in humans, there were 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. Afternoons were devoted largely to practical sessions that provided hands-on experiments in consultation with seminar leaders, rounded off by after-supper keynote lectures by leading scientists that reflected cutting-edge and future views related to our theme for that day. Ample breaks allowed time for informal interactions between lecturers and students.

This course was supported with funds provided by the Oliver Grace Fund.

PARTICIPANTS

Bayliss, A., Ph.D., University of Wales, Bangor, United Kingdom
Belmonte, M., Ph.D., University of Cambridge, United Kingdom
Bengtsson, S., Ph.D., University College London, United Kingdom
Bennett, T., Ph.D., McMaster University, Hamilton, Canada
Bishop, S., Ph.D., Cambridge University, United Kingdom
Griffin, A., B.S., University of Texas, Austin
Hagan, C., B.S., University of York, United Kingdom
Halligan, S., Ph.D., University of Reading, United Kingdom
Hassett, J., B.S., Emory University, Atlanta, Georgia
Kipps, C., Ph.D., University of Cambridge, United Kingdom
Krusemark, E., B.S., University of Georgia, Athens
Lee, L., B.S., University of York, United Kingdom
Marco, E., Ph.D., University of California, San Francisco
Matheson, A., B.S., Institute of Child Health, London, United Kingdom
Molnar-Szakacs, I., Ph.D., Ecole Polytechnique Federale de Lausanne, Switzerland
Moore, H., Ph.D., Columbia University, New York
Norris, C., Ph.D., University of Wisconsin, Madison
Oswald, T., B.S., University of Oregon, Eugene
Overy, K., Ph.D., University of Edinburgh, United Kingdom
Ruz, M., Ph.D., University of Oxford, United Kingdom
Southgate, V., Ph.D., Birkbeck College, London, United Kingdom
Thompson, S., B.S., University College London, United Kingdom
Uddin, L., B.S., New York University, New York
Williams, K., Ph.D., University of Edinburgh, United Kingdom
Yang, P., Ph.D., Kaoshiung Medical University, Taiwan

SEMINARS

Adolphs, R., California Institute of Technology, Pasadena: The amygdala.
Adolphs, R., California Institute of Technology, Pasadena: Review and summary.
Adolphs, R., California Institute of Technology, Pasadena: Eye tracking and psychophysiology.
Newmann, D., California Institute of Technology, Pasadena: Workshop: Eye tracking and psychophysiology.
Anderson, D., California Institute of Technology, Pasadena: Model systems for studying fear and anxiety.
Blakemore, S., University College London, United Kingdom: Worskhop: Social cognition from adolescence to adulthood.
Cahill, L., University of California, Irvine: Sex differences in social cognition.
Couzin, I., Oxford University, United Kingdom: Workshop: The emergence of collective behavior–From locusts and birds to fish and human crowds.
Crews, D., University of Texas, Austin: Development of social phenotyes and mating systems.
Crow, T., Oxford University, United Kingdom: Evolution of language and the origins of psychosis.
Hauser, M., Harvard University, Cambridge, Massachusetts: The moral mind.
Huhman, K., Georgia State University, Atlanta: Conditioned defeat in hamsters.
Insel, T., National Institutes of Mental Health, Bethesda, Maryland: The future of social cognition.
Kidd, K., Yale University, New Haven, Connecticut: Introduction to complex genetics.
Parr, L., Emory University, Atlanta, Georgia: Emotional communication in chimpanzees.
Pfaff, D., The Rockefeller University, New York: Molecular mechanisms for the development of aggression.
Phelps, E., New York University, New York: The interaction of emotion and cognition.
Raine, A., University of Southern California, Los Angeles: Psychopathy.
Saxe, R., Massachussets Institute of Technology, Boston: Imaging how we think about people.
Schultz, R., Yale University, New Haven, Connecticut: Neuroimaging and neuroanatomy of social cognition.
Sebat, J., Cold Spring Harbor Laboratory: Approaches to discovering genes that influence social cognitive traits.
Skuse, D., University College London, United Kingdom: Welcome, introduction, and overview.
Skuse, D., University College London, United Kingdom: Endophenotypes in social cognitive research.
Skuse, D., University College London, United Kingdom: X-linked genes and social cognition.
Skuse, D., University College London, United Kingdom: Review and summary.
Tager-Flusberg, H., Boston University, Massachusetts: Autism spectrum disorders and Williams syndrome.
Young, L., Emory University, Atlanta, Georgia: Neurobiology of pair bonding in voles.

Workshop on Schizophrenia and Related Disorders

July 22–31

INTRUCTORS **D. Lewis,** University of Pittsburgh
D. Porteous, University of Edinburgh, United Kingdom
D. Weinberger, National Institute of Mental Health

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 10-day workshop, students learned about the clinical aspects of schizophrenia, schizoaffective disorder, and bipolar disorder, as well as explored in detail the genetic and neurobiological underpinnings of these complex psychiatric disorders.

The Workshop was divided into four main sessions: Clinical Overview; Genetics and Genomics; Developmental Neurobiology and Neural Circuits, and Cognitive Systems. 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.

This workshop was supported with funds provided by the Oliver Grace Fund.

PARTICIPANTS

Barnett, J., B.S., University of Cambridge, United Kingdom
Bergman, O., B.S., Goteborg University, Gothenburg, Sweden
Bordelon, J., B.S., Emory University, Atlanta, Georgia
Burdick, K., Ph.D., North Shore Long Island Jewish Health System, Glen Oaks, New York
Den Ouden, H., B.S., University College London, United Kingdom
Deo, A., B.S., New York University, New York
Duncan, C., B.S., Garvan Institute of Medical Research, Sydney, Australia
Eyjolfsson, E., B.S., Norwegian University of Science and Technology, Trondheim, Norway
Gaisler-Salomon, I., Ph.D., Columbia University, New York
Hajos, M., Ph.D., Pfizer Global Research and Development, Groton, Connecticut
Heimer, H., B.S., Schizophrenia Research Forum, Providence, Rhode Island
Heinrich, J., Ph.D., Wyeth Research, Monmouth Junction, New Jersey
Horvath, S., B.S., University of Szeged, Hungary
Lin, P.-I, Ph.D., Cold Spring Harbor Laboratory
March, D., B.S., Columbia University, New York
McCatthy, S., Ph.D., Cold Spring Harbor Laboratory
Meyer, K., B.S., Northwestern University, Chicago, Illinois
Osumi, N., Ph.D., Tohoku University, Sendai, Japan
Panos, J., B.S., Western Michigan University, Kalamazoo
Rinaldi, T., B.S., Ecole Polytechnique Federale de Lausanne, Switzerland
Sachs, N., Ph.D., Johns Hopkins University, Baltimore, Maryland
Sosa Machado, Y., B.S., North Dakota State University, Fargo
Stevens, B., Ph.D., University of Florida College of Medicine, Gainesville
Van Beek, J., Ph.D., H. Lundbeck A/S, Valby-Copenhagen, Denmark
Vogels, T., B.S., Columbia University, New York
Wass, C., B.S., University of Goteborg, Sweden

SEMINARS

Abi-Dargham, A., Columbia University, New York: Dopamine system.
Bilder, R., David Geffen School of Medicine at UCLA, California: Cognition in schizophrenia.
Caron, M., Duke University Medical Center, Durham, North Carolina: Animal models.
Coyle, J., Harvard University/McLean Hospital, Belmont, Massachusetts: Glutamate system.
Kane, J., Zucker Hillside Hospital, Glen Oaks, New York: Diagnosis and clinical phenomenology. Neuropharmacology and current treatment approaches.
Lawrie, S., Royal Edinburgh Hospital, United Kingdom: Neuroanatomy and imaging in schizophrenia. Challenges; new technologies.
Levitt, P., Vanderbilt University, Nashville, Tennessee: Developmental neurobiology. Development of cortical circuitry and disorders of processing.
Lewis, D., University of Pittsburgh, Pennsylvania: GABAergic system. Summary of neurobiology.
Manji, H., National Institutes of Health, Bethesda, Maryland: Neuronal plasticity cascades: Genes to behavior pathways in the pathophysiology and treatment of schizophrenia and mood disorders.
McKay, R., National Institutes of Health, Bethesda, Maryland: Do stem cells matter? Defining dopamine neuron development and function.
Murray, R., Institute of Psychiatry, London, United Kingdom: History and clinical description. Neurodevelopment and schizophrenia. Summary of session 1.
O'Donnell, P., Albany Medical College, New York: Peri-adolescent maturation of prefrontal cortical circuits.
O'Donovan, M., University of Wales, Cardiff, United Kingdom: Schizophrenia genetics: Concepts, methods, current status. Specific genes 1 (dysbindin/G72, neuregulin 1, the VCFS region).
Porteous, D., University of Edinburgh, United Kingdom: Specific genes II: DISC-1. Specific genes III (NPAS, GRIK4). Summary of genetics.
Robbins, T., University of Cambridge, United Kingdom: Modeling neural circuits: Cognition. Modeling neural circuits: Positive symptoms. Preclinical models of schizophrenia and their utility. Future directions.
Role, L., and Talmage, D., Columbia University, New York: Neuregulin signaling, smoking, and schizophrenia.
Rutter, M., Institute of Psychiatry, Kings College, London, United Kingdom: Overview of psychiatric genetics. Gene environment.
Sebat, J., Cold Spring Harbor Laboratory: Genomic approaches (ROMA, etc.).
Sesack, S., University of Pittsburgh, Pennsylvania: How the brain is wired: Principles of functional neural circuits.
Susser, E., Columbia University, New York: Epidemiology. Epidemiology: Part 2.
Tsuang, M., University of California, San Diego: Gene expression of blood and brain: Toward identifying biomarkers for schizophrenia.
Weinberger, D., NIMH/St. Elizabeth's Hospital, Bethesda, Maryland: Introduction: What we've learned so far. Future Directions.

Eukaryotic Gene Expression

July 25–August 14

INSTRUCTORS
M. Bulger, University of Rochester School of Medicine, New York
T. Oelgeschlager, Eukaryotic Gene Regulation, United Kingdom
A. Shilatifard, St. Louis University School of Medicine, St. Louis, Missouri
L. Tora, IGBMC, Illkirch, France

ASSISTANTS
G. Fromm, University of Rochester, New York
T. Hilton, IGBMC, Illkirch, France
B. Malecova, Transcription Laboratory, United Kingdom
J. Schneider, St. Louis University School of Medicine, Missouri
M. Steward, St. Louis University School of Medicine, Missouri

This course was designed for students, postdocs, and principal investigators who have recently ventured into the exciting area of gene regulation. The coursel focused on state-of-the-art strategies and techniques employed in the field. Emphasis was placed both on in vitro and in vivo protein–DNA interactions and on novel methodologies to study gene regulation. Students made nuclear extracts, expressed and purified DNA-binding transcriptional activators, 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. This included activator-stimulated histone acetyltransferase assays, chromatin footprinting, and chromatin remodeling assays, as well as histone methyltransferase 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.

PARTICIPANTS

Ahlskog, J., M.S., Abo Akademi University, Turku, Finland
Dimitrova, N., Ph.D., Philips Research/CSHL, Briarcliff Manor, New York
Donaldson, Z., B.S., Emory University, Atlanta, Georgia
Fung, I., B.Sc., University of Toronto, Canada
Gafencu, A., Ph.D., Institute of Cellular Biology and Pathology, "Nicolae Simionescu," Bucharest, Romania
Goh, A., Ph.D., Institute of Molecular and Cell Biology, Singapore
Helenius, K., M.S., Biomedicum Helsinki, Finland
Hufnagel, L., Ph.D., Kavli Institute for Theoretical Physics, Santa Barbara, California
Koschubs, T., M.A., University of Munich, Germany
Laybourn, C., M.S., Minnetonka High School, Minnetonka, Minnesota
Levine, J., Ph.D., California Institute of Technology, Pasadena
Maheshwari, A., M.S., Cornell University, Ithaca, New York
Malone, J., M.S., University of Texas, Arlington
Myburg, H., Ph.D., North Carolina State University, Raleigh
Navratilova, P., M.S., Sars International Centre, Bergen, Norway
Samten, B., M.S., The University of Texas Health Center, Tyler

SEMINARS

Attardi, L., Stanford University School of Medicine, California: Using mouse models to dissect the function of the p53 transactivator in tumor suppression.
Bentley, D., University of Colorado Health Sciences Center, Denver: Transcription and RNA processing.
Bulger, M., University of Rochester School of Medicine, New York: Hyperacetylated domains and tissue-specific gene expression in erythroid cells.
Gasser, S., Friedrich Miescher Institute, Basel, Switzerland: Chromosomal context and patterns of gene expression.
Hahn, S., Frederick Hutchinson Cancer Center, Seattle, Washington: Pol II basal transcription factors and gene expression.
Hannon, G., Cold Spring Harbor Laboratory: RNAi and gene silencing.
Kraus, L., Cornell University, Ithaca, New York: Chromatin, nuclear receptors, and transcription.
Levine, M., University of California, Berkeley: Transcription and development.
Mellor, J., University of Oxford, United Kingdom: Histone modifications and gene expression.
Oeglgeschlager, T., Marie Curie Research Institute, United Kingdom: Basal transcription factors/gene expression.
Peterson, C., University of Massachusetts Medical School, Worcester: Chromatin and nuclear function.
Roeder, R., The Rockefeller University, New York: Transcriptional regulation by RNA polymerase II.
Shilatifard, A., St. Louis University Medical Center, Missouri: Histone methylation and the regulation of gene expression.
Tora, L., Institut de Genetique et de Biologie Moleculaire et Cellulaire (IGBMC), France: Variation for a theme, or regulation of initiation of RNA polymerase II transcripton.
Tyler, J., University of Colorado Health Sciences Center, Denver: Chromatin, transcription, replication, and repair.
Yamamoto, K., University of California, San Francisco: Trancriptional control via the intracellular receptors.

Imaging Structure and Function in the Nervous System

July 25–August 14

INSTRUCTORS
F. Engert, Harvard University, Cambridge, Massachusetts
M. Hubener, Max-Planck Institute of Neurobiology, Martinsried, Germany
D. Kleinfeld, University of California, San Diego
J. Waters, Northwestern University, Evanston, Illinois

ASSISTANTS
F. Albineau, Harvard University, Cambridge, Massachusetts
D. DiGregorio, University of California School of Medicine, Los Angeles
A. Kampff, Harvard University, E. Aurora, New York
V. Nagerl, Max-Planck Institute of Neurobiology, Munich, Germany
V. Staiger, Volker, Max-Planck Institute of Neurobiology, Bavaria, Germany
A. Trevelyan, Columbia University, New York
P. Tsai, University of California, San Diego
R. Vislay, Harvard University, Cambridge, Massachusetts

Advances in light microscopy, digital image processing, and the development of a variety of powerful fluorescent probes present expanding opportunities for investigating the nervous system, from synaptic spines to networks in the brain. This intensive laboratory and lecture course provided participants with the theoretical and practical knowledge to utilize emerging imaging technologies. The primary emphasis of the course was on vital light microscopy. Students learned the principles of light microscopy, as well as use of different types of electronic cameras, laser-scanning systems, functional fluorophores, delivery techniques, and digital image-processing software. In addition to transmitted light

microscopy for viewing cellular motility, the course examined a variety of molecular probes of cell function, including calcium-sensitive dyes, voltage-sensitive dyes, photo-activated ("caged") compounds, 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. We used a spectrum of neural and cell biological systems, including living animals, brain slices, and cultured cells. Applicants had a strong background in the neurosciences or in cell biology.

Lecturers included Wolfhard Almers (Oregon Health & Science University), William Betz (University of Colorado Medical School), Winfried Denk (Max-Planck Institute for Medical Research), Anna Devor (MGH/Harvard Medical School), Fred Lanni (Carnegie-Mellon University), Greg Law (Perkin-Elmer), Jeff Lichtman (Harvard University), Jerome Mertz (Boston University), Venkatesh Murthy (Harvard University), Mark Schnitzer (Stanford University), Steve Vogel (NIH/NIAAA/DICBR), and Katrin Willig (Max-Planck Institut for Biophysical Chemistry.

This course was supported with funds provided by the National Institute of Mental Health, National Institute on Drug Abuse, and the Howard Hughes Medical Institute.

PARTICIPANTS

Anantharam, A., B.A., Cornell University Medical College, New York
Duguid, I., Ph.D., University College London, United Kingdom
Encalada, S., Ph.D., University of California, San Diego
Gordon, G., B.Sc., University of Calgary, Canada
Kalmbach, A., B.S., University of Pittsburgh, Pennsylvania
Karrow, K., Ph.D., Harvard Medical School, Boston, Massachusetts
Lien, C.-C., Ph.D., University of California, Berkeley
Luo, W., Ph.D., Duke University Medical Center, Durham, North Carolina
Murphy, G., Ph.D., HHMI/University of Washington, Seattle
Rumpel, S., Ph.D., Cold Spring Harbor Laboratory
Sundberg, K., B.S., The Salk Institute, La Jolla, California
Wu, W., Ph.D., Oregon Health & Science University, Portland

SEMINARS

Almers, W., Oregon Health & Science University, Portland: TIRF.
Betz, W., University of Colorado Medical School, Denver: FM.
David, V., Spectra-Physics, Mountain View, California: Ultrafast lasers.
Denk, W., Max-Planck Institute for Medical Research, Heidelberg, Germany: 2-photon and serial EM.
Devor, A., MGH/Harvard Medical School, Charlestown, Massachusetts: Blood flow imaging.
DiGregorio, D., University of California School of Medicine, Los Angeles: Uncaging.
Engert, F., Harvard University, Cambridge, Massachusetts: Basics of 2-photon imaging. Home brew 2-photon overview. Tadpoles rock.
Griesbeck, O., Max-Planck Institute of Neurobiology, Martinsried, Germany: Genetically encoded sensors.
Hubener, M., Max-Planck Institute of Neurobiology, Martinsried, Germany: Primer on intrinsic optical signals. Frequent domain methods for intrinsic signals.
Kampff, A., Harvard University, E. Aurora, New York: ImageJ.
Kilborn, K., Intelligent Imaging Innovations, Inc., Santa Monica, California: Deconvolution.
Kleinfeld, D., University of California, San Diego: Denoising and Fourier. Ultra-high-resolution blood flow and stroke. Voltage-sensitive dyes.
Lanni, F., Carnegie-Mellon University, Pittsburgh, Pennsylvania: Basic microscopy. Basic microscopy diffraction theory. Light sources. DIC, dot tube, apotome, demo.
Law, G., Perkin-Elmer: Spinning disk.
Lichtman, J., Harvard University, Cambridge, Massachusetts: Confocal microscopy. Brainbow multilabels.
Mertz, J., Boston University, Massachusetts: Nonlinear techniques.
Murthy, V., Harvard University, Cambridge, Massachusetts: Synaptophluorin imaging.
Nagerl, V., Max-Planck Institute of Neurobiology, Munich, Germany: Bring on da noise.
Schnitzer, M., Stanford University, California: Deep imaging.
Tille, S., Zeiss, Thornwood, New York: Overview of the Zeiss Meta and Pascal.
Tsai, P., University California, San Diego: Basic optical design. Basic design scanning.
Vogel, S., NIH/NIAAA/DICBR, Bethesda, Maryland: FRET. FRET and lifetime imaging in 2-photon microscopy.
Waters, J., Northwestern University, Evanston, Illinois: Quantitative modeling of organic Ca indicator signals. Advanced aspects (loading, quantitative modeling) of organic Ca indicators.
Willig, K., Max-Planck Institute for Biophysical Chemistry, Germany: Ultramicroscopy.

Yeast Genetics and Genomics

July 25–August 14

INSTRUCTORS **F. Luca**, University of Pennsylvania, Philadelphia
J. Strathern, National Cancer Institute, Frederick, Maryland
M. Whiteway, National Research Council of Canada, Montreal

ASSISTANTS **A. Farley,** Vanderbilt University, Nashville, Tennessee
M. Martchenko, Montreal, Quebec, Canada
G. Santoyo, National Cancer Institute, Frederick, Maryland

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 prehets, construction and analysis of gene fusions, and generation of mutations in cloned genes, were also emphasized.

Students used classical and molecular approaches to gain experience in identifying and interpreting various kinds of genetic interactions including suppression and synthetic lethality. Students were immersed in yeast genomics and performed and interpreted experiments with DNA arrays. Students gained first-hand experience in modern cytological approaches such as epitope tagging and imaging yeast cells using indirect immunofluorescence, GFP-protein fusions, and a variety of fluorescent indicators for various subcellular organelles. Lectures on fundamental aspects of yeast genetics were presented along with seminars given by outside speakers on topics of current interest.

This year's speakers included David Amberg (SUNY Upstate Medical University), Linda Breeden (Fred Hutchinson Cancer Research Center), Dan Burke (University of Virginia), Kara Dolinski (Princeton University), Beverly Errede (University of North Carolina, Chapel Hill), Daniel Gottschling (Fred Hutchinson Cancer Research Center), Michael Gustin (Rice University), Alan Hinnebusch (NICHD/NIH), Chris Kaiser (Massachusetts Institute of Technology), James Konopka (SUNY Stony Brook), Rodney Rothstein (Columbia University College of Physicians and Surgeons), Pamela Silver (Harvard Medical School/Dana Farber Cancer Institute), Michael Snyder (Yale University), and George Sprague (University of Oregon).

This course was supported with funds provided by the Howard Hughes Medical Institute.

PARTICIPANTS

Cheney, C., Ph.D., Pomona College, Claremont, California
Docking, R., B.Sc., McGill University, Montreal, Canada
Geijer, C., M.Sc., Goteborg University, Sweden
Kraft, C., Ph.D., ETH Honggerberg, Zurich, Switzerland
Kuo, D., B.S./M.S., University of California, San Diego
Lopez, A., Ph.D., University of Toronto, Canada
Magnuson, R., Ph.D., University of Alabama in Huntsville
Otero, J.M., M.Eng., BioCentrum-DTU, Denmark
Ranjan, A., Ph.D., National Cancer Institute, Bethesda, Maryland
Rudalska, R., M.S., Euopean Institute of Oncology, Milan, Italy
Samoilov, M., Ph.D., HHMI/University of Berkeley, California.
Sasaki, M., M.S., Cornell University, New York
Siddiqui, K., B.Sc., Cold Spring Harbor Laboratory
Vogelsang, M., B.S., National Institute of Chemistry Slovenia, Ljubljana, Slovenia
Wurtele, H., B.S., University of Montreal, Canada
Yu, E., B.S., Brandeis University, Madison, Wisconsin

SEMINARS

Amberg, D., SUNY Upstate Medical University, Syracuse, New York: Actin cytoskeleton.
Breeden, L., Fred Hutchinson Cancer Research Center, Seattle, Washington: The transcriptional program that starts the budding yeast cell cycle.
Burke, D., University of Virginia Medical Center, Charlotteville: Mitotic regulation.
Dolinski, K., Princeton University, New Jersey: SGD.
Errede, B., University of North Carolina, Chapel Hill: MAP kinase signaling in yeast.
Gottschling, D., Fred Hutchinson Cancer Research Center, Seattle, Washington: Yeast as a model for aging.
Gustin, M., Rice University, Houston, Texas: Ups and downs of osmosensing MAP kinase pathways in yeast.
Hinnebusch, A., NICHD/NIH, Bethesda, Maryland: General amino acid control.
Kasier, C., Massachusetts Institute of Technology, Cambridge: ER processing and disulfide bond formation.
Konopka, J., SUNY at Stony Brook, New York: Genetic analysis of the yeast pheromone response.
Rothstein, R., Columbia University College of Physicians and Surgeons, New York: Yeast recombination.
Silver, P., Harvard Medical School/Dana Farber Cancer Institute, Boston, Massachusetts: Synthetic biology.
Snyder, M., Yale University, New Haven, Connecticut: Genomic analysis of regulatory networks in yeast.
Sprague, G., University of Oregon, Eugene: Cell-type regulation and novel posttranslational modifications.

C. elegans

July 27–August 14

INSTRUCTORS **M. de Bono,** MRC/Cambridge, United Kingdom
A. Desai, University of California, San Diego
M. Labouesse, Institut de Génétique et de Biologie Moléculaire et Cellulaire, France
K. Oegema, University of California, San Francisco

ASSISTANTS **A. Benedetto,** Institut de Génétique et de Biologie Moléculaire et Cellulaire, France
A. Dammermann, University of California, San Diego
B. Olofsson, MRC/Cambridge, United Kingdom

This course was designed to familiarize investigators with *C. elegans* as an experimental system, with an emphasis on both classical genetic analysis reverse genetic approaches and protein purification. A major goal was to teach students how to successfully exploit the information generated by the *C. elegans* genome project. The course was suited both for those who have a current training in molecular biology and some knowledge of genetics, but have no experience with *C. elegans*, as well as students with some prior worm experience who wished to expand their repertoire of expertise.

The following topics were covered both in the laboratory and by lectures from experts in the field: worm pushing, *C. elegans* databases and worm bioinformatics, anatomy and development, forward genetics, chemical and transposon mutagenesis, generation of transgenic animals, expression pattern analysis, reverse genetics, construction and screening of deletion libraries, RNA interference, antibody

coimmunoprecipitation, TAP-tag purification, and worm liquid culture. The course was designed to impart sufficient training to students in the most important attributes of the *C. elegans* system to enable them to embark on their own research projects after returning to their home institutions.

Speakers included Raffi Arolan (University of California, San Diego), Kaveh Ashrafi (Washington University School of Medicine), Joe Culotti (Mount Sinai Hospital Research Institute), Abby Dernburg (Lawrence Berkeley National Laboratory), Lesilee Rose (University of California, Davis), Hitoshi Sawa (Center for Developmental Biology, RIKEN), Tim Schedl (Washington University School of Medicine), Shai Shaham (The Rockefeller University), Frank Slack (Yale University), Ralf Sommer (Max-Planck Institute for Developmental Biology), and Mei Zhen (Mount Sinai Hospital, University of Toronto).

This course was supported with funds provided by the National Institute of Child Health and Human Development.

PARTICIPANTS

Au, C., B.S., Vanderbilt University Medical Center, Nashville, Tennessee
Cowen, L., Ph.D., Whitehead Institute/Massachusetts Institute of Technology, Cambridge
David, D., Ph.D., University of California, San Francisco
Fensgard, O., M.Sc., University of Oslo, Norway
Goodchild, R., Ph.D., Columbia University, New York
Husson, S., M.Sc., K.U. Leuven, Belgium
Kaplan, F., Ph.D., University of Florida, Gainesville
Li, W., Ph.D., University of Texas Southwestern Medical Center, Dallas
Pinto, S.M., M.Sc., University of Zurich, Switzerland
Olahova, M., M.Sc., University of Newcastle Upon Tyne, United Kingdom
Pike, B., Ph.D., Friedrich Miescher Institute, Basel, Switzerland
Pruyne, D., Ph.D., Cornell University, Ithaca, New York
Siedel, H., B.A., Princeton University, New Jersey
van Wolfswinkel, J., M.S., Hubrecht Laboratorium, Utrecht, The Netherlands
Yin, J., M.S., Queens College at CUNY, Flushing, New York
Williams, C., B.S., University of Alabama, Birmingham

SEMINARS

Arolan, R., University of California, San Diego: Nematicidal Bt crystal proteins: From antiparasitics to innate immunity.
Ashrafi, K., University of California, San Francisco: *C. elegans* fat and feeding regulatory circuits.
Culotti, J., Samuel Lunenfeld Research Institute, Canada: Genetic identification of axon guidance and cell migration signaling pathways: Evidence for the growth cone "setpoint" hypothesis.
de Bono, M., MRC Laboratory of Molecular Biology, United Kingdom: Foraging by a blind worm.
Dernburg, A., Lawrence Berkeley National Laboratory, California: Analysis of meiosis in *C. elegans*.
Desai, A., University of California, San Diego: Cracking the kinetochore using the *C. elegans* embryo.
Labouesse, M., Institut de Génétique et de Biologie Moléculaire et Cellulaire, France: Using *C. elegans* to analyze epithelial morphogenesis.
Oegama, K., University of California, San Francisco: Using *C. elegans* to dissect cell division mechanisms.
Rose, L., University of California, Davis: Cell polarity and spindle positioning in the embryo.
Sawa, H., Riken Institute, Japan: Regulation of asymmetric division by Wnt signaling.
Schedl, T., Washington University, Seattle: Control of *C. elegans* germ line development.
Shaham, S., The Rockefeller University, New York: Glia-neuron interactions in the *C. elegans* nervous system.
Slack, F., Yale University, New Haven, Connecticut: Developmental timing of seam cells.
Sommer, R., Max-Planck Institute Tubingen, Germany: Nematode evolution: Two species is more than one.
Zhen, M., Samuel Lunenfeld Research Institute, Canada: Channel and active zone formation at the *C. elegans* synapse.

Stem Cells

August 3–16

INSTRUCTORS
R. McKay, National Institutes of Health, Bethesda, Maryland
A. McClaren, University of Cambridge, United Kingdom
A. Spradling, Carnegie Institute of Washington, Baltimore, Maryland
A. Surani, Wellcome, CRC Institute, Cambridge, United Kingdom
M. Wicha, University of Michigan, Ann Arbor

ASSISTANTS
J. Boyd, National Institutes of Health, Bethesda, Maryland
P. Tesar, National Institutes of Health, Bethesda, Maryland

This 2-week lecture course brought together leading researchers in the stem cell field with a small group of international students. Stem cells construct organs in development. They sustain tissues in the adult and restore them after injury. Because of these properties, isolating and manipulating stem cells has become a major new element in biomedical science. This lecture and discussion course covered a series of subjects including the cells of the early embryo, the nature of germ cells, the mechanisms that control the number of stem cells, their stability, and transformation into other cell types. The clinical potential and political impact of stem cell technology were also presented in depth by invited speakers. A key feature of the course was the easy access to the instructors and the invited lecturers for informal discussion. The purpose of the course was to provide participants with an opportunity to achieve an advanced understanding of the scientific and clinical importance of stem cells.

This course was supported with funds provided by the Howard Hughes Medical Institute.

PARTICIPANTS

Alexander, M., Ph.D., University of Texas Southwestern Medical Center, Dallas
Alexandrina, B., Ph.D., Institute of Cellular Biology & Pathology, Bucharest, Romania
Forde, S., B.S., University of Oxford, United Kingdom
Gakhar, S., Ph.D., Maharshi Dayanand University, Rohtak, India
Hirabayashi, Y., Ph.D., University of Tokyo, Japan
Houbaviy, H., Ph.D., Rutgers University, Piscataway, New Jersey
Huang, T.-s., Ph.D., University of Bergen, Norway
Jonsson, M., B.S., Lund University, Sweden
Lattanzi, A., Ph.D., INBB, Civitanova Marche, Italy
Ma, J., B.S., University of California, Davis
Pepe, A.E., Ph.D., University of Bologna, Italy
Powers, R., B.S., University of Washington, Seattle
Stuelten, C., Ph.D., National Cancer Institute, Bethesda, Maryland
Wilson, A., Ph.D., Boston University, Massachusetts
Yuan, J., Ph.D., University of California, San Diego

SEMINARS

Ahn, S., National Institutes of Health, Bethesda, Maryland and Zervas, M., Brown University, Providence, Rhode Island: Genetic lineage and stem cells: Linking development, disease, and physiology of the brain.
Battey, J., National Institutes of Health, Bethesda, Maryland: Stem cell politics and medicine.
Bernstein, B., Harvard University, Boston, Massachusetts: Epigenetic mechanisms of pluripotency.
Brickman, J., University of Edinburgh, United Kingdom: What can a frog teach an ES cell or an ES cell a frog? ES cells as a model for gastrulation.
Cantley, L., Harvard Medical School, Boston, Massachusetts: Biochemical basis of mammalian cell growth.
Civin, C., Johns Hopkins University School of Medicine, Baltimore, Maryland: Biology of hematopoietic stem cells.
Cooke, H., MRC, Edinburgh, United Kingdom: Germ cells to stem cells and back again.
Doetsch, F., Columbia University, New York: Natural stem cells and their role in the adult mammalian brain.
Gotoh, Y., University of Tokyo, Japan: Signaling pathways, regulation neural precursor cell fate, and apoptosis.
Greiner, E., Evotech AG, Hamburg, Germany: Small-molecule chemistry and drug screening technologies.
Keller, G., Mount Sinai School of Medicine, New York: Lineage-specific differentiation of embryonic stem cells.
Krek, W., Friedrich Miescher Institute, Basel, Switzerland: von-Hippel-Lindau tumor suppressor functions: From oxygen sensing to ciliary signaling.
Lee, C.-Y., University of Michigan, Ann Arbor: Genetic regulation of self-renewal and differentiation in *Drosophila*.
McKay, R., National Institutes of Health, Bethesda, Maryland: Introduction to stem cells. Stem cells and medicine in the central nervous system.
McLaren, A., University of Cambridge, United Kingdom: How mice got where they are today.
Mercola, M., The Burnham Institute, La Jolla, California: Heart differentiation in vivo and in vitro.
Nakafuku, M., Cincinnati Children's Hospital Research Foundation, Ohio: Stem cells and brain repair: Theory and some practices.
Niederhuber, J., National Cancer Institute, Bethesda, Maryland: The tissue stem cells as a target for cancer initiation.
Rafii, S., Cornell University, Ithaca, New York: Interaction between vascular and organ-specific stem cells.
Segal, R., Harvard Medical School, Boston, Massachusetts: Neural differentiation and synapse formation.
Shen, M., UMDNJ–Robert Wood Johnson Medical School, Piscataway, New Jersey: Axis formation in the mouse embryo.
Spradling, A., Carnegie Institute of Washington, Baltimore, Maryland: Stem cells and their niches: What are they and how do you find them? Germ cells and epithelial stem cells in *Drosophila*.
Surani, A., Wellcome Trust, Cambridge, United Kingdom: Imprinting, epigentics, and germ cells.
Wicha, M., University of Michigan, Ann Arbor: Stem cells and cancer: A fresh look at an old idea.
Wilson, V., Edinburgh University, United Kingdom: The node and its role in the patterning of the embryo.

X-Ray Methods in Structural Biology

October 16–31

INSTRUCTORS **W. Furey,** V.A. Medical Center and University of Pittsburgh, Pennsylvania
G. Gilliland, Centorcor, Inc., Radnor, Pennsylvania
A. McPherson, University of California, Irvine
J. Pflugrath, Rigaku Americas Corporation, The Woodlands, Texas

ASSISTANT **J. Cary,** Northern Illinois University, DeKalb

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 is designed for scientists with a working knowledge of protein structure and function, but who are new to macromolecular crystallography. Topics covered included basic diffraction theory, crystallization (proteins, nucleic acids and complexes), crystal characterization, X-ray sources, and optics, synchrotrons, crystal freezing, data collection, data reduction, multiple isomorphous replacement, multiwavelength anomalous diffraction, molecular replacement, solvent flattening, noncrystallographic symmetry averaging, electron density interpretation, molecular graphics, structure refinement, structure validation, coordinate deposition, and structure presentation. Participants learned through extensive hands-on experiments. One or more proteins were crystallized and the structure(s) determined by several methods, in parallel with lectures on the theory and informal discussions behind the techniques. Applicants were familiar with the creation and editing of simple text files on Linux workstations using a screen-based editor (either vi or emacs).

Speakers included Paul Adams, Martin Caffrey, Serge Cohen, Morten Kjeldgaard, Gerard Kleywegt, Duncan McRee, Randy Read, David Richardson, Robert Sweet, Thomas Terwilliger, Dale Tronud, David Waugh, and John Wesbrook.

This course was supported with funds provided by the National Cancer Institute and the Howard Hughes Medical Institute.

PARTICIPANTS

Barnard, T., Ph.D., NIH/NIDDK, Bethesda, Maryland
Braddock, D., Ph.D., Yale University School of Medicine, New Haven, Connecticut
Brelidze, T., Ph.D., University of Washington, Seattle
Eriksson, H., M.Sc., Massachusetts Institute of Technology, Cambridge
Gretes, M., B.Sc., University of British Columbia, Vancouver, Canada
Guettler, S., M.S., Cancer Research UK London Research Institute, United Kingdom
Guo, S., M.S., Eli Lilly, Indianapolis, Indiana
Gupta, D., M.Sc., University of Cambridge, United Kingdom
Harwood, I., B.S., University of California, San Francisco
Hoefer, N., M.S., University of Limerick, Columbus, Ohio
Krajewski, W., B.S., Uppsala University, Sweden
Malojcic, G., M.Sc., ETH Zurich, Switzerland
Mans, J., M.Sc., NIAID/NIH, Bethesda, Maryland
Reddy, R., Ph.D., Metabsis Therapeutics, Inc., La Jolla, California
Stavropoulos, P., B.S., The Rockefeller University, New York
Xue, Y., B.S., Northwestern University, Evanston, Illinois

SEMINARS

Adams, P., Lawrence Berkeley Laboratory, California: Introduction to CNS and PHENIX. Structure Refinement.
Caffrey, M., University of Limerick, Castletroy, Ireland: Introduction to membrane protein crystallization.
Cohen, S., Netherlands Cancer Institute, Amsterdam, The Netherlands: Automated model building and refinement with ARP/WARP.
Furey, W., V.A. Medical Center and University of Pittsburgh, Pennsylvania: Patterson group therapy. Isomorphous replacement and anomalous scattering. MAD phasing: A classical approach. Solvent flattening/phase combination. Solving structures with BnP. Noncrystallographic symmetry averaging.
Gilliland, G., Centorcor Inc., Radnor, Pennsylvania: Crystallization databases and strategies.
Joshua-Tor, L., Cold Spring Harbor Laboratory: Structure presentation. Argonaute: The secret of slicer.
Kjeldgaard, M., Aarhus University, Denmark: Electron density fitting from A to O.
Kleywegt, G., University of Uppsala, Sweden: Just because it's in Nature, doesn't mean it's true...(macromolecular structure validation).
McPherson, A., University of California, Irvine: Crystallization of macromolecules I. Crystallization of macromolecules II. Waves, vectors, and complex numbers. Symmetry, periodicity, unit cells, space groups, Miller planes, and lattices. Fundamental diffraction relationships and Bragg's law. Patterson techniques. Fourier transforms and the electron density equation. Heavy atoms and anomalous scatterers. Mechanisms of crystal growth.
McRee, D., ActiveSight, San Diego, California: MIAutoStructure: An industrial strength package for solving, fitting, and scripting ligand-bound protein structures.
Pflugrath, J., Rigaku Americas Corporation, The Woodlands, Texas: Data collection: Design and setup I. Scaling and merging synchrotron data. Away from the edge: Sulfur SAD with chromium radiation.
Read, R., University of Cambridge, United Kingdom: Molecular replacement: From Pattersons to likelihood. Likelihood and experimental phasing.
Richardson, D. and J., Duke University Medical Center, Durham, North Carolina: Detection and repair of model errors using MolProbity.
Sweet, R., Brookhaven National Laboratory, Upton, New York: Fundamentals of crystallography. X-ray sources and optics.
Terwilliger, T., Los Alamos National Laboratory, New Mexico: Automated structure solution and model building.
Tronrud, D., University of Oregon, Eugene: Macromolecular refinement I. Macromolecular refinement II. Difference electron density maps.
Waugh, D., National Cancer Institute, Frederick, Maryland: Protein engineering for X-ray crystallographers.
Westbrook, J., Rutgers University, The State University of New Jersey, Piscataway: Automating PDB deposition.

Programming for Biology

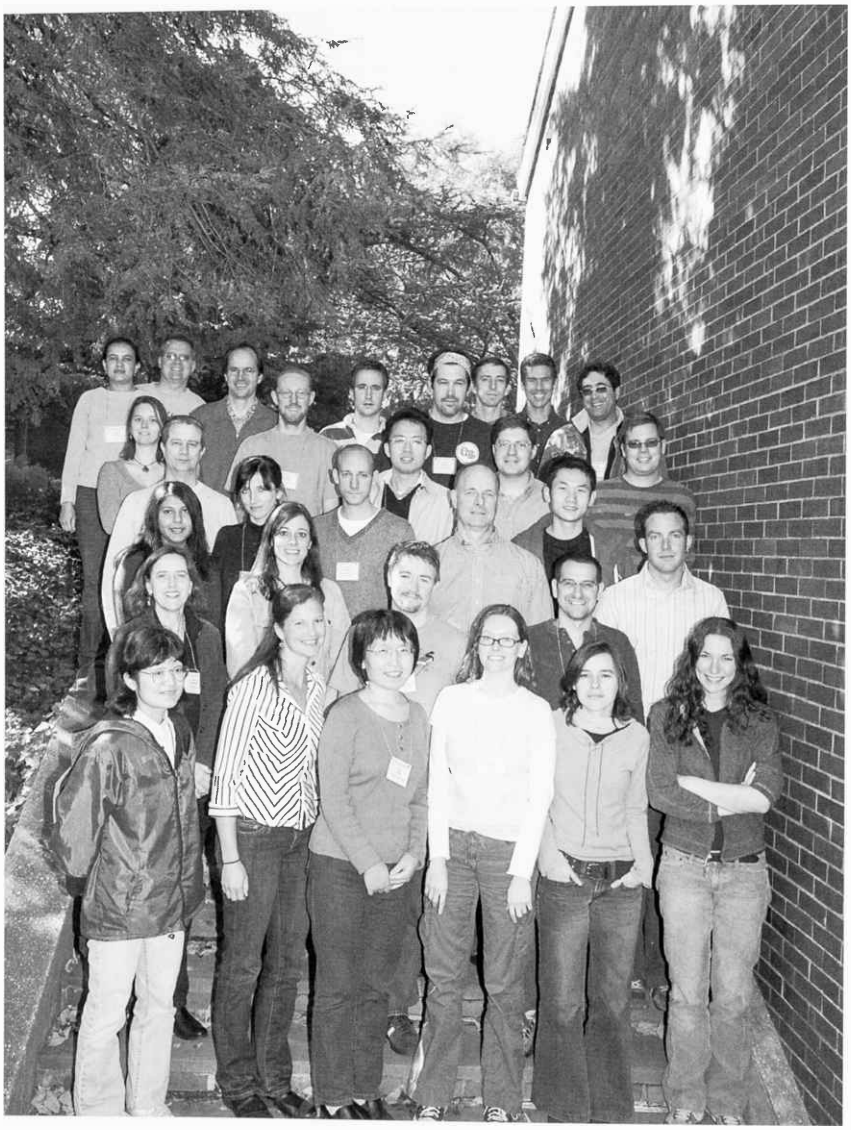
October 18–31

INSTRUCTORS

S. Lewis, University of California, Berkeley
S. Prochnik, DOE Joint Genome Institute/University of California, Berkeley
L. Stein, Cold Spring Harbor Laboratory
J. Tisdall, DuPont Experimental Station, Wilmington, Delaware

ASSISTANTS

J. Babayev, Pioneer Hi-Bred, Johnston, Iowa
D. Curiel, Pioneer Hi-Bred, Johnston, Iowa
C. Maher, Cold Spring Harbor Laboratory
T. Marques, Universitat Pompeu Fabra, Barcelona, Spain
D. Messina, Washington University School of Medicine, St. Louis, Missouri
S. Robb, University of Utah, Salt Lake City
C. Schmidt, University of Delaware, Newark



Today, the computer is an indispensable part of a research biologist's tool kit. The success of the human and other organism genome projects has created terabytes of data on everything from genetic linkage mapping, to nucleotide sequences, to protein structures, stashed away in databases around the globe. Large-scale technologies such as DNA microarrays and high-throughput genotyping have transformed the nature of laboratory experimentation. Furthermore, even when biologists are not generating large data sets of their own, they will want to collect and analyze data from myriad sources in the pursuit of novel candidates or even entire research avenues. A few years ago, it might have been sufficient to use Excel spreadsheets for managing laboratory data and canned Web interfaces for searching, but as the volume of data grows and the subtlety of analysis increases, these techniques, even supplemented by some simple programming skills, have become inadequate. Modern biologists must be adept at juggling disparate data sets in order to pursue their research.

Designed for students and researchers with some prior programming experience, this 2-week program gave biologists the expanded bioinformatics skills necessary to construct computational systems that can exploit this increasingly complex information landscape, with an emphasis on fitting the wide range of existing analysis tools into extensible bioinformatics systems. The course combined formal lectures with hands-on sessions in which students work to solve a series of problem sets covering common scenarios in the acquisition, validation, integration, analysis, and visualization of biological data. For their final projects, students posed problems using their own data and worked with each other and the faculty to solve them. The prerequisites for the course was basic knowledge of UNIX, procedural Perl programming, HTML document creation and the database query language, SQL. Lectures and problem sets covering this background material are available online and students can study 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 Genomics course.

Speakers included Emina Begovic (University of California, Berkeley), Peter Brokstein (DOE Joint Genome Institute), Roderic Guigo (Institut Municipal d'Investigacio Medica, Spain), Winston Hide (University Western Cape, South Africa), Gabor Marth (Boston College), Sheldon McKay (Cold Spring Harbor Laboratory), Chris Mungall (Berkeley *Drosophila* Genome Project), Lior Pachter (University of California, Berkeley), William Pearson (University of Virginia), Jason Stajich (Duke University), Paul Thomas (Applied Biosystems), and Olga Troyanskay (Princeton University).

This course was supported by the National Human Genome Research Institute.

PARTICIPANTS

Akagi, K., Ph.D., Mouse Cancer Genetics Program/National Cancer Institute, Frederick, Maryland
Aman, J., B.A., Mount Desert Island Biological Laboratory, Bar Harbor, Maine
Badhwar, A.P., B.Sc., McGill University, Montreal, Canada
Crosson, S., Ph.D., The University of Chicago, Illinois
Fell, M., M.Sc., Roslin Institute, Edinburgh, United Kingdom
Fernando, O., M.S., Universitat Pompeu Fabra, Barcelona, Spain
Fritz-Laylin, L., B.A., University of Berkeley, California
Hubbard, E.J., Ph.D., New York University, New York
Jeddeloh, J., Ph.D., Orion Genomics, LLC., St. Louis, Missouri
Kramer, M., B.S., Cold Spring Harbor Laboratory
Lawson, H., B.A., Pennsylvania State University, State College
Magrini, V., Ph.D., Washington University/Genome Sequencing Center, St. Louis, Missouri
McGovern, M., B.S., New York University, New York
Pettersson, E., M.Sc., Royal Institute of Technology, Stockholm, Sweden
Riesenfeld, C., B.S., Desert Research Institute, Reno, Nevada
Ross, E., B.S., HHMI/University of Utah, Salt Lake City
Sandhu, S., M.S., Ohio State University, Wooster
Wang, K., M.S., CBS, Technical University of Denmark, Lyngby
Zhang, X., Ph.D., Michigan State University, East Lansing
Zhou, Y., Ph.D., Fox Chase Cancer Center, Philadelphia, Pennsylvania

SEMINARS

Cain, S., Cold Spring Harbor Laboratory: Gbrowse.
Curiel, D., Pioneer Hi-Bred, Johnston, Iowa: Introduction to Javascript.
Guigo, R., Center for Genomic Regulation, Barcelona, Spain: Gene prediction.
Hartzell, G., University of California, Berkeley: Good programming practices and version control.

Marth, G., Boston College, Chestnut Hill, Massachusetts: Sequence variation analysis: SNPs, haplotypes.

McKay, S., Cold Spring Harbor Laboratory: Querying SQL, databases with DBS.pm. Biological database design.

Pearson, W., University of Virginia, Charlottesville: Aligning proteins: Local/global alignment algorithms (Smith-Waterman and blastp, etc.) and substitution matrices.

Prochnik, S., Berkeley *Drosophila* Genome Project, California: Designing and running bioinformatics pipelines with Perl: Job tracking, dependencies.

Rokas, A., The Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge, Massachusetts: Studying molecular evolution with Perl. Molecular evolution.

Stajich, J., University of California, Berkeley: Introduction to Bioperl: The organization and the basic classes. Bioperl II: Using Bioperl: More classes and using them.

Stein, L., Cold Spring Harbor Laboratory: Perl review I. Perl review II. Perl review III. Perl IV. Perl III. Perl review IV (writing OOP modules).

Thomas, P., Applied Biosystems, Foster City, California: Functional assignment, protein domain assignment, COGs, panther.pfam.

Tisdall, J., DuPont Experimental Station, Wilmington, Delaware: Algorithms in bioinformatics.

Immunocytochemistry, In Situ Hybridization, and Live Cell Imaging

October 23–November 5

INSTRUCTORS
A. Dernburg, Lawrence Berkeley National Laboratory, Berkeley, California
J. Murray, University of Pennsylvania School of Medicine, Philadelphia
J. Swedlow, University of Dundee, United Kingdom

ASSISTANT INSTRUCTORS
W.G. Cox, Invitrogen Molecular Probes Detection Technology, Eugene, Oregon
K. Hu, Indiana University, Bloomington
P. LeBaccon, Institut Curie, Paris, France
J. Peng, University of California, Berkeley
J. Waters, Harvard Medical School, Boston, Massachusetts

ASSISTANTS
I. Porter, University of Dundee, United Kingdom
B. Sutton, Invitrogen Molecular Probes Detection Technology, Eugene, Oregon

This course focused on specialized techniques in microscopy, in situ hybridization, immunocytochemistry, and live cell imaging related to localizing DNA, RNA, and proteins in fixed cells, as well as protein and RNA dynamics in living cells. It emphasized the use of the latest equipment and techniques in fluorescence microscopy, including confocal laser-scanning microscopy, deconvolution methods, digital image processing, and time-lapse imaging of living specimens. The curriculum was designed to present students with state-of-the-art technology and scientific expertise in the use of light microscopy to address basic questions in cellular and molecular biology. It was developed for the molecular biologist who is in need of microscopic approaches and for the cell biologist who is not familiar with the practical application of the advanced techniques presented in the course.

Among the methods presented were the preparation of tagged nucleic acid probes; fixation methods; detection of multiple DNA sequences in single nuclei or chromosome spreads; comparative genomic hybridization; cellular localization of RNA; localization of nucleic acids and proteins in the same cells; use of a variety of reporter molecules and nonantibody fluorescent tags; indirect antibody labeling; detection of multiple proteins in a single cell; and the use of GFP variants to study protein expression, localization, and dynamics. In each method, several experimental protocols were presented allowing the students to assess the relative merits of each and to relate them to their own research. Students were encouraged to bring their own nucleic acid, protein, or antibody probes to the course, which were used in addition to those provided by the instructors. The laboratory exercises were supplemented with lectures given by invited distinguished scientists, who presented up-to-the-minute reports on current methods and research using the techniques being presented.

Speakers included W. Gregory Cox, Rich Day, Arshad Desai, Patricia LaBaccon, J. Richard McIntosh, Tom Ried, David Spector, and Jennifer Waters.

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Basu, J., M.Sc., Baylor College of Medicine, Houston, Texas
Boldingh Debernard, K., M.S., University of Oslo, Norway
Buran, B., B.S./B.A., Massachusetts Eye and Ear Infirmary, Boston
Carter, K., B.S./B.A., Yale University, New Haven, Connecticut
Dudnakova, T., Ph.D., MRC Human Genetics Unit, Edinburgh, United Kingdom
Dus, M., B.S., Cold Spring Harbor Laboratory/Watson School of Biological Sciences, Cold Spring Harbor
Garcia, L., Lic., Instituto de Estudios Avanzados, Baruta, Michigan
Houwing, S., B.S., Hubrecht Laboratory, Utrecht, The Netherlands
Hwang, Y.-C., B.S., University of California, Riverside
Janes, K., Ph.D., Harvard Medical School, Boston, Massachusetts
Jorgensen, S., M.S., University of Copenhagen, Kobenhavn, Denmark
Kimura, M., Ph.D., Gifu University School of Medicine, Gifu, Japan
Martins, A., B.S., University of Cambridge, United Kingdom
Tradewell, M., B.Sc., McGill University, Montreal, Canada
Wallace, J., Ph.D., NIH/NIDDK, Bethesda, Maryland
Wu, J., Ph.D., Wellcome Trust Sanger Institute, Cambridgeshire, United Kingdom

SEMINARS

Cox, W.G., Invitrogen Molecular Probes Detection Technology, Eugene, Oregon: Basics and applications of RNA FISH.
Day, R., University of Virginia, Charlottesville: Seeing colors: Applications and limitations of the fluorescent proteins.
Dernburg, A., Lawrence Berkeley National Laboratory/University of California: Basics of DNA FISH.
Desai, A., University of California, San Diego: Studies on mitosis.
Hu, K., Scripps Research Institute, La Jolla, California, and Murray, J., University of Pennsylvania School of Medicine, Philadelphia: Basic introduction to light and fluorescence microscopy.
McIntosh, J.R., University of Colorado, Boulder: Current practice and future prospects for EM.
Murray, J., University of Pennsylvania School of Medicine, Philadelphia: Immunocytochemistry. How to build a parasite: Live cell imaging of *Toxoplasma gondii*.
Ried, T., NCI/NIH, Bethesda, Maryland: Mechanisms and consequences of chromosomal aberrations in cancer cells.
Spector, D., Cold Spring Harbor Laboratory: Localization of gene expression by FISH and in living cells.
Waters, J., Harvard Medical School, Boston, Massachusetts, and LeBaccon, P., Institut Curie, Paris, France: Digital detectors and digital imaging fundamentals.

Phage Display of Proteins and Peptides

November 7–20

INSTRUCTORS **C. Barbas,** Scripps Research Institute, La Jolla, California
D. Siegel, University of Pennsylvania School of Medicine, Philadelphia
G. Silverman, University of California, La Jolla

ASSISTANTS **S. Kacir,** University of Pennsylvania, Philadelphia
K. Noren, New England BioLabs, Beverly, Massachusetts
M. Roman, University of California, San Diego
C. Tuckey, New England BioLabs, Beverly, Massachusetts

Recent advances in the generation and selection of antibodies from combinatorial libraries allow for the rapid production of antibodies from immune and nonimmune sources. This intensive laboratory/lecture course focused on the construction of combinatorial antibody libraries expressed on the surface of phage and selection of desired antibodies from the library. Students learned the theoretical and practical aspects of constructing combinatorial libraries from immune and nonimmune sources, as well as the construction of synthetic antibody libraries. Antibodies were selected from the library by panning. Production, purification, and characterization of Fab fragments expressed in *E. 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, retroviral and cell display libraries, immunobiology of the antibody response, and recent results on the use of antibodies in therapy. The theory and practical implications for selection from phage displayed libraries of random peptides, cDNA products, and semisynthetic proteins were explored.

Seminar speakers included Angela Belcher (Massachusetts Institute of Technology), Marilena Hall (Stonehill College), Henry Lowman (Genentech, Inc.), Christopher Noren (New England BioLabs), Sachdev Sidhu (Genentech, Inc.), George Smith (University of Missouri), Ian Wilson (Scripps Research Institute), and K. Dane Wittrup (Massachusetts Institute of Technology).

This course was supported with funds provided by the Howard Hughes Medical Institute.

PARTICIPANTS

Adedoyin, J., M.S., Karolinska Institute, Stockholm, Sweden
Barderas, R., Ph.D., Spanish National Cancer Research, Madrid, Spain
Cheng, Z., B.Sc., University of Alberta, Edmonton, Canada
Cordier, W., B.S., Egea Biosciences, San Diego, California
Cosic, J., B.S., Dyax Corporation, Cambridge, Massachusetts
Hearty, S., B.Sc., Dublin City University, Republic of Ireland
Mininni, T.L., B.A., Wyeth Research, Pearl River, New York
Nicola, A., M.D., Albert Einstein College of Medicine, Bronx, New York
O'Rourke, S., Ph.D., University of California, Santa Cruz
Pitaksajjakul, P., M.Sc., Mahidol University, Bangkok, Thailand
Plana, E., M.S., Polytechnic University of Valencia, Spain
Rochette, S., M.S., University of Nottingham, United Kingdom
Rouziere, A.-S., Ph.D., University of Glasgow, United Kingdom
Seiler, T., M.D., The Feinstein Institute for Medical Research, Manhasset, New York
Shults, M., Ph.D., Illumina, Inc., San Diego, California
Wu, P., Ph.D., HHMI/University of California, Berkeley

SEMINARS

Barbas, C., Scripps Research Institute, La Jolla, California: Software and hardware for genomes: Polydactyl zinc finger proteins and the control of endogenous genes.
Belcher, A., Massachusetts Institute of Technology, Cambridge: Phage as a tool kit for the synthesis and assembly of materials for electronics and energy.
Hall, M., Stonehill College, Easton, Massachusetts: Target-unrelated phage.
Lowman, H., Genetech, Inc., San Francisco, California: SAR of peptides using phage.
Noren, C., New England BioLabs, Beverly, Massachusetts: Phage peptide libraries: The PhD for peptides.
Sidhu, S., Genetech, Inc., San Francisco, California: Antibody phage display and chemical diversity in antigen recognition.
Siegel, D., University of Pennsylvania Medical Center, Philadelphia: Cell surface selection of combinatorial Fab libraries.
Silverman, G., University of California, San Diego: Repertoire cloning of SLE autoantibodies.
Smith, G., University of Missouri, Columbia: Phage display of peptides.
Wilson, I., The Scripps Research Institute, La Jolla, California: Structural biology of the immune system.
Wittrup, K.D., Massachusetts Institute of Technology, Cambridge: Yeast display libraries.

Computational and Comparative Genomics

November 8–14

INSTRUCTORS **W. Pearson,** University of Virginia, Charlottesville
R. Smith, GlaxoSmithKline, King of Prussia, Pennsylvania

ASSISTANT **B. Cantarel,** University of Virginia, Charlottesville

Beyond BLAST and FASTA—This course presented a comprehensive overview of the theory and practice of computational methods for gene identification and characterization from DNA sequence data. The course focused on approaches for extracting the maximum amount of information from protein and DNA sequence similarity through sequence database searches, statistical analysis, and multiple sequence alignment. Additional topics included gene recognition (exon/intron prediction), identifying signals in unaligned sequences, and integration of genetic and sequence information in biological databases.

The course combined lectures with hands-on exercises; students were encouraged to pose challenging sequence analysis problems using their own data. The course makes extensive use of local WWW pages to present problem sets and the computing tools to solve them. Students used Windows and Mac workstations attached to a UNIX server; participants were comfortable using the Unix operating system and a Unix text editor.

The course was designed for biologists seeking advanced training in biological sequence analysis, computational biology core resource directors and staff, and scientists in other disciplines, such as computer science, who wish to survey current research problems in biological sequence analysis.

The primary focus of this course was the theory and practice of algorithms used in computational biology, with the goal of using current methods more effectively and developing new algorithms. Students more interested in the practical aspects of advanced software development are encouraged to apply to the course on Programming for Biology.

Speakers included Stephen Altschul (National Library of Medicine), Judith Blake (The Jackson Laboratory), Xose Fernandez (EMBL-EBI), Ross Hardison (Pennsylvania State University), Eric Sayers (National Library of Medicine), and James Taylor (Courant Institute of Mathematics, New York University).

This course was supported by the National Human Genome Research Institute.

PARTICIPANTS

Anzola, J., B.Sc., Texas A&M University, College Station, Texas
Berglund, L., M.S., Royal Institute of Technology, Stockholm, Sweden
Brisson, J., Ph.D., Princeton University, New Jersey
Brown, S., Ph.D., Kansas State University, Manhattan
Chandler, E., B.A., VIMS/College of William and Mary, Gloucester Point, Virginia
Counterman, B., B.S., Duke University, Durham, North Carolina
Dranginis, A., Ph.D., St. John's University, Queens, New York
Ernst, S., Ph.D., Tufts University, Medford, Massachusetts
Gonzalez, M., B.A., University of Maryland Baltimore County, Baltimore
Hirlyur Nagaraj, S., M.Sc., Macquarie University, Sydney, Australia
Leonardo, T., Ph.D., Princeton University, New Jersey
Milac, A., Ph.D., National Institutes of Health, Bethesda, Maryland
Molinaro, A., Ph.D., Yale School of Medicine, New Haven, Connecticut
Oliveira, A., Ph.D., University of Georgia, Athens
Raby, B., M.D., Brigham & Women's Hospital/Harvard Medical School, Boston, Massachusetts
Sandhu, S., M.S., Ohio State University, Wooster
Skjaeveland, A., M.S., University of Oslo, Norway
Yampolsky, L., Ph.D., East Tennessee State University, Johnson City, Tennessee
Zhao, Q., B.S., University of Virginia, Charlottesville
Zuccolo, A., Ph.D., Arizona Genomics Institute/University of Arizona, Tucson

SEMINARS

Altschul, S., National Library of Medicine, Bethesda, Maryland: Statistics of sequence similiarity scores. Iterated protein database searches with PSI-BLAST.
Blake, J., The Jackson Laboratory, Bar Harbor, Maine: An introduction to the gene ontology.
Fernandez, X., EMBL-EBI, Hinxton, United Kingdom: The ENSEMBL database of genomes. ENSMART and genome computation.
Hardison, R., Pennsylvania State University, University Park: Comparative genomics I: Tools for comparative genomics.
Pearson, W., University of Virginia, Charlottesville: Protein evolution and sequence similarity searching. Algorithms for biological sequence comparison. Hidden Markov models and protein profiles. Finding consensus sites.
Sayers, E., National Library of Medicine, Bethesda, Maryland: NCBI resources for bioinformatics and computational biology. NCBI genome resources.
Smith, R., GlaxoSmithKline, King of Prussia, Pennsylvania: Approaches to multiple sequence alignment.
Taylor, J., Pennsylvania State University, University Park: Genome computation and gene regulation.

The Genome Access Course

April 25–26, August 29–30, November 29–30

TRAINERS **T. Fiedler**, Cold Spring Harbor Laboratory
G. Howell, The Jackson Laboratory
B. King, The Jackson Laboratory

Initiated in 2002, this course is an intensive 2-day introduction to bioinformatics that was held three times in 2006, training 63 participants in total. The core of the course was designed to cover the manipulation and analysis of sequence information. The course was broken into modules designed to give a broad overview of a given topic, with ample time for examples chosen by the instructors. Each module included three parts, consisting of a discussion of theory and methods, coverage of software and Web resources, and use of selected tools with examples (including those supplied by the students). The modular design allowed the instructors to tailor the presentation to the interests of the students. Modules included Electronic Sequence Information; Pairwise Sequence Comparisons; Multiple Sequence Alignments; Gene Prediction; Genome Analysis; Sequence Variation; Protein Classification and Structural Analysis; Proteomics; and Phylogenetic Analysis.

Applications to the course were open to all on a first-come, first-served basis, subject to basic eligibility requirements. The course was held at the Laboratory's newly opened Genome Research Center at Woodbury located seven miles south of the main Laboratory campus. Each student was provided with a PC laptop with wireless modem for the duration of the course. Students were encouraged to supply problem sets and sequences of interest to the trainers for possible incorporation as examples in the modules. Materials were made available on the Web, and students continued to ask questions of the trainers as they applied what they had learned in their individual endeavors.

In collaboration with the Wellcome Trust Open Door Workshops, this short course was also run in Sao Paolo, Brazil, and Mexico City, Mexico, in February. The rationale for running these courses for the regional scientific communities in South and Central America was borne out by significant attendance in both venues. This effort is featured in the spring issue of the Laboratory's *Harbor Transcript*.

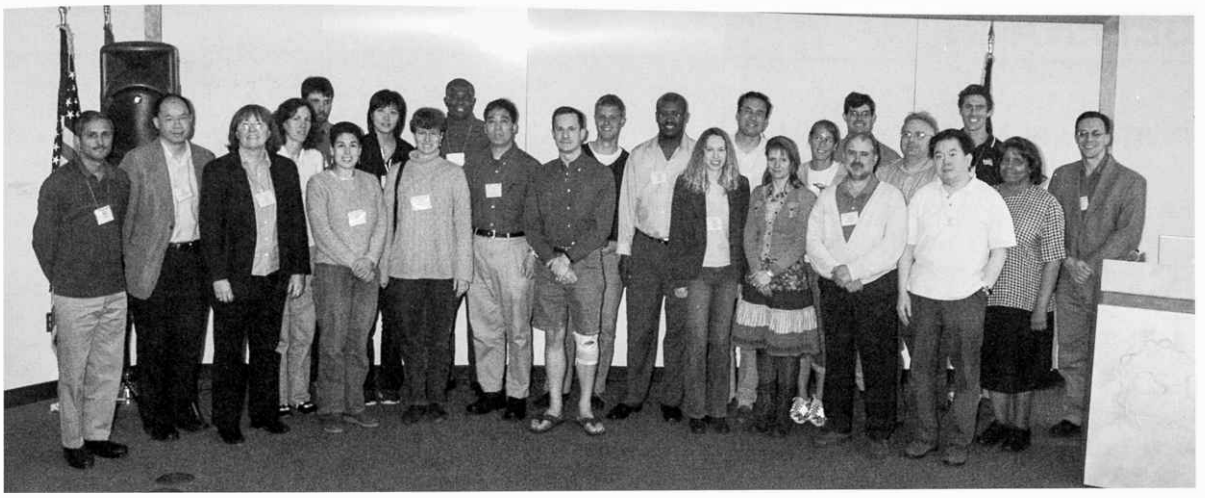
April 25–26

Dr. M. Aldave, The Jules Stein Eye Institute, University of Los Angeles
Dr. M. Boccio, The University Hospital of Columbia and Cornell, New York
Dr. H. Brorson, NatImmune A/S Copenhagen, Denmark
Dr. J. Brown, Dana-Farber Cancer Institute, Boston Massachusetts
Dr. J. Diaz, Louisiana State University, Baton Rouge

Dr. T. Graham, North Carolina State University, Raleigh
Ms. J. Hill, Center for Health and Technology, Deer Park, New York
Dr. T. Hoy, University of Florida, Gainesville
Dr. M. Lee, Columbia University, New York
Mr. J. Majewski, The Walter and Eliza Hall Institute, Melbourne, Australia
Dr. S. Nazar, Cancer Institute of New Jersey, New Brunswick
Dr. Z. Otero, Technical University of Denmark, Lyngby
Ms. C. Ponaka, GE Healthcare, Piscataway, New Jersey
Dr. F. Singhal, Pacific Northwest National Laboratory, West Richland, Washington
Dr. B. Stein, Roswell Park Cancer Institute, Buffalo, New York
Dr. I. Stoeckel, United States Geological Survey, Columbus, Ohio
Dr. A. Sutula, University of Wisconsin, Madison
Mr. B. Symer, National Cancer Institute, Frederick, Maryland
Dr. D. Wang, University of Rochester, New York

August 29–30

Mr. M. Abedi, Roger Williams Hospital, Providence, Rhode Island
Dr. D. Adekoya, University of Tromsoe, Norway
Dr. T. Banck, Mount Sinai School of Medicine, New York
Dr. D. Bandera, Eli Lilly and Co., Indianapolis, Indiana
Dr. C. Beutler, Mount Sinai School of Medicine, New York
Dr. A. Buerckstuemmer, Center for Molecular Medicine, Vienna, Austria
Ms. S. Dollard, Canadian Food Inspection Agency, Ottawa, Canada
Dr. M.A. Gaylord, Eli Lilly and Co., Indianapolis, Indiana
Ms. C. Gunturi, Centocor Inc., Raritan, New Jersey
Dr. T. Hao, The Campbell Family Institute for Breast Cancer, Toronto, Canada
Mr. S. Hsieh, Cleveland Clinic Foundation/Lerner College of Medicine, Cleveland, Ohio
Mr. F. Abdishakur, Imumorin Spelman College, Atlanta, Georgia
Ms. J. Knoechel, University of California, San Francisco
Dr. G. Lohr, University of California, San Francisco
Dr. M. Millham, Pfizer Inc., Groton, Connecticut
Mr. B. Mynarcik, SUNY at Stony Brook, New York
Dr. J. Pajaro, Mayo Clinic, Jacksonville, Florida
Mr. I. Piscopo, University of California, San Francisco
Dr. N. Poon, Brigham and Women's Hospital, Boston, Massachusetts
Dr. A. Meeran Sanders, Brown University/Rhode Island Hospital, Providence
Dr. D. Tegay, Cold Spring Harbor Laboratory
Mr. J. Tilahun, Amherst College, Massachusetts
Dr. R. Tseng, University of Pennsylvania, Philadelphia
Dr. A. Versalovic, Baylor College of Medicine, Houston, Texas
Ms. M. Yoon, Brigham and Women's Hospital, Boston, Massachusetts



November 29–30

Dr. M. Bharti, Rutgers, The State University of New Jersey, Piscataway
Dr. O. Bonini, University of Pennsylvania, Philadelphia
Ms. S. Butler, DuPont, Wilmington, Delaware
Dr. M. Devare, Cornell University, Ithaca, New York
Ms. L. Fellay, Duke University, Durham, North Carolina
Dr. A. Greenstein, Multiple Sclerosis Research Institute, Philadelphia, Pennsylvania
Dr. A. Harris, Helicos Biosciences, Cambridge, Massachusetts
Dr. N. Hastings, Dana-Farber Cancer Institute, Boston, Massachusetts
Ms. K. He, Temple University, Philadelphia, Pennsylvania
Dr. M. Hong, Tulane University Health Sciences Center, New Orleans, Lousianna
Dr. J. Kohli, University of Rochester, New York
Dr. T. Noonan, Massachusetts General Hospital for Children, Boston
Dr. J. Pandelova, Oregon State University, Coravallis
Dr. J. Ramabhadran, United States Environmental Protection Agency, Research Triangle Park, North Carolina
Dr. T. Taccioli, Ohio State University, Columbus
Ms. J. Vasenkova, Open Biosystems Inc., Huntsville, Alabama
Dr. Q. Welch, Ohio University, Athens
Dr. Y. Yang, Mount Sinai School of Medicine, New York
Dr. G. Zhang, Case Western Reserve University, Cleveland, Ohio

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Agilent Technologies
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Applied Scientific Instrumentation, Inc.
Astro-Med Inc.
Axon Instruments
Becton Dickinson Labware
Berthold Technologies USA, LLC
Bioptechs Inc.
Bio-Rad Laboratories
Burleigh Instruments, Inc.
Cambrex Bio Science Rockland Inc.
Carl Zeiss, Inc.
Charles River Laboratories Inc.
Chroma Technology Corporation
Dagan Corporation
David Kopf Instruments
Delaware Diamond Knives
Denville Scientific Inc.
Diagnostic Instruments
Drummond Scientific Company
Epicentre Technologies
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Nikon Inc.
Olympus America Inc.
Pel Freez Biologicals
Perkin Elmer Life and Analytical Sciences
Pierce Chemical Co.
Promega Corp.
QIAGEN Inc.
Q-Imaging Inc
Richard Allan Scientific
Rigaku/MSC
Roche Applied Science
SageN Research
Scigene
Sigma-Aldrich
Siskiyou Corporation
Spectra-Physics Lasers Inc.
Stratagene
Sutter Instruments
Tecan US, Inc.
The Jackson Laboratory
ThermoFinnigan
Thermo Labsystems
The Vibratome Company
T.I.L.L. Photonics GmbH
Torrey Pines Scientific
Universal Imaging Corporation
VDS Vosskuhler GmbH
Vector Laboratories
Warner Instruments
Waters Corp
Zeitz-Instrumente Vertriebs GmbH

SEMINARS

INVITED SPEAKER PROGRAM

Each year, Cold Spring Harbor Laboratory invites speakers from outside the institution to present their findings on a weekly basis. These seminars keep the CSHL staff current on the latest developments and broaden their perspectives. Graduate students and postdoctoral fellows meet with the seminar speakers for lunch immediately after the seminar, allowing an opportunity for the exchange of ideas in an informal setting.

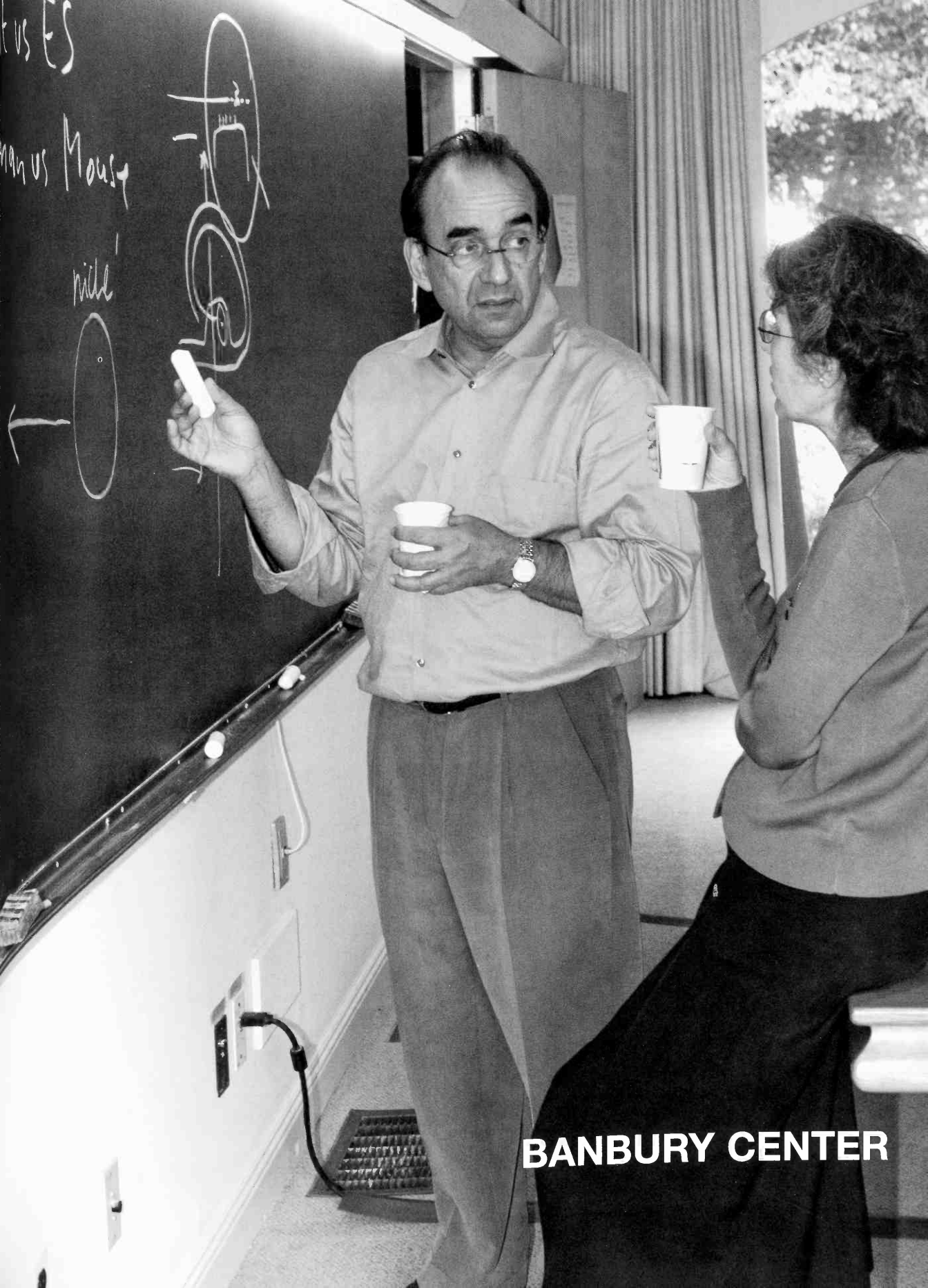
	Title	Host
January		
Dr. Ronald Evans, Department of Gene Expression, Salk Institute for Biological Studies	PPARs: Running around obesity.	Terri Grodzicker
Dr. David Cox, Chief Scientific Officer, Perlegen Sciences, Inc., Mountain View, California	Common human DNA variation and complex human traits: Success stories.	W. Richard McCombie
February		
Dr. Gail Mandel, Howard Hughes Medical Institute, Stony Brook University	The rise and fall of REST: Creating the nervous system.	Holly Cline
Dr. Robert Williams, Department of Anatomy and Neurobiology, University of Tennessee, Health Science Center	A systems genetics approach to study complex biological systems from the level of gene variants through to behavior and disease susceptibility.	Josh Dubnau
March		
Dr. Douglas Hanahan, Department of Biochemistry and Biophysics, Diabetes Center and Comprehensive Cancer Center, University of California, San Francisco	Targeting the tumor microenvironment.	Vivek Mittal
Dr. Anne Brunet, Department of Genetics, Stanford University School of Medicine	FOXO transcription factors in the control of longevity.	Michael Zhang
Dr. David Van Vactor, Department of Cell Biology, Harvard Medical School	Control of synapse morphogenesis by the receptor tyrosine phosphatase LAR.	Senthil Muthuswamy
Dr. Daniel R. Weinberger, Clinical Brain Disorders Branch, National Institute of Mental Health	Schizophrenia: From genes to pathways.	Holly Cline
April		
Dr. Rebecca Doerge, Department of Statistics, Purdue University	Functional genomics of quantitative traits: Expression level polymorphisms of QTLs affecting disease resistance pathways in *Arabidopsis*.	Holly Cline
September		
Dr. Michael Hengartner, Institute of Molecular Biology, University of Zurich	Roads to ruin: Apoptotic pathways in the nematode *C. elegans*.	Mona Spector
Dr. Yang Shi, Harvard Medical School	Dynamic regulation of histone methylation by demethylases.	Michael Zhang
October		
Dr. Jeff Dangl, University of North Carolina at Chapel Hill	The molecular logic of the plant immune system.	Wolfgang Lukowitz
Dr. Richard Morimoto, Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University	Stress and protein misfolding in aging and neurodegenerativedisease.	William Tansey
Dr. Paolo Sassone-Corsi, Department of Pharmacology, University of California, Irvine	A chromatin remodeling CLOCK.	David Spector
November		
Dr. Aneel Aggarwal, Mount Sinai School of Medicine	Beyond Watson and Crick: Translesion DNA synthesis in eukaryotes.	Leemor Joshua-Tor

	Title	Host
Dr. Robert D. Goldman, Department of Cell and Molecular Biology, Feinberg School of Medicine, Northwestern University	Nuclear lamin functions are being revealed by a plethora of human diseases.	David Spector
Dr. Andrew Chess, Department of Medicine, Massachusetts General Hospital and Harvard Medical School	Unusual mechanisms specifying cell identity.	Holly Cline
Dr. Freda Miller, The Hospital for Sick Children Research Institute, University of Toronto	Neural stem cells: Novel signals and sources.	Alea Mills
December		
Dr. Pamela Silver, Department of Systems Biology, Harvard Medical School	Discovery and design of biological networks.	William Tansey
Dr. Brenda Schulman, St. Jude Children's Research Hospital, Howard Hughes Medical Institute	Structural insights into ubiquitin-like protein transfer cascades.	William Tansey

IN-HOUSE SEMINAR PROGRAM

Cold Spring Harbor In-House Seminars were initiated to provide a semiformal avenue for communication between the various research groups at the Laboratory. The seminars also afford a necessary opportunity for the graduate students and postgraduate staff to develop their skills in organizing, presenting, and defending their research.

	Title
January	
Dominik Duelli (Lazebnik Lab)	Cell fusion, viruses, and oncogenic transformation.
David Jackson	Making sense of the signals in plant development.
February	
Cordula Schulz	Stem cells and their niches: An organismal approach in the male gonad of *Drosophila melanogaster*.
Ivan Ho (Zhong Lab)	Neurofibromatosis type 1's involvement in learning and memory.
Bill Keyes (Mills Lab)	Investigating the role of p63 in senescence, aging, and cancer.
March	
Adriana Hemerly (Stillman Lab)	A novel gene network controlling G_1/S transition in plants.
Jannic Boehm (Malinow Lab)	AMPA-receptor phosphorylation during synaptic plasticity: All good things come in threes.
Fabiola Rivas (Hannon Lab)	RISC proteomics and microRNA-mediated repression or wag the mRNA: A poly(A) tale.
Rob Lucito	Characterizing genomic alterations and analyzing their role in cancer.
Chris Maher (Stein Lab)	The expanding microRNA world: Evolution and expression of plant regulatory RNAs.
April	
Mike Myers	Rapid analysis of protein complexes.
October	
Ingrid Bureau (Svoboda Lab)	Mapping the neuronal circuits of the developing mouse barrel cortex.
Rotem Karni (Krainer Lab)	Alternative splicing and cancer.
Eric Enemark (Joshua-Tor Lab)	A coordinated team of escorts with discriminating fingers promote unzipping.
Adam Kepecs (Mainen Lab)	Uncertainty in decision-making: Why is it useful and how is it represented in the rat brain?
November	
Yi-Jun Sheu (Stillman Lab)	Protein kinase docking activates the initiation of DNA replication.
Patrick Paddison	An RNAi screen for modifiers of Nanog expression in mouse embryonic stem cells.
Lee Henry	Label retaining cells in the mouse gustatory system.
December	
Julius Brennecke (Hannon Lab)	Transposon silencing in the *Drosophila* germ line: piRNAs dance flamenco.
Min Yu (McCombie Lab)	3D vs. 2D: Culture influences gene expression of mammary epithelial cells.


BANBURY CENTER

BANBURY CENTER
EXECUTIVE DIRECTOR'S REPORT

The Banbury Center Annual Report has followed the same format since the first Annual Report in 1976 (although the 1976 report was only 2 pages long compared with the 52-page 2005 report!). This year, however, the format has changed. Instead of a long introductory director's report describing the meetings, followed by the individual programs, a short description of each meeting now precedes its program.

Banbury Center continues to have a very full schedule of meetings. Even though 2006 was relatively quiet compared with the record-breaking year 2005, we nevertheless held 17 scientific meetings, a typical number. Of the 607 scientists who attended the scientific meetings, 82% came from the United States, a proportion that has remained remarkably constant in recent years. These participants came from 35 states, with California, Maryland, Massachusetts, and New York providing 45% of the total number of participants. One hundred fourteen scientists from 19 countries participated in meetings, for a total of 114. Canada, France, Germany, Sweden, and the United Kingdom provided more than 50% of these scientists. In addition, the Center was used for 15 other events, including six courses, group meetings for Laboratory scientists, two week-long courses for the Watson School of Biological Sciences, and two meetings for local nonprofit groups. Altogether, there were 32 events here in 2006.

The distribution of topics was similar to that previous years, although most of the meetings dealt with neurological and mental disorders. This reflects the intrinsic importance of these topics and the Laboratory's increasing research interest in these areas. Two meetings were held on the genetics of mental disorders (autism and bipolar disorder) and four on degenerative disorders where considerable progress has been made in understanding their molecular pathogenesis (fragile-X syndrome, spinal muscular atrophy, Parkinson's disease, and amyotrophic lateral sclerosis). In addition, neuroscience was the focus of three meetings. One reviewed the experimental analysis of neurogenesis in the adult brain, a topic of critical importance if therapies are to be developed for stroke and other forms of brain trauma. Another dealth with memory and whether recalled memories are "labile" and subject to modification before being "reconsolidated." This phenomenon may be important in assessing the validity of "recovered memories." One meeting reviewed the interactions between physiological and psychological correlates of feeding and appetite. This meeting followed up on a Banbury Center meeting in 1998

Banbury Center conference room, summer

Banbury Center conference room, winter

on obesity. At that time, leptin had just been identified and that meeting was concerned with what had been learned of the physiological basis of obesity.

There were two meetings relating to infectious diseases. The meeting on Lyme disease, one of a long series of Banbury Center meetings on the topic, reviewed the controversial issue of "chronic" Lyme disease, that is, the persistence of Lyme-disease-like symptoms in the absence of infection. The second meeting discussed the latest findings on innate, as opposed to acquired, immunity. This, it seems, is found in all multicellular organisms, and a notable feature of the meeting was that the participants worked on plants, invertebrates, and vertebrates.

The meeting on innate immunity included plants, but the Banbury plant meeting itself discussed the integration of hormonal and genetic regulation of plant development. These have been regarded as separate fields of research, but it has become clear that the two broad developmental pathways are closely linked. Participants examined the extent to which hormones regulate known genetic pathways and vice versa.

There have been repeated attempts to establish a theoretical biology that would provide a firm conceptual framework for experimental research, performing a role analogous to that of theoretical physics. Theoretical analysis and modeling have, perhaps, played a greater role in neuroscience research than in other biological disciplines, and we held a meeting that reviewed computational approaches to modeling brain function. A second meeting dealt with more general features of biological systems, examining whether organisms have employed common engineering principles in their evolution. It drew on participants from a most varied set of disciplines, including mathematics, engineering theory, and biology.

Even during a relatively quiet year, the operation of Banbury Center still requires a major effort by many people. That our meetings run so smoothly is a tribute to the hard work of Sydney Gary, Bea Toliver, Ellie Sidorenko, and Barbara Polakowski. The Center's grounds crew staff Mike Peluso and Joe Ellis keep the Banbury environment a beautiful resource for our participants. Our hectic schedule places a great burden on the Food Services and Housekeeping departments whose staffs must cope with ever-changing schedules and rapid turnarounds. I thank all of these people and the scientists at the Laboratory who continue to support the Banbury Center activities.

Jan Witkowski
Executive Director

MEETINGS

The Evolving Role of the Board-certified Medical Geneticist

February 14–16

FUNDED BY **American College of Medical Genetics and individual participants**

ARRANGED BY **B.R. Korf,** University of Alabama, Birmingham

BACKGROUND

As a result of the advances in gene mapping using, for example, single-nucleotide polymorphisms (SNPs) and genome-wide tools such as microarrays, we now know the genes underlying some 2000 human inherited disorders. However, despite this dramatic increase in our ability to use molecular techniques for diagnosis, applications of this knowledge have been limited. This discussion meeting focused on defining the role of the medical geneticist in rare and common disorders.

Introduction: **J.A Witkowski,** Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Setting the Stage

B.R. Korf, University of Alabama, Birmingham
D.H. Ledbetter, Emory University School of Medicine, Atlanta, Georgia
M.F. Murray, Harvard Medical School, Boston, Massachusetts: Welcome and overview of meeting.
J. Zonana, University of Oregon Health Science University, Portland: Roles of medical geneticists outside of the United States.
G. Feldman, Wayne State University, Detroit, Michigan: Roles of medical geneticists in the United States.
R. Bachman, Kaiser Permanente Hospital, Oakland, California: Genetic services: The HMO model.
M. Blitzer, University of Maryland: Genetic workforce study.

SESSION 2: Role of the Medical Geneticist

B.R. Korf, University of Alabama, Birmingham: Pediatrics.
M.F. Murray, Harvard Medical School, Boston, Massachusetts: Adult medicine.
J.L. Simpson, Baylor College of Medicine, Houston, Texas: Prenatal diagnosis.
M. Scheuner, RAND Corporation, Santa Monica, California: Public health.

General Discussion: Action items and planned follow-up

Research on Genetics of Bipolar Disorder: Current Approaches and Future Directions

February 22

FUNDED BY **The Stanley Foundation**

ARRANGED BY **J.D. Watson,** Cold Spring Harbor Laboratory
S. Gary, Banbury Center, Cold Spring Harbor Laboratory

BACKGROUND

This discussion meeting was held to assess the current state of research on bipolar disorder. As with many psychiatric disorders, research on the genetics of bipolar disorder has been slow to identify genes underlying the condition. Many factors contribute to this lack of progress, including the heterogeneity of the disorder and the absence of a definitive diagnostic classification scheme. The meeting focused on genetic approaches, and participants reviewed lessons learned from past collaborative efforts and discussed what is needed to establish a new collaborative project. The meeting concluded with a discussion on future experiments and the importance of sharing resources and data.

Welcome: J.D. Watson, Cold Spring Harbor Laboratory

J.R. DePaulo and J. Potash, Johns Hopkins University School of Medicine, Baltimore, Maryland: Overview of clinical aspects of bipolar disorder.

E.S. Gershon, University of Chicago, Illinois: Current status of research on genetics of bipolar disorder.

J. Sebat, Cold Spring Harbor Laboratory: ROMA analysis of autism.

Discussion of Future Research Directions

Questions for discussion

What patient groups are currently represented in the available DNA collections?

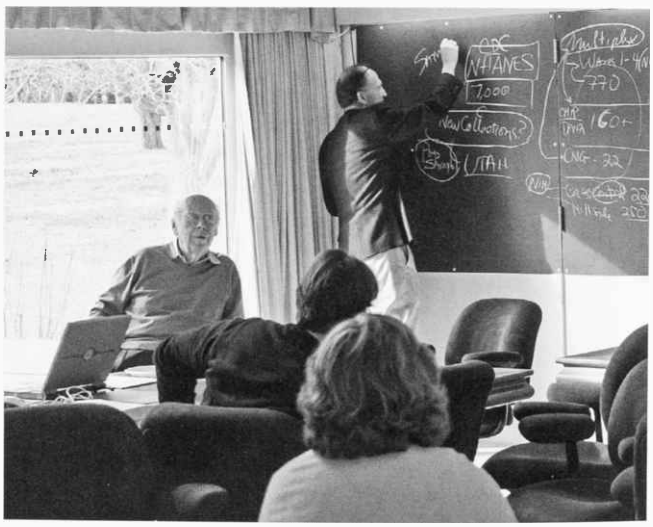
What information is important in collecting future samples?

What are the concerns and suggestions regarding overlap between bipolar and schizophrenia?

Limit analysis to sib pairs? Other approaches?

Future directions? How best to proceed?

A. Malhotra, T. Marr, E. Gershon



J. Watson, R. DePaulo

Neurogenesis in the Adult Brain

February 26–March 1

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **F. H. Gage,** The Salk Institute for Biological Studies, San Diego, California
G. Enikolopov, Cold Spring Harbor Laboratory

BACKGROUND

Research efforts on neurogenesis in the adult brain have grown substantially in recent years. Improved methods allow researchers to observe the birth and function of new neurons in the adult, leading to some exciting reports on the role of neurogenesis not only in the normal adult brain, but also in certain neurological disorders such as depression and stroke. This meeting examined such questions as What are the molecular and morphological characteristics of the cells as they transition from stem cells to mature neurons? What are the key molecular, cellular and electrophysiological mechanisms that regulate different aspects of the maturation process? Are changes in neurogenesis responsible for disease-related changes in behavior?

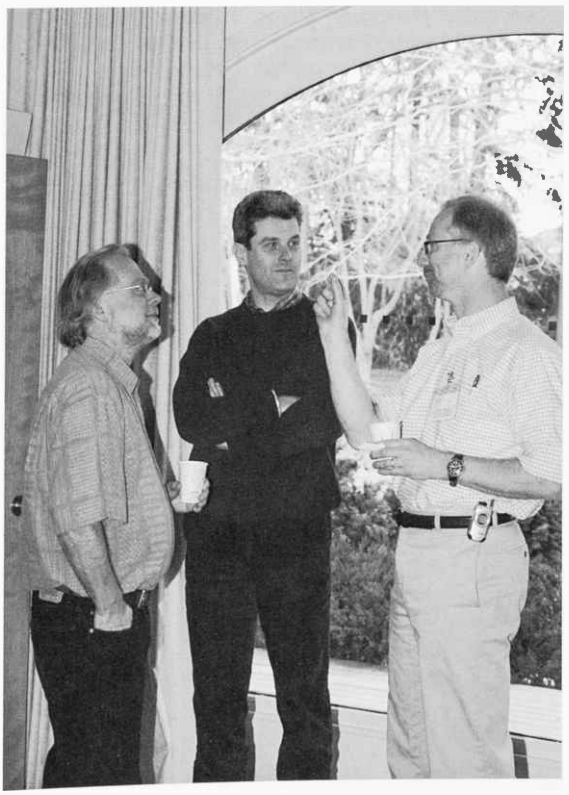
Introduction: **S. Gary,** Banbury Center, Cold Spring Harbor Laboratory
F.H. Gage, The Salk Institute for Biological Studies, San Diego, California

SESSION 1: Basic Processes of Adult Neurogenesis
Chairperson: J.D. Macklis, Harvard Medical Center, Boston, Massachusetts

D. Van Der Kooy, University of Toronto, Ontario, Canada: The origin of adult neurons from stem and progenitor cells.
G. Enikolopov, Cold Spring Harbor Laboratory: Differentiation cascade in the hippocampus.
C. Lie, Institute for Developmental Genetics, Munich, Germany: Role of Wnt signaling in hippocampal neurogenesis.
A. Schinder, Leloir Institute Foundation, Buenos Aires, Argentina: Functional convergence of neurons generated in the developing and adult hippocampus.

SESSION 2: Molecular Mechanisms Regulating Neurogenesis
Chairperson: R. McKay, National Institute of Neurological Disorders and Stroke/NIH, Bethesda, Maryland

F. Doetsch, Columbia University, New York: MicroRNA regulation of adult neurogenesis.
H.G. Kuhn, Goteburg University, Sweden: Peripheral growth factors as stimulants for adult neurogenesis.
J. Bischofberger, Physiologisches Institut der Universitat Freiburg, Germany: Calcium signaling and synaptic plasticity in newly generated hippocampal granule cells.
H. Song, Johns Hopkins University School of Medicine, Baltimore, Maryland: Molecular mechanisms regulating synaptic integration and plasticity of newly generated neurons in the adult brain.
D.A. Steindler, University of Florida Brain Institute, Gainesville: Tying together cell and molecular interactions that regulate neurogenesis.



R. Hen, G. Kempermann, J. Macklis

SESSION 3: Regulation of Adult Neurogenesis
Chairperson: P. Rakic, Yale University School of Medicine, New Haven, Connecticut

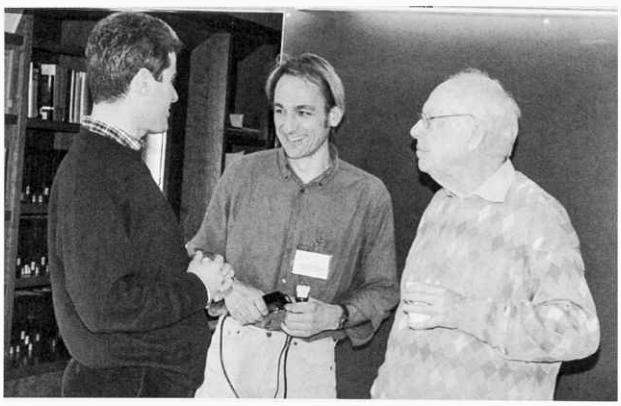
G. Kempermann, Max Delbrück Center for Molecular Medicine, Berlin, Germany: Natural variation and genetic covariance in adult hippocampal neurogenesis.
H. van Praag, Salk Institute for Biological Studies, San Diego, California: Regulation of neurogenesis by exercise in rodents.
H.A. Cameron, National Institute of Mental Health/NIH, Bethesda, Maryland: Regulation of new neuron survival by learning.
S.A. Small, Columbia University, New York: MRI correlates of neurogenesis in mice and humans.
D.N. Abrous, Institute Francois Magendie, Bordeaux, France: Comments and discussion on "environmental regulation of adult neurogenesis."

SESSION 4: Functional Significance of Adult Neurogenesis
Chairperson: F.H. Gage, The Salk Institute for Biological Studies, San Diego, California

R. Hen, Columbia University, New York: Hippocampal neurogenesis contributes to contextual fear learning and antidepressant response.
T.J. Shors, Rutgers University, Piscataway, New Jersey: Learning and neurogenesis: What is it about learning that rescues new neurons from death?
J.M. Wojtowicz, University of Toronto, Ontario, Canada: Radiation-induced inhibition of neurogenesis interferes with hippocampus-dependent memory function.
S. Jessberger, The Salk Institute for Biological Studies, La Jolla, California: Aberrant neurogenesis contributes to cognitive impairment following seizure activity.

SESSION 5: Role for Neurogenesis in Disease
Chairperson: P. Eriksson, Sahlgrenska Academy Goteburg University, Sweden

R.S. Duman, Yale University School of Medicine, New Haven, Connecticut: Neurogenic actions of antidepressants.
A. Pieper, University of Texas Southwestern Medical Center, Dallas: NPAS3 and neurogenesis in schizophrenia.
O. Lindvall, Lund University Hospital, Sweden: Neurogenesis after ischemic and epileptic insults in adult brain.
S.A. Goldman, The University of Rochester Medical Center, New York: Hb9 enhancer-based isolation and targeting of human motor neurons.



G. Kempermann, J. Bischofberger, J. Watson

S. Jessberger, F. Gage

A Critical Assessment of Autism Genetics

March 12–15

FUNDED BY **Autism Speaks; Cure Autism Now; McLaughlin Centre for Molecular Medicine; Nancy Lurie Marks Family Foundation; National Alliance for Autism Research; Simons Foundation**

ARRANGED BY **A.P. Monaco,** University of Oxford, United Kingdom
S.W. Scherer, The Hospital for Sick Children, Toronto, Canada
A.J. Bailey, University of Oxford, United Kingdom

BACKGROUND

Autism is known to have a strong genetic etiology, but even after many years of molecular genetic studies, it has been difficult to identify susceptibility genes for autism that are influencing a large majority of patients. However, there is increasing optimism that this is about to change. The new tools of genomics that, for example, enable high-density genetic marker studies of individual regions and the whole genome at reasonable cost, combined with larger families being studied, will hopefully yield new data. Participants in the meeting critically assessed the current state of research and looked for the leads that should be followed in the future.

Introduction: **S. Gary,** Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Clinical Aspects
Chairperson: A.P. Monaco, University of Oxford, United Kingdom

P. Szatmari, McMaster University, Ontario, Canada: Historical and clinical introduction.
A.J. Bailey, University of Oxford, United Kingdom: Utilizing the whole autism phenotype.
S. Baron-Cohen, University of Cambridge, United Kingdom: Hypersystemizing, assortative mating, and androgens.
D. Skuse, Institute of Child Health, London, United Kingdom: Potential X-linked mechanisms influencing susceptibility to autistic traits.
E. Fombonne, Montreal Children's Hospital, Canada: Recent trends in the epidemiology of autism and hypotheses about environmental exposures.

Key Points and Discussion: P. Szatmari, McMaster University, Ontario, Canada

SESSION 2: Whole-genome Genetic Studies
Chairperson: S.W. Scherer, The Hospital for Sick Children, Toronto, Canada

V.J. Vieland, University of Iowa, Iowa City: The incredible shrinking LOD: How increasing the sample size can actually obscure true linkage peaks, and what we can do about this.
D. Geschwind, University of California School of Medicine, Los Angeles: Approaching heterogeneity in autism: Endophenotypes and candidate pathway analysis.
I. Jarvela, University of Helsinki, Finland: Molecular genetics of Asperger syndrome, the under-diagnosed endophenotype among autism spectrum disorders.
J. Lamb, University of Oxford, United Kingdom: Gender and parent of origin affects in linkage data from IMGSAC.
G.D. Schellenberg, Seattle V.A. Medical Center, Washington: Recently completed genome scan of 225 families.
A. Chakravarti, Johns Hopkins University School of Medicine, Baltimore, Maryland: A linkage and association scan for autism genes.

Key Points and Discussion: M.A. Pericak-Vance, Duke University Medical Center, Durham, North Carolina

SESSION 3: Candidate Genes and Regional Association Studies
Chairperson: A.J. Bailey, University of Oxford, United Kingdom

J. Sutcliffe, Vanderbilt University, Nashville, Tennessee: Autism susceptibility and dysregulation of serotonin.
J. Lamb, University of Oxford, United Kingdom, and E. Maestrini, University of Bologna, Italy: A high-density SNP association study of the autism susceptibility loci on chromosomes 2q24-q32 and 7q21-q33.
J. Buxbaum, Mt. Sinai School of Medicine, New York: Chromosome 2q in autism.
A.L. Beaudet, Baylor College of Medicine, Houston, Texas: A mixed genetic and epigenetic, and mixed de novo and inherited model for autism.
T. Bourgeron, Institut Pasteur, Paris, France: Autism spectrum disorders and synaptic plasticity.

Key Points and Discussion: M. Gill, St. James' Hospital, Dublin, Ireland

SESSION 4: Chromosomal Abnormalities/Copy-number Variants
Chairperson: M.A. Pericak-Vance, Duke University Medical Center, Durham, North Carolina

C. Lee, Brigham and Women's Hospital, Boston, Massachusetts: Array-based comparative genomic hybridization and copy-number variation in the human genome.
J. Sebat, Cold Spring Harbor Laboratory: A high-resolution scan for genome copy-number variation in autism.
E.E. Eichler, University of Washington, Seattle: Structural variation, mental retardation, and autism.
S.W. Scherer, The Hospital for Sick Children, Toronto, Canada: Cytogenomic investigations into the molecular etiology of autistic spectrum disorder.
N.C. Schanen, University of Delaware, Wilmington: Autism spectrum disorders in duplication chromosome 15 syndrome.
S. Muthuswamy, Cold Spring Harbor Laboratory: CNP allelic imbalance in autism.

Key points and discussion: G.D. Schellenberg, Seattle V.A. Medical Center, Washington

SESSION 5: Statistical Genetics
Chairperson: V.J. Vieland, University of Iowa, Iowa City

B. Devlin, University of Pittsburgh School of Medicine, Pennsylvania: Linkage genome scan for the autism genome project.
R.M. Cantor, University of California School of Medicine, Los Angeles: Evidence of gene on 17q contributing to autism in males.
E.M. Wijsman, University of Washington School of Medicine, Seattle: Multilocus models and analysis of component phenotypes.
M. Daly, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Genome-wide association prospects in the face of the great genetic complexity of autism.

Key points and discussion: J.L. Haines, Vanderbilt University Medical Center, Nashville, Tennessee

Summary: A.P. Monaco, University of Oxford, United Kingdom

The Phenomenology of Reconsolidation

March 26–29

FUNDED BY **Volkswagen Foundation and Marie Robertson Memorial Fund**

ARRANGED BY **Y. Dudai,** Weizmann Institute of Science, Rehovot, Israel
B. Everitt, University of Cambridge, United Kingdom
K. Nader, McGill University, Montreal, Canada

BACKGROUND

We would all like to know more about our memories. How is it that our brains retain just a few memories out of the myriad events that we experience each moment of the day, and how is it that some of these memories are kept for decades? It seems that newly formed memory remains in a dynamic or "labile" form for a short time after which the memory trace is stabilized or "consolidated." During the past several years, it has emerged that after a memory undergoes consolidation, it may return to a labile state under certain circumstances, requiring it to be reconsolidated. There are important theoretical and practical implications of these findings, for example, with respect to a variety of mental illnesses, such as posttraumatic stress disorder, phobia, and drug addiction.

Welcome: S. Gary, Banbury Center, Cold Spring Harbor Laboratory

History of the Phenomenon: S.J. Sara, Université Pierre & Marie Curie, Paris, France

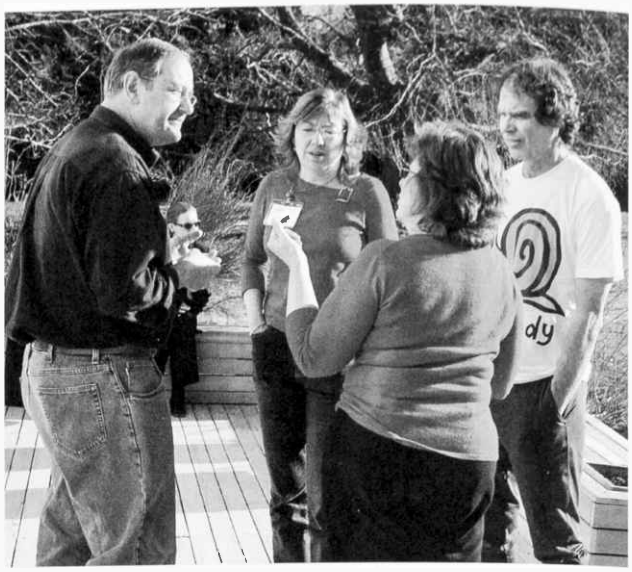
SESSION 1: The Nature of Amnesia
Chairperson: S.J. Sara, Université Pierre & Marie Curie, Paris, France

Topic 1: Why isn't there a resolution to the nature of amnesia? What are the issues?

D. Riccio, Kent State University, Ohio: Reconsolidation: A bit of history, a bit of (contrarian?) theory.
P.E. Gold, University of Illinois, Urbana-Champaign: Memories and amnesias.

Topic 2: New empirical approaches to the issue

L. de Hoz, Medical University Charite, Berlin, Germany: Memory loss and reminding in the spatial domain.
O. Hardt, University of Arizona, Tucson: A novel approach to identifying the nature of amnesia.



P. Gold, C. Rankin, S. Sara, K. Lukowiak

SESSION 2: Boundary Conditions on Reconsolidation and Other New Findings
Chairperson: M. Walker, Harvard Medical School, Boston, Massachusetts

Brief Presentations
Extinction
Strength of Training
Directly Reactivated versus Indirectly Reactivated Memories
Age of Memories
New Findings in Reconsolidation

SESSION 3: Reconsolidation Across Species and Tasks
Chairperson: K.P. Giese, University College of London, United Kingdom

M. Walker, Harvard Medical School, Boston, Massachusetts: Role of sleep in memory consolidation and reconsolidation.
S. Davis, CNRS and University Paris Sud, France: Cellular and molecular mechanisms of consolidation and reconsolidation.
B.W. Balleine, University of California, Los Angeles: Reconsolidation of appetitive memories.
K. Lukowiak, University of Calgary, Alberta, Canada: Reconsolidation and a well-rehearsed memory.
K. Anokhin, Russian Academy of Medical Sciences, Moscow: Reconsolidation and memory recovery in mice and chicks: A comparative perspective.

SESSION 4: Molecular and Cellular Mechanisms
Chairperson: B.S. Everitt, University of Cambridge, United Kingdom

R. Fonseca, Julio de Matos Hospital, Lisbon, Portugal: Neuronal activity determines the protein synthesis dependence of L-LTP.
C. Rankin, University of British Columbia, Vancouver, Canada: Blocking memory reconsolidation in *C. elegans* reverses behavioral expression of memory as well as establishes changes in glutamate receptor expression.
S. Kida, Tokyo University of Agriculture, Japan: Mechanisms of interaction between memory reconsolidation and extinction.
K.P. Giese, University College of London, United Kingdom: Reconsolidation as partial recapitulation of consolidation.
J.L.C. Lee, University of Cambridge, United Kingdom: The molecular mechanisms of fear memory consolidation and reconsolidation are doubly dissociable.
C. Albernini, Mt. Sinai School of Medicine, New York: Why does memory undergo reconsolidation? A working hypothesis from studies on inhibitory avoidance memory.
K. Nader, McGill University, Quebec, Canada: A neural manifestation of the overtraining boundary conditions on reconsolidation.

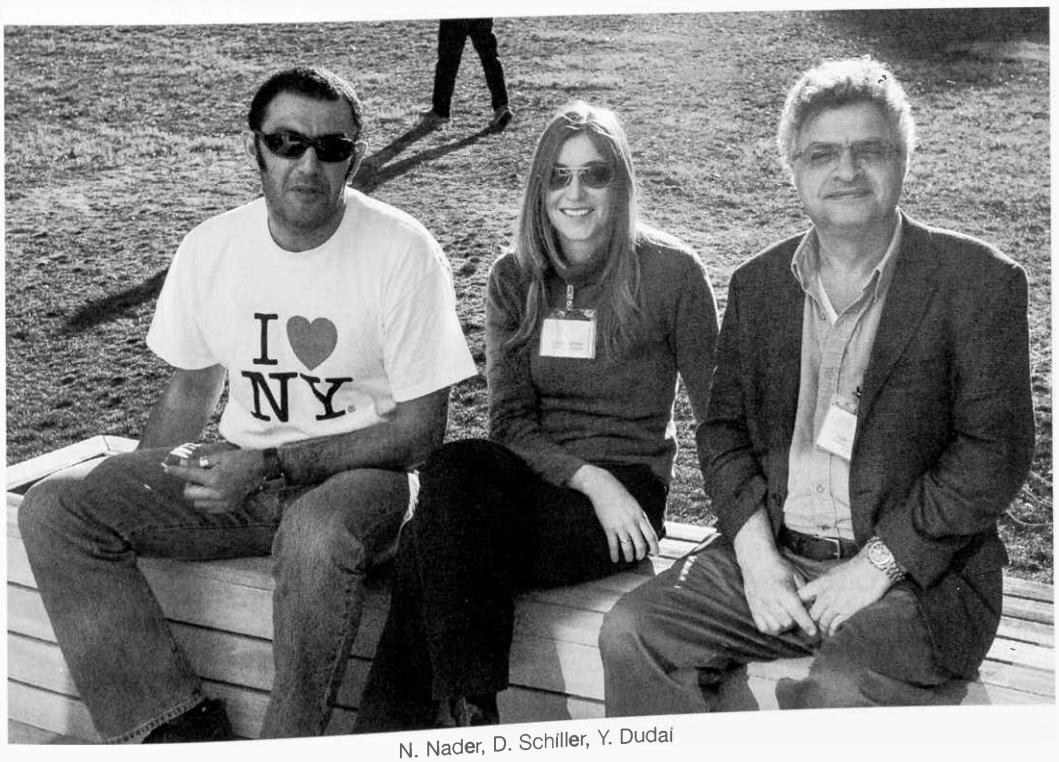
SESSION 5: Reconsolidation in Normal and Psychopathological Memories
Chairperson: L. Nadel, University of Arizona, Tucson

S.J. Sara, Universite Pierre and Marie Curie, Paris, France: Learning-related activation of locus coeruleus during slow wave sleep: A new player on the reactivation scene?
M. Altemus, Cornell University, New York: Fear conditioning in healthy humans: Effects of propranolol.
J. Gorman, McLean Hospital, Belmont, Massachusetts: The significance of reconsolidation of fear memories for the treatment of anxiety disorders.
E. Phelps, New York University: Reconsolidation in humans? Specific challenges.
R.K. Pitman, Harvard Medical School, Charlestown, Massachusetts: Translational model of PTSD updated: Role of reconsolidation and treatment implications.
K. Myers, Emory University, Atlanta, Georgia: Extinction shortly following fear acquisition may erase conditioned fear.
B.S. Everitt, University of Cambridge, United Kingdom: Memory reconsolidation as a therapeutic target in drug addiction.

SESSION 6: Interpretations and New Directions
Chairperson: B.S. Everitt, University of Cambridge, United Kingdom

L. Nadel, University of Arizona, Tucson: Reconsolidation: Challenging fundamental concepts of memory.
Y. Dudai, The Weizmann Institute of Science, Rehovot, Israel: Reconsolidation: Taping into trace persistence and use.

Group Discussion
Mediator: Y. Dudai, The Weizmann Institute of Science, Rehovot, Israel



N. Nader, D. Schiller, Y. Dudai

Computational Approaches to Cortical Functions

April 2–5

FUNDED BY **The Swartz Foundation**

ARRANGED BY **L.F. Abbott,** Columbia University, New York
H. Cohen, The Swartz Foundation, Scarborough, New York
R.M. Shapley, New York University

BACKGROUND

The meeting focused on the dynamics of large-scale computational models of the cerebral cortex, including both theoretical and experimental results concerning large-scale neural systems. Participants discussed modeling requirements at the synaptic, neuronal, and circuit levels, as well as the effects of network size and scaling rules. Other topics covered included propagation and gating of signals, self-sustained oscillations and reverberatory states, spike and local field relationships, chaotic activity, and other phenomena exhibited by these models and their experimental counterparts.

Introduction: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory

SESSION 1

X.-J. Wang, Brandeis University, Waltham, Massachusetts: Neural mechanisms of feature-based attention in a two-network model of sensory processing.
W. Maass, Technical University of Graz, Austria: A model for computation in cortical microcircuits.
O. White, Massachusetts Institute of Technology, Cambridge: Signal reconstruction from recurrent neuronal networks.
K. Rajan, College of Physicians & Surgeons, New York: Controlling neural networks dynamics.
A. Aersten, Albert Ludwigs Universitat, Freiburg, Germany: Variability and precision in cortical networks.

SESSION 2

C. Geisler, Rutgers University, Newark, New Jersey: Behavior-dependent phase rescaling of hippocampal pyramidal cells.
T. Vogels, Columbia University, New York: Signal propagation and switching in networks.
M. Diesmann, Albert-Ludwigs University, Freiburg, Germany: Spike-timing-dependent plasticity in balanced random networks.
B. Pesaran, New York University: Free choice increases synaptic interactions between frontal and parietal cortex.
R. Yuste, Columbia University, New York: Internal dynamics determine the cortical response to thalamic stimulation.

SESSION 3

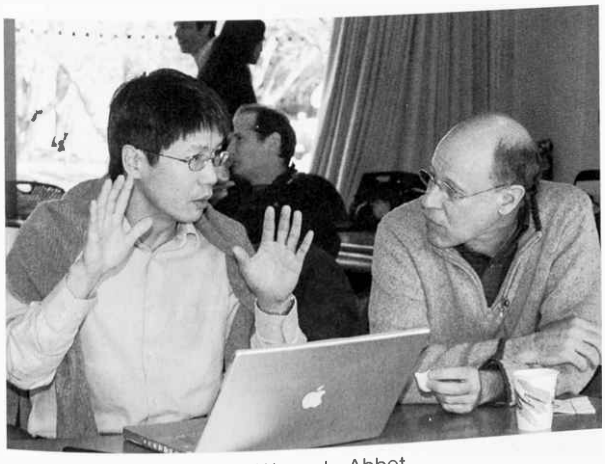
D. Cai, New York University: Spontaneous cortical activity in V1.
M.V. Tsodyks, Weizmann Institute of Science, Rehovot, Israel: Information encoding and processing via spatiotemporal patterns in cortical networks.
C. van Vreeswijk, Rene Descartes University, Paris, France: Role of dendritic shunting inhibition.
W. Gerstner, Ecole Polytechnique Federale de Lausanne, Switzerland: Ultra-short-term information buffering in a random network of spiking neurons: Macroscopic versus microscopic effects.
B. Knight, The Rockefeller University, New York: Neuron population dynamics in a simple canonical form.

SESSION 4

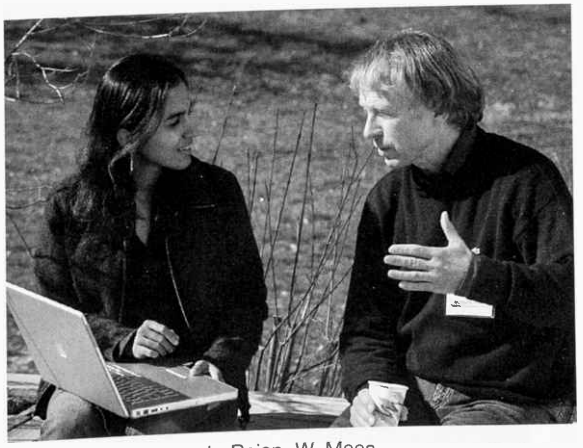
D. Tranchina, New York University: Try to save computation time in large-scale neural network modeling with population density methods, or just fuhgeddaboudit?
R.D. Traub, SUNY Downstate Medical Center, Brooklyn, New York: Gap junctions and fast oscillations in hippocampus and neocortex.
R.M. Shapiro, Cape Visions, Inc. New York, New York: Integrate and fire neural network: VLSI chip design.
A. Henrie, University of California, Los Angeles: Coherence of activity in primate V1.
D. Plenz, National Institute of Mental Health/NIH, Bethesda, Maryland: Neuronal avalanches in superficial layers of neocortex.

SESSION 5

M.J. Shelley, New York University: V1 dynamics and sparsity and multiple feature maps.
B. Murphy, University of California, San Francisco: Spontaneous activity and orientation maps in balanced cortical networks with structured V1-like connectivity.
A. Rangan, New York University: Cortical correlates underlying the line-motion-illusion phenomenon in primary visual cortex.
T. Thiagarajan, National Institute of Mental Health/NIH, Bethesda, Maryland: Precise propagation of initial neuronal group activity in neuronal avalanches in cortex.



X.-Y. Wang, L. Abbot



L. Rajan, W. Maas

Fragile-X Syndrome: Basic Mechanisms and Treatment Implications

April 9–12

FUNDED BY **NIH–National Institute of Mental Health (through a grant to the University of Illinois)**

ARRANGED BY **W.T. Greenough,** University of Illinois, Urbana
B.A. Oostra, Erasmus Universiteit Rotterdam, The Netherlands
E. Berry-Kravis, Rush University Medical Center, Chicago, Illinois
K. Clapp, FRAXA Research Foundation, Newburyport, Massachusetts

BACKGROUND

This meeting was the seventh in a series of annual Banbury Center conferences on fragile X, although fragile X was discussed at Banbury long before then, in the early days of applying recombinant DNA techniques to mapping and cloning genes involved in inherited disorders. This meeting took a broad perspective on fragile X, reviewing what is known of *FMR1*/RNA interactions, FMRP cellular biology and the proteins that interact with it, synaptic physiology, and how fragile X affects the nervous system in animal models and humans. A final session discussed progress in the all-important task of developing treatments.

Introduction: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Basic Mechanisms of the *FMR1* Gene/RNA Interactions/Cargo RNAs
Chairperson: B.A. Oostra, Erasmus Universiteit Rotterdam, The Netherlands

J.R. Fallon, Brown University, Providence, Rhode Island: Axonal FMRP in the developing vertebrate brain.

J. Darnell, The Rockefeller University, New York: G-quadruplex and kissing complex RNA ligands of the fragile-X family of RNA-binding proteins.

S. Ceman, University of Illinois, Urbana: Identification and characterization of the methyl arginines in the fragile-X mental retardation protein.

B. Bardoni, Faculte de Medecine, Nice, France: FMRP and RNA: Old and novel friends.

Y. Feng, Emory University, Atlanta, Georgia: Translation regulation by FMRP during neuronal development.

SESSION 2: FMRP Interacting Proteins and FMRP Cellular Biology Including Transport, Signal Transduction, and Mechanisms of Translational Regulation
Chairperson: S.T. Warren, Emory University School of Medicine, Atlanta, Georgia

G.J. Bassell, Emory University, Atlanta, Georgia: The stimulating travels and function of FMRP.
P.W. Vanderklish, Scripps Research Institute, La Jolla, California: Novel physiological and proteomic characterization of the *Fmr1* KO mouse.
M. Hayashi, Massachusetts Institute of Technology, Cambridge: A physical and functional interaction between p21-activated kinase and the fragile-X mental retardation protein.
M.V. Catania, Institute of Neurological Sciences, Catania, Italy: mGlu5 receptor expression and interaction with Homer proteins in a mouse model of FRAXA syndrome.
I.J. Weiler, University of Illinois, Urbana-Champaign. FMRP: Some aspects of translational control.
H. Cline, Cold Spring Harbor Laboratory: Control of experience-dependent structural plasticity by local protein synthesis.
E. Klann, Baylor College of Medicine, Houston, Texas: Translational and proteasomal regulation of FMRP during hippocampal mGluR-LTD.

SESSION 3: Synaptic Physiology and Function of FMRP Including Effects on LTP/LTD, Glutamate, and GABA Activity
Chairperson: W.T. Greenough, University of Illinois, Urbana

O. Steward, University of California, Irvine: Translation of mRNA at synapses in *Fmr1* knockout mice.
K.M. Huber, University of Texas Southwestern Medical Center, Dallas: Role for FMRP in synaptic function and plasticity.
M.F. Bear, Massachusetts Institute of Technology, Cambridge: Tests of the mGluR theory.
S. Chattarji, National Centre for Biological Sciences, Bangalore, India: mGluR- and NMDAR-dependent synaptic plasticity: A contrasting view from the amygdala.
B.A. Oostra, Erasmus Universiteit Rotterdam, The Netherlands: Cerebellar function and the FMR1 knockout mouse
C.L. Cox, University of Illinois, Urbana: Altered synaptic plasticity in sensory neocortex associated with FMRP knockout.
J.R. Larson, University of Illinois, Chicago: Age-dependent impairment of LTP in olfactory cortex of mice lacking FMRP.

SESSION 4: Brain Phenotypes Mediated by FMRP Including Dendritic Morphology, Neural Networks/Seizure Generation, Learning, and Behavioral Measures in FXS Models
Chairperson: J. Darnell, The Rockefeller University, New York

W.T. Greenough, University of Illinois, Urbana: Spine/synapse phenotype: Context-dependent expression.
I. Bureau, Cold Spring Harbor Laboratory: Defects of functional and anatomical connectivity in the barrel cortex of *fmr1* KO mice.
R.K.S. Wong, SUNY–Health Science Center, Brooklyn, New York: Cellular mechanisms of mGluR-induced epileptogenesis.
M. Zhuo, University of Toronto, Ontario, Canada: Synaptic and behavioral studies of prefrontal cortical potentiation in a mouse model for fragile X.
R.E. Paylor, Baylor College of Medicine, Houston, Texas: Behavior of *Fmr1* KO mice: Genetic interactions.
C.B. Smith, National Institute of Mental Health/NIH, Bethesda, Maryland: In vivo studies of regional brain metabolism in the fragile-X mouse.
D.L. Nelson, Baylor College of Medicine, Houston, Texas: Circadian derangement in *Fmr1/Fxr2* knockout mice: A role for FMR1 in clock control?

SESSION 5: A Look at Progress in Moving toward the Goal of Treatment in Humans
Chairperson: M.R. Tranfaglia, FRAXA Research Foundation, Newburyport, Massachusetts

S.T. Warren, Emory University School of Medicine, Atlanta, Georgia: A 2000-compound drug screen using *dFmr1*-deficient *Drosophila*.
G. Bilbe, Novartis Institutes for BioMedical Research, Basel, Swtizerland: Exploratory therapeutic approaches to FRX.
F. Gasparini, Novartis Pharma AG, Basel, Switzerland: Identification and characterization of PET-imaging ligands for the mGlu5 receptor.
W. Spooren, Hoffmann-La Roche Ltd., Basel, Switzerland: Fenobam, a clinically validated non-benzodiazepine anxiolytic, is a potent, selective, and noncompetitive mGlu5 receptor antagonist with inverse agonist activity.
E. Berry-Kravis, Rush University Medical Center, Chicago, Illinois: Progress in clinical trial design for FXS treatment trials.
S.C. Landis, National Institute of Neurological Disorders and Stroke/NIH, Bethesda, Maryland: Brief comments about importance of fragile X from NIH's perspective.

Appetite and Feeding

April 30–May 3

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **J. Friedman,** The Rockefeller University, New York
S. O'Rahilly, University of Cambridge, United Kingdom

BACKGROUND

There is a complex interaction between physiology and psychology in determining when and how much we eat. Biochemical and physiological processes regulate food intake, but this is modified by psychological stimuli relating to the senses of taste, smell, and sight. These studies involve researchers in diverse areas of study and one of the goals of the meeting was to bring together these investigators who may not otherwise meet. The success of the meeting was evident from the range of organisms and topics discussed. These included studies of round worms, fruit flies, snails, and human beings and of smell and taste. One talk was titled "Disgust Discussed"!

Introduction: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Studies in Invertebrates
Chairperson: E.J. Nestler, University of Texas Southwestern Medical Center, Dallas

C.I. Bargmann, The Rockefeller University, New York: Food quality and the regulation of food preference.
G. Ruvkun, Massachusetts General Hospital, Boston: Functional genomic analysis of *C. elegans* fat control.
L. Vosshall, The Rockefeller University, New York: Genetic control of olfactory perception in *Drosophila*.
R. Gillette, University of Illinois, Urbana-Champaign: Neural circuits integrating appetite, sensation, and experience in foraging decisions.

SESSION 2: Feeding in Vertebrates
Chairperson: G. Barsh, HHMI/Stanford University School of Medicine, California

M.W. Schwartz, Harborview Medical Center, Seattle, Washington: Integration of homeostatic and satiety signals in food intake regulation.
J.S. Flier, Beth Israel Deaconess Medical Center, Boston, Massachusetts: Hypothalamic neurogenesis and the regulation of energy balance.
J.K. Elmquist, University of Texas Southwestern Medical Center, Dallas: CNS pathways underlying coordinated body weight and glucose homeostasis.
E.J. Nestler, University of Texas Southwestern Medical Center, Dallas: Regulation of brain reward regions by feeding peptides in animal models of addiction and depression.
M.A. Cowley, Oregon Health and Science University, Portland: Cannabinoid antagonists regulate energy balance through the ventral tegmental area, not the melanocortin circuits.

SESSION 3: Feeding in Vertebrates
Chairperson: J.S. Flier, Beth Israel Deaconess Medical Center, Boston, Massachusetts

R. Cone, Oregon Health Sciences University, Portland, Oregon: Genetics of energy homeostasis in a vertebrate model system.
G. Barsh, HHMI/Stanford University School of Medicine, California: Transcriptional regulation of Agrp expression.
E. Maratos-Flier, Beth Israel Deaconess Medical Center, Boston, Massachusetts: Appetite regulation and drug sensitization: Role of melanin-concentrating hormone in regulating dopaminergic tone.
H. Grill, University of Pennsylvania, Philadelphia: The neural control of feeding and energy expenditure is distributed with nodes in hypothalamus and caudal brainstem.
A.E. Kelley, University of Wisconsin, Madison: Energy, action, and reward: Neural control of food motivation.
B.E. Levin, V.A. Medical Center, East Orange, New Jersey: Metabolic sensing neurons and the control of feeding: The final common pathway to obesity.

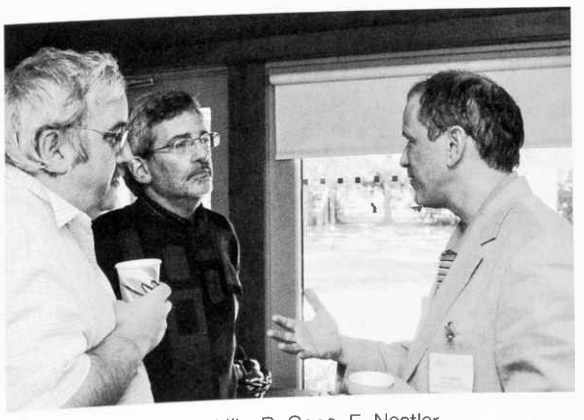
SESSION 4: Taste, Smell, and Gut Hormones
Chairperson: S. O'Rahilly, University of Cambridge, United Kingdom

U. Boehm, University Hamburg, Germany: Feedback loops link odor and pheromone signaling with reproduction.
M. Zoller, Senomyx, Inc., La Jolla, California: Using human taste receptors to discover novel taste modulators.
S.R. Bloom, Imperial College London, United Kingdom: Mouth, bowel, and brain—Are they connected?
J.M. Friedman, HHMI/The Rockefeller University, New York: Leptin and the neural circuit regulating appetite.

SESSION 5: Reward, Addiction, Disgust, and Human Studies
Chairperson: J.M. Friedman, HHMI/The Rockefeller University, New York

E. Rolls, University of Oxford, United Kingdom: Neurophysiological and fMRI analyses of the processing of taste and olfactory information in cortical areas related to the control of appetite.
A. Calder, MRC Cognition and Brain Science Unit, Cambridge, United Kingdom: Disgust discussed.
A. Del Parigi, Pfizer Global R&D, Groton, Connecticut: Neuroimaging markers of eating behavior in humans.
S. O'Rahilly, University of Cambridge, United Kingdom: Genetics and human eating behavior.

A. Calder, E. Rolls



S. O'Rahilly, R. Cone, E. Nestler

Design Principles In Biological Systems

May 7–10

FUNDED BY **The Dart Foundation**

ARRANGED BY **P.P. Mitra,** Cold Spring Harbor Laboratory
J. Doyle, California Institute of Technology, Pasadena
R.M. Murray, California Institute of Technology, Pasadena

BACKGROUND

Living organisms have been conventionally studied following a reductionist strategy, using the tools of genetics, biochemistry, and cell biology. However, there are also likely to be more general principles involved, and a new theoretical biology is currently taking shape with more formal emphasis on design or engineering principles. For this research program to succeed, biology researchers will need to be trained in the concepts and mathematics relevant to engineering theories, and engineering theorists and physical scientists need training in biological problems. This meeting was a bold start to achieving these goals.

Introduction: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Control Theory
Chairperson: M.E. Csete, Emory Healthcare, Atlanta, Georgia

R.M. Murray, California Institute of Technology, Pasadena: Tutorial.

Examples: Cellular Networks
M. Khammash, University of California, Santa Barbara: Feedback control and heat shock response in bacteria.
M.A. Savageau, University of California, Davis: Feedback control in cellular metabolism.

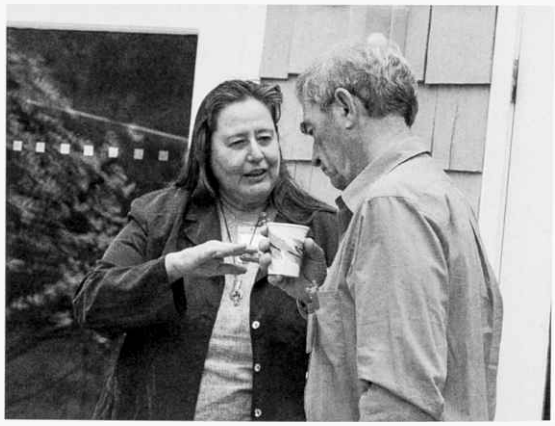
Examples: Insect Locomotion
M.H. Dickinson, California Institute of Technology, Pasadena: Control mechanisms in insect flight.
P. Holmes, Princeton University, New Jersey: Dynamics and control in insect walking.

SESSION 2: Control Theory
Chairperson: B. Bamieh, University of California, Santa Barbara

Examples: Motor Control
S. Massaquoi, Massachusetts of Technology, Cambridge: Cerebellum as feedback controller.
D. Kleinfeld, University of California, San Diego: Active sensation in the rat vibrissae system.

Examples: Brain Architecture/Evolution
H.J. Karten, University of California, San Diego: Architectural principles of vertebrate brains and feedback control.
C.C. Hilgetag, International University Bremen, Germany: Feedback or feedforward? Analysis of visual cortical circuitry.

H. Samad, M. Kirschner



L. Caporale, H. Karten

SESSION 3: Control Theory—Human Applications
Chairperson: J.E. Spiro, Nature Publishing Group, New York

D. Prelec, Institute for Advanced Study, Princeton, New Jersey: Feedback control and economics.

Discussion
Leader: H. El-Samad, University of California, San Francisco

SESSION 4: Distributed Control and Games
Chairperson: H. Breiter, Massachusetts General Hospital and Harvard Medical School, Charlestown

M. Kearns, University of Pennsylvania, Philadelphia: Tutorial: Game theory
B. Bamieh, University of California, Santa Barbara: Tutorial: Distributed control.

Examples: Evolution of Design
M.W. Kirschner, Harvard Medical School, Boston, Massachusetts: Facilitated variation.
M. Levine, University of California, Berkeley: Genomic architecture.

SESSION 5: Communication Theory
Chairperson: S.K. Mitter, Massachusetts Institute of Technology, Cambridge

P. Mitra, Cold Spring Harbor Laboratory: Tutorial.

Examples: Animal Communication Systems
P. Narins, University of California, Los Angeles: Time and frequency division multiplexing in frog communication.
T. Fitch, University of St. Andrews, Fife, Scotland: Animal source codes: Vocal adaptations in animals.

Examples: Linguistic Communications
P. Niyogi, University of Chicago, Illinois: Human language as a communication system.

SESSION 6: Communication Theory—Human Applications
Chairperson: L.H. Caporale, Columbia University, New York

J. Doyle, California Institute of Technology, Pasadena: Comparing human technologies and biological systems.

Discussion
Leader: A. Sengupta, Rutgers, The State University of New Jersey

SESSION 7: Computation
Chairperson: S. Basu, National Science Foundation, Arlington, Virginia

Examples: Molecular Circuits
E. Winfree, California Institute of Technology, Pasadena: Automata and algorithms: Examples from in vitro biochemistry.
B. Mishra, New York University: Tutorial: Theory of computation.
H.S. Seung, Massachusetts Institute of Technology, Cambridge: Examples of automata in neural systems.
C.D. Smolke, California Institute of Technology, Pasadena: Examples from cellular biochemistry.

Recap and future planning
P.P. Mitra, Cold Spring Harbor Laboratory
J. Doyle, California Institute of Technology, Pasadena
R.M. Murray, California Institute of Technology, Pasadena

Parkinson's Disease: Insights from Genetic and Toxin Models

May 14–17

FUNDED BY **The Thomas Hartman Foundation for Parkinson's Research**

ARRANGED BY **N. Bonini,** University of Pennsylvania, Philadelphia
S.E. Przedborski, Columbia University, New York

BACKGROUND

Neither the cause nor the mechanism by which neurons degenerate in Parkinson's disease are completely understood. However, scientists now have experimental models developed through gene manipulation and the use of toxins in organisms as varied as primates, rodents, roundworms, and yeast. Cell culture and cell-free systems are also available for investigation of specific aspects of Parkinson's disease. Unfortunately, none of these models replicate all aspects of the disorder and this meeting took up the challenge of deciding how to choose the best model for a given question.

Introduction: **S. Gary,** Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Primate Models
Chairperson: P. Aebischer, Swiss Federal Institute of Technology, Lausanne, Switzerland

E. Bezard, Universite Victor Segalen-Bordeaux, France: Neuroprotection for Parkinson's disease: Call for clinically driven experimental design in animal models.
J.H. Kordower, Rush University Medical Center, Chicago, Illinois: Use of aged and MPTP-treated monkeys to study PD pathogenesis and experimental therapeutics.
D. Gash, University of Kentucky College of Medicine, Lexington: Toxin-induced and age-associated parkinsonism in rhesus monkeys.

S. Przedborski, M. Beal

SESSION 2: Rodent Toxin Model
Chairperson: R. McKay, National Institute of Neurological Disorders and Stroke/NIH, Bethesda, Maryland

R.S. Betarbet, Emory University School of Medicine, Atlanta, Georgia: The rotenone model and converging mechanisms in Parkinson's disease.
D.A. Di Monte, The Parkinson's Institute, Sunnyvale, California: Modeling environmental risk factors for Parkinson's disease.
S.E. Przedborski, Columbia University, New York: The MPTP mouse model of PD.
M.F. Beal, Cornell University, New York: Testing novel therapies in the MPTP model of PD.
P.K. Sonsalla, UMDNJ–RWJMS, Piscataway, New Jersey: Acute and chronic administration of MPP+: Models of Parkinson's disease.
M.J. Zigmond, University of Pittsburgh School of Medicine, Pennsylvania: Endogenous mechanisms of neuroprotection in cellular and animal models of Parkinson's disease.

SESSION 3: Rodent Models: Genetic
Chairperson: M.F. Beal, Cornell University, New York

J. Shen, Harvard Medical School, Boston, Massachusetts: Insights from multidisciplinary analysis of parkin, DJ-1, and PINK1 knockout mice.
X.W. Yang, David Geffen School of Medicine at University of California, Los Angeles: BAC transgenic mouse models of neurodegeneration.
P. Aebischer, Swiss Federal Institute of Technology, Lausanne, Switzerland: Use of viral vectors to create genetic models of Parkinson's disease.
T.C. Sudhof, University of Texas Southwestern Medical Center, Dallas: Toward a functional understanding of synucleins.
R.H. Edwards, University of California, San Francisco: The dynamics of α-synuclein at the nerve terminal.

SESSION 4: Invertebrate Models and Stem Cells
Chairperson: T.C. Sudhof, University of Texas Southwestern Medical Center, Dallas

N. Bonini, University of Pennsylvania, Philadelphia: Insights from *Drosophila* models for Parkinson's disease.
R. Nass, Vanderbilt University Medical Center, Nashville, Tennessee: Pharmacogenetic analysis in a novel model of Parkinson's disease: Identification of genetic and chemical modulators of dopamine neuron degeneration in *C. elegans*.
P. Muchowski, University of California, San Francisco: Genetic dissection of α-synuclein toxicity using yeast.
R. McKay, National Institute of Neurological Disorders and Stroke/NIH, Bethesda, Maryland: Nigro-striatal disease and the origin of dopamine neurons.
L. Studer, Memorial Sloan-Kettering Cancer Center, New York: Human embryonic stem cells.

SESSION 5: Cell and Cell-free Systems
Chairperson: D. Di Monte, The Parkinson's Institute, Sunnyvale, California

L.A. Greene, Columbia University, New York: Why neurons die in PD: Insights from a cellular model of the disease.
D. Sulzer, Columbia University, New York: Regulation of cytosolic dopamine in the SN.
P.T. Lansbury, Brigham and Women's Hospital, Cambridge, Massachusetts: Discovering new targets for old drugs: Parkinson's disease therapy in *Drosophila*.
M. Cookson, National Institutes of Health, Bethesda, Maryland: Contribution of kinase activity to the cellular phenotypes of LRRK2 mutants.
A. Maria Cuervo, Albert Einstein College of Medicine, Bronx, New York: Selective autophagy in the pathogenesis of PD.

Summary
S. E. Przedborski, Columbia University, New York
N. Bonini, University of Pennsylvania, Philadelphia
M.J. Zigmond, University of Pittsburgh School of Medicine, Pennsylvania

Spinal Muscular Atrophy: From RNA To Synapses

September 17–19

FUNDED BY **Spinal Muscular Atrophy Foundation**

ARRANGED BY **T.M. Jessell,** Columbia University, New York
C.E. Henderson, Columbia University, New York
C. Joyce, Spinal Muscular Atrophy Foundation, New York

BACKGROUND

Spinal muscular atrophy is another topic that has been covered in several Banbury Center meetings and it is a further example of how intensive research using the tools of modern experimental biology is leading to a detailed understanding of the molecular and cellular basis of the disorder. Sessions reviewed the latest progress made in analysis of the two survival motor neuron genes and the role of its encoded protein in processing and transport of mRNAs in neurons.

Introduction: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory

Welcome and Introductions: C.E. Henderson, Columbia University, New York, and **D. Singh,** Spinal Muscular Atrophy Foundation, New York

SMA Overview

D.C. De Vivo, The Neurological Institute, Columbia University Medical Center, New York: SMA clinical features.

K.H. Fischbeck, National Institute of Neurological Disorders and Stroke/NIH, Bethesda, Maryland: SMA research overview.

SESSION 1: RNA in Neurons

Chairpersons: K.C. Martin, Semel Institute for Neuroscience and Human Behavior, University of California, Los Angeles: Local translation at the synapse during synapse formation and neuronal plasticity; **S.R. Jaffrey,** Weill Medical College, Cornell University, New York: Intra-axonal mRNA translation and neuronal survival.

G.J. Bassell, Emory University, Atlanta, Georgia: Interactions of the SMN complex with localized mRNPs in neuronal processes and growth cones.

G. Dreyfuss, HHMI/University of Pennsylvania School of Medicine, Philadelphia: The SMN complex: Molecular functions and screening for small molecules that affect its expression.

C.E. Beattie, The Ohio State University, Columbus: A zebra fish model of the human motoneuron disease.

A. Krainer, Cold Spring Harbor Laboratory: Strategies for increasing inclusion of SMN2 exon 7.

Discussants: A. MacKenzie, Children's Hospital of Eastern Ontario, Canada; **C.E. Beattie,** The Ohio State University, Columbus; **U.R. Monani,** Columbia University Medical Center, New York

SESSION 2: The Axon and Synapse

Chairpersons: B.A. Barres, Stanford University School of Medicine, California: How are CNS synapses eliminated?; **M.E. Greenberg,** Children's Hospital, Boston, Massachusetts: Signaling networks that regulate synaptic development and cognitive function.

G.S. Battaglia, Neurological Institute, Milano, Italy: The axonal localization of SMN and the role of a-SMN in stimulating axon growth.

Z. He, Children's Hospital Boston, Massachusetts: Axon degeneration and regeneration in SMA.

W. Thompson, University of Texas, Austin: A preliminary examination of the physiology and innervation of hindlimb muscles in the mouse model of type II SMA.

C.C.J. Miller, University of London, United Kingdom: Axonal transport and neurodegeneration.

Discussants: S. Burden, Skirball Institute, New York University Medical School; **J. Pierre Julien,** Centre Hospitalier de l'Universite Laval, Quebec, Canada; **T. Gordon,** University of Alberta, Canada

SESSION 3: Survival of Neurons; Gene Therapy

Chairpersons: P. Aebischer, Swiss Federal Institute of Technology, Lausanne, Switzerland: The issue of delivering therapeutics to motor neurons; **M. Sendtner,** Universitat Wuerzburg, Germany: Characterization of axonal alteration in cell culture and mouse models of SMA.

L.L. Rubin, Curis, Inc., Cambridge, Massachusetts: Small-molecule screens for increased SMN levels in fibroblasts and motor neurons.

S. Artavanis-Tsakonas, Massachusetts General Hospital and Harvard Medical School, Charlestown: Genetic modifier screens for SMN function.

C.T. Sumner, National Institute of Neurological Disorders and Stroke/NIH, Bethesda, Maryland: Histone deacetylase inhibitors for treatment of SMA.

F. Saudou, Institut Curie, Orsay, France: Huntington's disease: Understanding and restoring Huntington function in axonal transport.

Discussants: B. McCabe, Columbia University, New York; **D.K. Gifford,** Massachusetts Institute of Technology, Cambridge

Closing Discussion and Recommendations

C.E. Henderson, Columbia University, New York

T.M. Jessell, HHMI/Columbia University, New York

D. Singh, Spinal Muscular Atrophy Foundation, New York

The Biology of Neuroendocrine Tumors

September 24–26

FUNDED BY **Verto Institute**

ARRANGED BY **A.J. Levine,** Institute of Advanced Studies, Princeton, New Jersey
E. Vosburgh, Verto Institute, Stamford, Connecticut

BACKGROUND

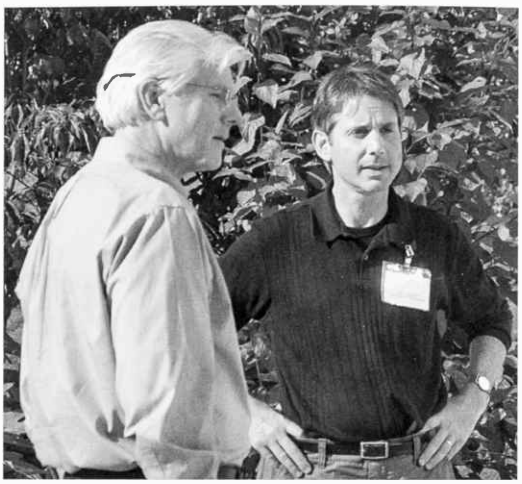
Neuroendocrine tumors are rare tumors with the characteristics of both neural and endocrine cells. Menin was identified as a tumor suppressor involved in multiple endocrine neoplasia type 1 and has been the subject of intensive research. It is a histone methyltransferase, and its role in neuroendocrine cell growth and differentiation and neural crest development was reviewed at this meeting. Data on the characterization of several new cells lines (essential tools for research) were presented, together with genomic analysis of tumor samples. Looking toward therapies, recent progress in clinical trials incorporating receptor tyrosine kinase, mTOR, and VEGF inhibitors was reviewed.

Introduction: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory

SESSION 1

Chairperson: A.J. Levine, Institute of Advanced Studies, Princeton, New Jersey

R.V. Lloyd, Mayo Clinic, Rochester, Minnesota: Characterization of a new carcinoid cell line (HC-45).
G. Van Buren, M.D. Anderson Cancer Center, University of Texas, Houston: The development and characterization of a human midgut carcinoid cell line.
M.L. Meyerson, Dana Farber Cancer Institute, Boston, Massachusetts: Menin function.
X. Hua, University of Pennsylvania, Philadelphia: Menin regulates hematopoiesis and leukemogenesis.
J.A. Epstein, University of Pennsylvania, Philadelphia: Menin has a critical role in neural crest development.
S.K. Kim, Stanford University School of Medicine, California: Calcineurin/NFAT signaling, an essential regulator of neuroendocrine cell growth and function.



E. Vosburgh, J. Epstein

SESSION 2

Chairperson: A.J. Levine, Institute of Advanced Study, Princeton, New Jersey

S. Karnik, Stanford University Medical Center, California: Epigenetic regulation of pathologic and facultative neuroendocrine cell growth.
F. Leu, Verto Institute, Cancer Institute of New Jersey, New Brunswick: Stepping the brake on carcinoid/neuroendocrine tumor by turning on the TGF-β-somatostatin circuitry.
C.J. Barnstable and J. Tombran-Tink, Yale University School of Medicine, New Haven, Connecticut: PEDF inhibits the growth of ovarian and breast cancers.
C. Harris, Verto Institute, Cancer Institute of New Jersey, New Brunswick: CINJ: L1 retrotransposition in neuroendocrine tumors.
D. Klimstra and L. Tang, Memorial Sloan-Kettering Cancer Center, New York: Prognostic classification and gene expression analysis of well-differentiated pancreatic endocrine neoplasms.
M.H. Kulke, Dana Farber Cancer Institute, Boston, Massachusetts: Single-nucleotide polymorphism array analysis of small-bowel carcinoid tumors.
J. Hoh, Yale University School of Medicine, New Haven, Connecticut: Preliminary report on a collaborative carcinoid case control study.

SESSION 3
Chairperson: E. Vosburgh, Verto Institute, Stamford, Connecticut

M. Essand, Uppsala University, Sweden: A novel chromogranin-a promoter-driven replication-selective oncolytic adenovirus as a therapeutic agent for carcinoid tumors.
K. Oberg, University Hospital, Uppsala, Sweden: 11C-HTP PET in carcinoid and NET.
L. Kvols, University of South Florida, Tampa: Targeted peptide receptor radiotherapy and SOM 230.
J. Yao, Gastrointestinal Medical Oncology, Houston, Texas: Streptizocin-based chemotherapy.
M.H., Kulke, Dana Farber Cancer Institute, Boston, Massachusetts: VEGF TK inhibitors, temazolamide, thalidomide, and lenalinamide therapies.
K. Oberg, University Hospital, Uppsala, Sweden: Biological therapy of NET.
J. Yao, Gastrointestinal Medical Oncology, Houston, Texas: Anti-VEGF and mTOR therapy.
L. Kvols, University of South Florida, Tampa: ENETS-NANETS: What is it and where is it going?

Chronic Lyme Disease Syndromes: New Avenues for Investigation

October 15–18

FUNDED BY **Aetos Technologies, Inc., Centers for Disease Control and Prevention (CDC); NIH–National Institute of Allergy and Infectious Diseases, National Institute of Arthritis and Musculoskeletal and Skin Diseases, and National Institute of Neurological Diseases and Stroke; U.S. Food and Drug Administration**

ARRANGED BY **S.E. Schutzer,** UMDNJ–New Jersey Medical School, Newark
P. Coyle, SUNY Stony Brook, New York
J. Dunn, Brookhaven National Laboratory, Upton, New York

BACKGROUND

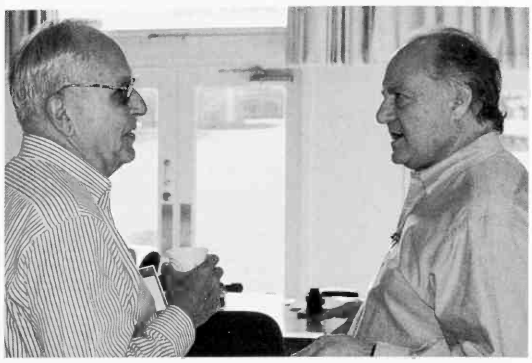
There have been significant advances in the treatment of acute Lyme disease, but many questions remain about those patients who appear to be pathogen-free but continue to exhibit a variety of symptoms. These have been ascribed to many different causes. This is a highly controversial subject and one of great practical importance for individuals who are suffering from these symptoms—How should they be treated? Participants in this meeting discussed three questions: Does the causative agent of Lyme disease, the spirochete *Borrelia burgdorferi,* persist in post-Lyme cases? Might there be other as yet unrecognized coinfections transmitted by the same tick vector? Might spirochete infection trigger long persisting inflammatory symptoms?

Welcoming Remarks, Goals and Objectives: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory; **S.E. Schutzer,** UMDNJ–New Jersey Medical School, Newark

SESSION 1: Can *B. burgdorferi* Persist? Is There an Autoimmune Component in Posttreatment Lyme Cases?

Chairpersons: P.J. Baker, National Institute of Allergy and Infectious Diseases/NIH, Bethesda, Maryland; **F.S. Kantor,** Yale University, New Haven, Connecticut

J.L. Benach, SUNY Stony Brook, New York: Discovery of *B. burgdorferi* in ticks. Did we miss other organisms and why?



P. Baker, S. Schutzer

A.C. Steere, Harvard Medical School, Massachusetts General Hospital, Boston: Discovery of Lyme disease in humans and antibiotic-refractory Lyme arthritis: Persistent infection or autoimmunity.
J.V. Ravetch, The Rockefeller University, New York: Autoimmune approaches in Lyme disease.
S.W. Barthold, University of California, Davis: Persistent infection in animal models and collagen-spirochete interaction and immune evasion.

SESSION 2: Postinfectious Responses
Chairpersons: C.B. Beard, Centers for Disease Control, Fort Collins, Colorado; **B.J. Luft,** SUNY Stony Brook, New York

S.D. Vernon, Centers for Disease Control and Prevention, CDC, Salida, Colorado: What the human genome can tell us once the pathogen's gone: Lessons learned from chronic fatigue syndrome.
E.S. Raveche, UMDNJ–New Jersey Medical School, Newark: In vivo studies of Bb infection in autoimmune-prone B-cell hyperactive NZB mouse strain.
J.L. Benach, SUNY Stony Brook, New York: Joint *Babesia* and *Borrelia* infections in mice.
L.K. Bockenstedt, Yale University School of Medicine, New Haven, Connecticut: Animal models for the study of *B. burgdorferi* persistence: What do we know and what can we learn?
T.H. Rider, Massachusetts Institute of Technology, Lexington: Novel methods of detecting and inactivating pathogens.
R. Salazar, CytoViva, Aetos Technologies, Inc., Auburn, Alabama: In situ identification and visualization spirochetal structures as *B. burgdorferi*.

SESSION 3: Genomics Part 1
Chairperson: E. Fikrig, Yale University School of Medicine, New Haven, Connecticut

D.J. Ecker, Ibis Biosciences, Isis Pharmaceuticals, Carlsbad, California: Universal biosensing for pathogen discovery.

SESSION 4: Insights from Chronic Lyme Disease Therapy Trials
Chairperson: P. Coyle, SUNY Stony Brook, New York

M.S. Klempner, Boston University Medical Campus, Massachusetts: Chronic symptoms following treatment of acute Lyme disease.
P. Coyle, SUNY Stony Brook, New York: Stony Brook trial.
B. Fallon, College of Physicians & Surgeons, New York: Placebo ceftriaxone encephalopathy trial.
A.R. Marques, National Institute of Allergy and Infectious Disease/NIH, Bethesda, Maryland: Framing questions from the clinical sphere.

SESSION 5: Microbes in the Tick
Chairperson: J.L. Benach, SUNY Stony Brook, New York

D. Fish, Yale University, New Haven, Connecticut: Prevalence and interactions among *I. scapularis*-borne pathogens.
K. Clay, Indiana University, Bloomington: Microbial diversity and interactions in ticks.

SESSION 6: Genomics Part 2
Chairperson: E. Fikrig, Yale University School of Medicine, New Haven, Connecticut

B.J. Luft, SUNY Stony Brook, New York: Immunologic and phylogenic analysis of OspC: Insights into depth of infection.

SESSION 7: Potential Nonmicrobial Action of Antibiotics
Chairperson: C.B. Beard, Centers for Disease Control, Fort Collins, Colorado

J.N. Adkins, Pacific Northwest National Laboratory, Richland, Washington: AMT tag proteomics: Application to disease pathogenesis and diagnosis.
J.D. Rothstein, Johns Hopkins University School of Medicine, Baltimore, Maryland: Neuroprotective and inflammatory potential of antibiotics in neurological disease.
D.C. Martz, Rocky Mountain Chronic Disease Specialists, Colorado Springs, Colorado: Motor neuron disease that is antibiotic-responsive.

SESSION 8: Rephrasing Questions Amenable to Research
Chairpersons: P. Coyle, SUNY Stony Brook, New York; **S.E. Schutzer,** UMDNJ–New Jersey Medical School, Newark

Outline of suggested blueprint for future research.
P.J. Baker, National Institute of Allergy and Infectious Diseases/NIH, Bethesda, Maryland; **M. Nunn,** National Institute of Neurological Disorders and Stroke/NIH, Bethesda, Maryland: Upcoming federal funding opportunities in the field.

New Horizons in Internet Site Development

October 22–25

FUNDED BY **The William and Flora Hewlett Foundation**

ARRANGED BY **D. Micklos and J. Connolly,** Dolan DNA Learning Center, Cold Spring Harbor Laboratory

BACKGROUND

The Internet and the Web are revolutionizing the provision and delivery of information of all kinds, and nowhere more dramatically than in education. However, the design and content presentation of education Web sites are almost certainly not optimal for their function. This workshop brought together workers in a variety of fields, including cognitive science, neuroscience, network theory, knowledge management, learning theory, and technology convergence. They discussed, for example, how knowledge of concept maps can be harnessed to develop Web sites that are more intuitive to use and explore.

Introduction: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Insights from Neural and Molecular Science
Chairperson: M. Smith, William and Flora Hewlett Foundation, Menlo Park, California

Opening Remarks: D. Micklos, Dolan DNA Learning Center, Cold Spring Harbor Laboratory: Introducing *G2C Online*.

J. Connolly, Dolan DNA Learning Center, Cold Spring Harbor Laboratory: Bridging genes and behavior.

H.S. Kurtzman, National Institute of Mental Health/NIH, Bethesda, Maryland: Some current trends in cognitive research.

T. Tully, Cold Spring Harbor Laboratory: Flies R Us revisited: The case for molecular cognition.

SESSION 2: Cognitive Science and Network Theory
Chairperson: M. Smith, William and Flora Hewlett Foundation, Menlo Park, California

R.M. Shiffrin, Indiana University, Bloomington: Levels of analysis in modeling cognition.
M. Buchanan, La Vignerie, Livarot, France: Evolutionary wisdom and anti-intelligent design.
P. McKercher, Knowledge Web, University of California, Santa Clara: The Knowledge Web as a dynamic knowledge repository.
C. Dietlin, WGBH Interactive West, Greenfield, Massachusetts: Building an Internet site for the classroom.

SESSION 3: Multimedia Learning and Education
Chairperson: E.F. Rover, Dana Foundation, New York

J.R. Jungck, BioQuest Curriculum Consortium, Beloit College, Wisconsin: Creative commons: Connecting collaborators, cooperating communities, crossing chasms, and celebrating complexity.
J. Bobe, DNA Direct, Inc., San Francisco, California: Citizen science and patient communities on the Web.
J. Kruper, Cardean Learning Group, Chicago, Illinois: What the "participation culture" offers (science) education.
K. Borner, Indiana University, Bloomington: Mapping the structure and evolution of science locally and globally.

SESSION 4: Knowledge Management
Chairperson: E.F. Rover, Dana Foundation, New York

J. Novak, Institute for Human and Machine Cognition, University of West Florida, Pensacola: Use of Cmap tools and a new model for education to facilitate learning about genes and cognition.
J. Beisty, SAP Knowledge Management, SAP America, Inc., Newtown Square, Pennsylvania: The business of knowledge management.
S. Buckingham, The Open University, Milton Keynes, United Kingdom: Science portals as hubs for hypermedia discourse.
L. Petrides, Institute for the Study of Knowledge Management in Education, Half Moon Bay, California: Education 2.0: A knowledge management approach.

SESSION 5: Emerging Technologies
Chairperson: K. Borner, Indiana University, Bloomington

W.A. Baer, Annenberg Center for Communication, University of Southern California, Los Angeles: Internet evolution, technological opportunities, institutional constraints.
K. Howell, Federation of American Scientists, Washington, D.C.: Designing games for learning.
H. Heimer, Schizophrenia Research Forum, Providence, Rhode Island: Research forums: Scientist resources and communities online.
L. Stark, University of Utah–Eccles Institute of Human Genetics, Salt Lake City: Exploragraphic: Learning designed for today's tech-savvy students.

Final Remarks: D. Micklos, Dolan DNA Learning Center, Cold Spring Harbor Laboratory

K. Borner, J. Connolly

C. Sosnowy, H. Ba

Axonal Dynamics and Synaptic Junctions

October 29–31

FUNDED BY **The ALS Association**

ARRANGED BY **E. Holzbaur,** University of Pennsylvania, Philadelphia
D.W. Cleveland, University of California, San Diego
L. Bruijn, The ALS Association, Palm Harbor, Florida

BACKGROUND

There is growing interest in the role of axonal dynamics in neurodegenerative diseases including amyotrophic lateral sclerosis (ALS). The recent discovery of dynein/dynactin abnormalities linked to motor neuron degeneration, changes in mitochondrial axonal trafficking in mutant SOD1-expressing mice, and studies showing neuromuscular junction abnormalities in ALS have focused more attention on this area. This workshop reviewed the role of axonal and synaptic junction abnormalities in disease and the potential of therapeutic interventions for ALS targeting these abnormalities.

Welcome and Introductory Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory; **L. Bruijn,** The ALS Association, Palm Harbor, Florida

SESSION 1: Axonal Structure and Function
Chairperson: S. Burden, New York University Medical School

D.W. Cleveland, University of California, San Diego: Overview of axonal dynamics: Motors, fast and slow axonal transport, and their cargoes.
E. Holzbaur, University of Pennsylvania, Philadelphia: Dynactin/dynein and ALS.
P.J. Hollenbeck, Purdue University, West Lafayette, Indiana: The life of mitochondria in the axon.
G. Banker, Oregon Health and Science University, Portland: Organelle trafficking in hippocampal neurons.
T.L. Schwarz, Children's Hospital, Boston, Massachusetts: Axonal traffic in *Drosophila*.
C.C.J. Miller, University of London, United Kingdom: Axonal transport and neurodegeneration.

L. Bruijn, G. Morfini

SESSION 2: Development and Maintenance of Neural Connectivity
Chairperson: E. Holzbaur, University of Pennsylvania, Philadelphia

J. Lichtman, Harvard University, Cambridge, Massachusetts: Developmental axonal reorganization: A connectomic approach.
S. Burden, New York University Medical School: A MuSK-centric view of neuromuscular synapse formation.
E.S. Levitan, University of Pittsburgh, Pennsylvania: Synaptic capture of transiting vesicles.
G. Morfini, University of Illinois at Chicago: Mutant SOD-1-induced alterations in fast axonal transport.
S. Finkbeiner, University of California, San Francisco: Molecular mechanisms of Huntington-induced neurodegeneration.
L.W. Enquist, Princeton University, New Jersey: Axon-mediated spread of alpha herpesvirus infections.

SESSION 3: Axonal Abnormalities in Neurodegenerative Diseases
Chairperson: D.W. Cleveland, University of California, San Diego

W. Mobley, Stanford University, California: Tracking signaling endosomes in axons to decipher neurodegenerative disease mechanisms.

P. Caroni, Friedrich Miescher Institute for Biomedical Research, Basal, Switzerland: Selective vulnerability of fast-fatiguable motoneuron axons.

SESSION 4: Model Systems
Chairperson: L. Bruijn, The ALS Association, Palm Harbor, Florida

M. Sendtner, Universitat Wuerzburg, Germany: Characterization of axonal alteration in cell culture and mouse models of SMA.

P. Wong, Johns Hopkins University, Baltimore, Maryland: Mechanism of mutant dynactin-induced motor neuron degeneration.

B. Zheng, University of California, San Diego: Axon regeneration in the CNS using mouse models of spinal cord injury.

J.D. Glass, Emory Center for Neurodegenerative Disease, Atlanta, Georgia: Axonal degeneration in neurodegenerative disease: A rational therapeutic target.

M. Coleman, The Babraham Institute, Cambridge, United Kingdom: Common mechanisms of axon degeneration revealed using the slow Wallerian degeneration (*Wlds*) mouse.

E.P. Pioro, The Cleveland Clinic, Ohio: Axonal degeneration and protection of *Wlds* in wobbler motor neuron disease.

L. Reichardt, University of California, San Francisco: Roles of cadherins and caenins at the synapse.

Integration of Hormonal and Genetic Regulation in Plant Development

November 5–8

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **M. Estelle,** Indiana University, Bloomington
T. Schmülling, Free University of Berlin, Institute of Biology/Applied Genetics, Germany
W. Lukowitz, Cold Spring Harbor Laboratory

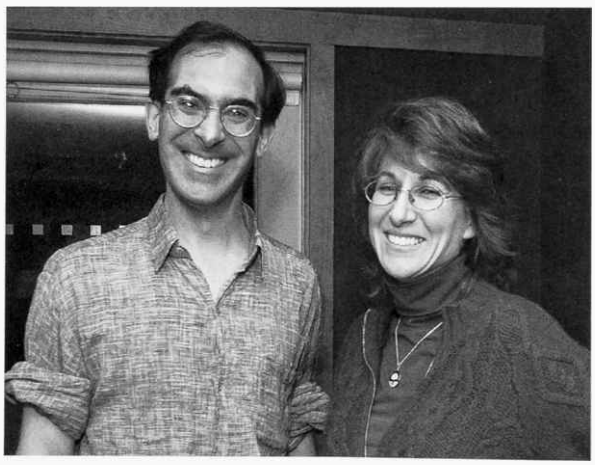
BACKGROUND

The role of plant hormones in growth development has traditionally been considered separately from the genetic programs that regulate pattern and form. However, with recent advances in our understanding of meristem function, organogenesis, and other developmental processes, it is clear that hormonal and genetic regulations of development are highly integrated. Participants in this meeting discussed questions such as What are the different mechanisms of hormone signaling in plants? How do different hormones act in a synergistic or antagonistic fashion? How do known key genetic players in shoot and root development regulate plant hormones?

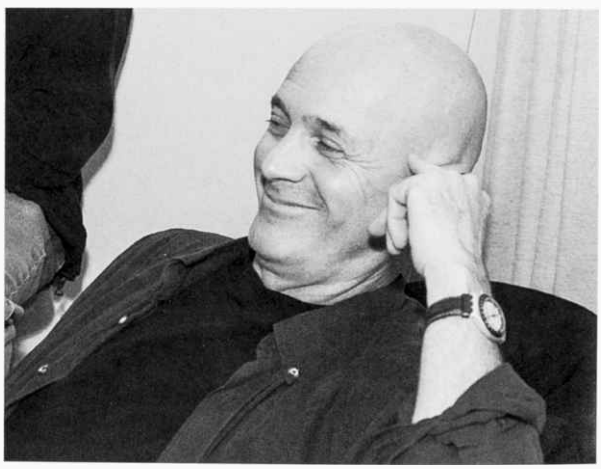
Introduction: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Pathways and Paradigms
Chairperson: W. Lukowitz, Cold Spring Harbor Laboratory

M. Estelle, Indiana University, Bloomington: Function of the TIR1/AFB auxin receptors in plant growth and development.
T. Schmülling, Free University of Berlin, Institute of Biology/Applied Genetics, Germany: Cytokinin receptor-mediated developmental pathways.
J. Chory, HHMI/The Salk Institute, La Jolla, California: Using knowledge gained from the Brassinosteroid signal transduction pathway to learn how plants grow.
J. Sheen, Massachusetts General Hospital, Boston: MAPK cascades in hormonal signaling.
G. Juergens, Universität Tübingen, Germany: What role for auxin in early embryogenesis?
S. Hake, U.S.D.A. Plant Gene Expression Center, Albany, California: KN1 targets: From maize to *Arabidopsis* and back again.



J. Reed, J. Malamy



D. Weiss

SESSION 2: New Players and Cross-talk
Chairperson: M. Estelle, Indiana University, Bloomington

J. Friml, ZMBP, Universität Tübingen, Germany: A novel pathway for a nongenomic action of plant hormone auxin.
S. Gilroy, Pennsylvania State University, University Park: C12+ and pH as integrators of plant growth and development.
Z. Yang, University of California, Riverside: ROP GTPases in hormone signaling.
T. Kakimoto, Osaka University, Japan: Paptide mediators that regulate epidermal cell patterning.
W. Lukowitz, Cold Spring Harbor Laboratory: A GATA factor mediating axis and root formation in the early embryo.
J. Long, The Salk Institute for Biological Sciences, La Jolla, California: A role for TOPLESS in embryonic polarity.
P. McCourt, University of Toronto, Canada: Using chemical genetics to map phenotype onto genotype.

SESSION 3: Root and Vascular Development
Chairperson: J. Chory, HHMI/The Salk Institute for Biological Studies, La Jolla, California

D. Weijers, Wageningen University, The Netherlands: Cell specification and auxin signaling in early embryogenesis.
Y. Helariutta, University of Helsinki, Finland: Integration of hormonal and genetic regulation during plant vascular morphogenesis.
J. Malamy, University of Chicago, Illinois: Hormonal regulation of lateral root formation.
M.J. Bennett, University of Nottingham, Loughborough, United Kingdom: Dissecting the hormonal control of root growth in *Arabidopsis*.
T. Berleth, University of Toronto, Ontario, Canada: Polar signals and gene regulation in vascular development.
J.M. Alonso, North Carolina State University, Raleigh: Ethylene and auxin signaling and response pathways: A paradigm for hormone interaction.

SESSION 4: Shoot Development I: Pathway Integration
Chairperson: T. Schmülling, Free University of Berlin, Institute of Biology/Applied Genetics, Germany

J. Kieber, University of North Carolina, Chapel Hill: Role of cytokinin signaling in growth and development.
T. Werner, Free University of Berlin, Germany: Cytokinin catabolism regulates the activity of plant meristems.
J. Lohmann, Universität Tübingen, Germany: Regulatory networks of meristem development.
D. Weiss, Hebrew University of Jerusalem, Rehovot, Israel: Role of O-GlcNAc transferase in the interaction between gibberelin and cytokinin response.
J.W. Reed, University of North Carolina, Chapel Hill: Functions of auxin response factors in vegetative and reproductive development.

SESSION 5: Shoot Development II: Phyllotaxis and Modeling
Chairperson: S. Hake, U.S.D.A. Plant Gene Expression Center, Albany, California

D. Jackson, Cold Spring Harbor Laboratory: Integration of hormonal signals and phyllotaxy.
C. Kuhlemier, University of Bern, Switzerland: Auxin and phyllotaxis.
M. Heisler, California Institute of Technology, Pasadena: Local and global control of auxin transport patterns in the shoot apical meristem.
P. McSteen, Pennsylvania State University, University Park: Genetic and hormonal regulation of axillary meristem initiation during maize inflorescence development.
E. Mjolsness, University of California, Irvine: Integrative mathematical modeling frameworks for plant development.
P. Prusinkiewicz, University of Calgary, Canada: Computational models of auxin-flow-induced patterning in plants.

Comparative Biology of Innate Immune Systems

November 27–30

FUNDED BY **Cold Spring Harbor Corporate Sponsor Program**

ARRANGED BY **J. Dangl,** University of North Carolina, Chapel Hill
D.A. Portnoy, University of California, Berkeley
B.J. Staskawicz, University of California, Berkeley

BACKGROUND

Immunity refers to the ability of an organism to resist attack by pathogens. We usually think of immunity as acquired immunity, the response mediated by the vertebrate immune system. However, organisms without immune systems can mount defenses against pathogens through innate immune systems, which, after much neglect, are also the subject of intensive research in human beings. This meeting was notable for bringing together scientists working on innate immunity in plants and animals, to discuss the mechanisms of innate immunity.

Introduction: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Plant Intracellular Receptors
Chairperson: B.J. Staskawicz, University of California, Berkeley

J. Dangl, University of North Carolina, Chapel Hill: Indirect recognition of pathogen virulence factors by plant NB-LRR disease resistance proteins.

P. Schulze-Lefert, Max-Planck Institute for Plant Breeding Research, Koln, Germany: Molecular links between PAMP- and NB-LRR-triggered immune responses.

J. Ellis, CSIRO, Canberra, Australia: Direct receptor-ligand interaction is the basis of gene-for-gene specificity in the flax-flax rust resistance avirulence gene interaction.

SESSION 2: Mammalian Bacterial Pathogenesis
Chairperson: B.J. Staskawicz, University of California, Berkeley

D.A. Portnoy, University of California, Berkeley: The mammalian innate immune system: A bacterial perspective.

R.R. Isberg, Tufts University School of Medicine, Boston, Massachusetts: Manipulation of host cell death and survival pathways by *Legionella pneumophila*.

S.I. Miller, University of Washington, Seattle: *Salmonella* interactions with the innate immune system.

SESSION 3: Signaling Systems in Plant Innate Immunity
Chairperson: J. Dangl, University of North Carolina, Chapel Hill

B.J. Staskawicz, University of California, Berkeley: Host-microbe interactions shaping the evolution of plant innate immunity.
G.B. Martin, Cornell University, Ithaca, New York: Roles of *Pseudomonas* type III effectors AvrPto and AvrPtoB in plant disease susceptibility.
J. Rathjen, John Innes Centre, Colony Norwich, United Kingdom: Specific recognition of AvrPto and AvrPtoB by the Pto/Prf complex in tomato.

SESSION 4: TLRs and Host Response
Chairperson: J. Dangl, University of North Carolina, Chapel Hill

K. Fitzgerald, University of Massachusetts Medical School, Worcester: Regulation and counter-regulation of type I interferon responses.
L. O'Neill, Trinity College, Dublin, Ireland: Toll-like receptor signal transduction.
D. Golenbock, University of Massachusetts Medical School, Worcester: Anatomy of Toll 9 and its relationship to disease.

SESSION 5: Bacterial Type-three Secretion Systems
Chairperson: D.A. Portnoy, University of California, Berkeley

J. Galan, Yale University School of Medicine, New Haven, Connecticut: The type III secretion system of *S. enterica*: Decoding its function.
J.E. Dixon, University of California, San Diego School of Medicine, La Jolla: Novel type III pathogenic effectors.
M.B. Mudgett, Stanford University, California: The *Xanthomonas* XopD protease: A generalist or specialist?
U. Bonas, Martin-Luther University, Halle, Germany: Control of *Xanthomonas* type-III secretion and dual activity of the effector AvrBs3 in the plant.
S. Yang He, Michigan State University, East Lansing: Suppression of host innate immune responses by the bacterial pathogen *P. syringae*.
J.T. Greenberg, University of Chicago, Illinois: *P. syringae* type-III effectors: Their functions in host-range determination and the disease process.

SESSION 6: Intracellular Receptors II
Chairperson: J. Galan, Yale University School of Medicine, New Haven, Connecticut

G. Nunez, University of Michigan Medical School, Ann Arbor: Function of NLRs in innate immunity.
J. Ting, University of North Carolina, Chapel Hill: CATERPILLARs R us: Immune defense genes conserved from plants to mammals.
F.L.W. Takken, University of Amsterdam, The Netherlands: The NB-ARC domain: An NTP-hydrolyzing molecular switch.
R. Innes, Indiana University, Bloomington: Molecular mechanisms underlying the activation of plant NB-LRR proteins.
S. Dinesh-Kumar, Yale University, New Haven, Connecticut: Role of TIR domain in recognition of pathogen-derived elicitors.
J.D.G. Jones, John Innes Centre, Norwich, United Kingdom: Novel approaches to understanding coevolution between plants and filamentous pathogens.

SESSION 7: Signaling Systems in Plant and Animal Innate Immunity
Chairperson: J.D.G. Jones, John Innes Centre, Norwich, United Kingdom

S. Robatzek, Max-Planck Institute for Plant Breeding Research, Cologne, Germany: Ligand-induced internalization of pattern-recognition receptors in *Arabidopsis* innate immunity.
F.M. Ausubel, Massachusetts General Hospital, Boston: The *C. elegans* innate immune response.
J. Sheen, Massachusetts General Hospital, Boston: Intracellular signaling in plant innate immunity.
R.W. Michelmore, University of California, Davis: Comparative analysis of plant-*Pseudomonas* interactions.
S. Somerville, Carnegie Institution, Stanford, California: The plant cell wall, the first line of defense.
N. Silverman, University of Massachusetts, Worcester: Intracellular recognition of pathogens in *Drosophila*.

J. Jones, J. Ellis, G. Martin

Redox Regulation of Signal Transduction

December 3–6

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **T. Finkel,** National Heart, Lung, and Blood Institute/NIH, Bethesda, Maryland
S.G. Rhee, National Heart, Lung, and Blood Institute/NIH, Bethesda, Maryland
N.K. Tonks, Cold Spring Harbor Laboratory

BACKGROUND

Cellular production of reactive oxygen species (ROS) has been associated with a number of disease states as well as the rate of organismal aging. For many years, it was assumed that oxidants functioned within cells in a random and solely destructive manner. In the last decade, evidence has accumulated that ROS can also function within the cell as part of normal signal transduction pathways. This meeting reviewed findings on the specific intracellular targets of oxidants, the range of redox-dependent pathways observed, and the physiological and pathophysiological process specifically regulated by ROS.

Introduction: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Sensing ROS Levels
Chairpersons: T. Finkel, National Heart, Lung, and Blood Institute/NIH, Bethesda, Maryland; **M. Murphy,** University of Cambridge, United Kingdom

C. Chang, University of California, Berkeley: New chemical approaches to study peroxide biology.
L.B. Poole, Wake Forest University, Winston-Salem, North Carolina: Cysteine sulfonic acids in catalysis and regulation.
V.N. Gladyshev, University of Nebraska, Lincoln: Thiols, selenols, and redox signaling networks.
R. Sitia, Universita Vita-Salute San Raffaele, Milan, Italy: Redox regulation in the endoplasmic reticulum.
J.D. Helmann, Cornell University, Ithaca, New York: Mechanisms of peroxide sensing in bacteria.
M.B. Toledano, LSOC, CEA-Saclay, Gif-sur-Yvette, France: H_2O_2 sensing and signaling by thiol-based peroxidases.

SESSION 2: ROS Generating/Regulating Systems
Chairpersons: N.K. Tonks, Cold Spring Harbor Laboratory; **C.F. Nathan,** Weill Medical College of Cornell University, New York

A. Holmgren, Karolinska Institute, Stockholm, Sweden: Thioredoxin and glutaredoxin systems in redox signaling.
P.T. Schumacker, Northwestern University, Chicago, Illinois: Mitochondrial oxidant generation in response to hypoxia: Compartmental specificity.
M. Murphy, University of Cambridge, United Kingdom: Role of mitochondria in redox signaling.
U.G. Knaus, Scripps Research Institute, La Jolla, California: Regulation and function of epithelial NADPH oxidases.
M.R. Williams, University of Maryland School of Medicine, Baltimore: Expression and function of NADPH oxidases in T lymphocytes.

SESSION 3: pTyr Signaling
Chairperson: L.B. Poole, Wake Forest University, Winston-Salem, North Carolina

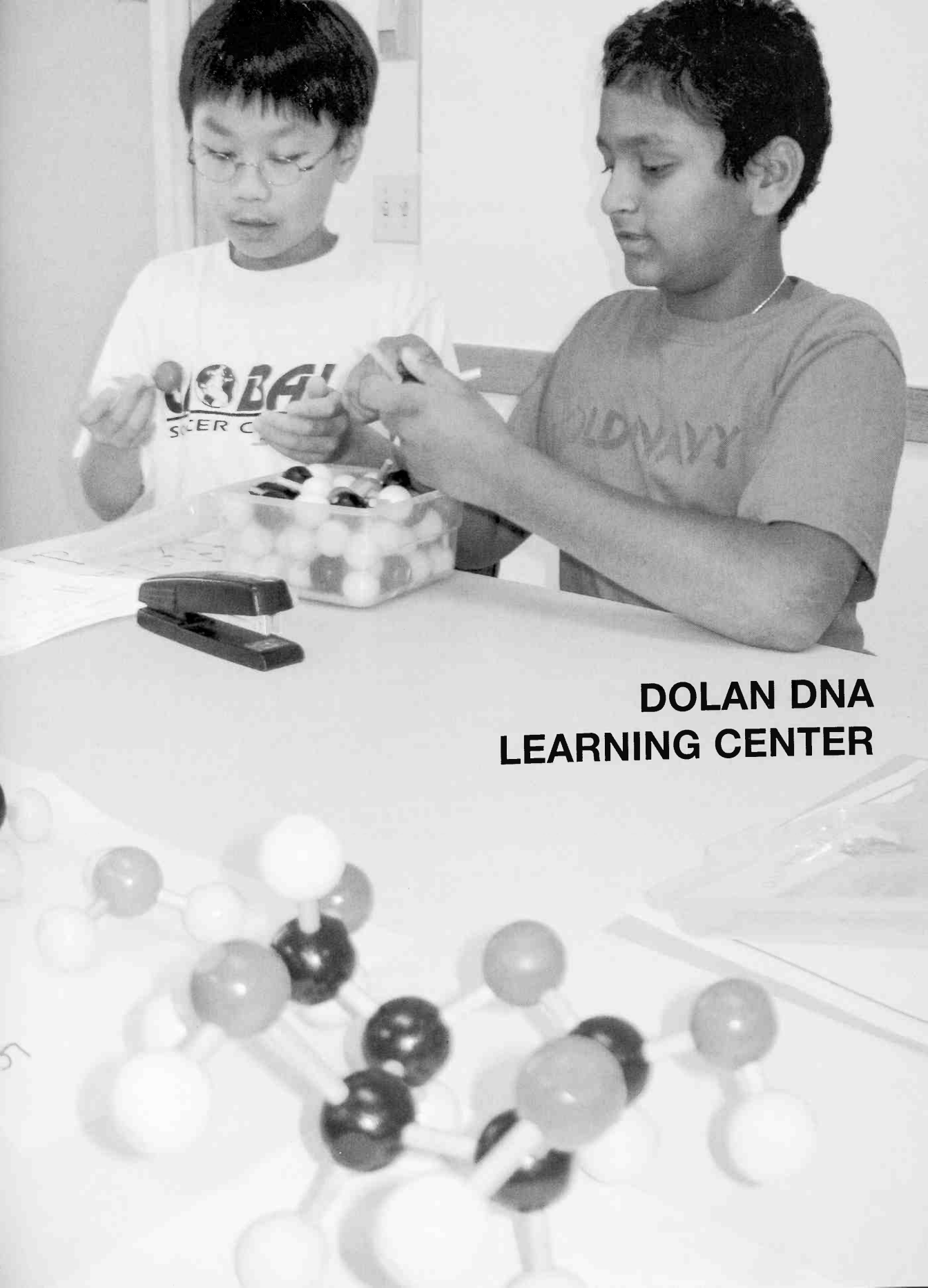
N.K. Tonks, Cold Spring Harbor Laboratory: Regulation of protein tyrosine phosphatase function by reversible oxidation.
A. Ostman, Karolinska Institute, Stockholm Sweden: Regulation of PTPs by hypoxia and ROS.
T.-C. Meng, Institute of Biological Chemistry, Taipei, Taiwan: Redox regulation of protein tyrosine phosphatases by reactive nitrogen species: From molecular identification to signaling implication.
P. Downes, University of Dundee, United Kingdom: Redox regulation of PTEN tumor suppressor amplifies growth-factor-dependent PI3-kinase signaling.
P. Chiarugi, University of Florence, Italy: Hydrogen peroxide: A key messenger for ligand-independent *rtk* activation.
A. Toker, Beth Israel Deaconess Medical Center, Boston, Massachusetts: Protein kinase D signaling in oxidative stress responses.

SESSION 4: Signaling and Disease
Chairpersons: **P.T. Schumacker,** Northwestern University, Chicago, Illinois; **U.G. Knaus,** Scripps Research Institute, La Jolla, California

G. Gilliland, HHMI/Harvard Medical School, Boston, Massachusetts: FoxO mediates resistance of hematopoietic stems cells to physiologic oxidative stress.
E.V. Avvedimento, University of Naples Federico II, Ancona, Italy: PDGF, ROS, and Ras signals in systemic sclerosis.
K. Griendling, Emory University, Atlanta, Georgia: Regulation of VSMC migratory signals by reactive oxygen species.
J.F. Engelhardt, University of Iowa, Iowa City: Nox-dependent signaling by redox-active endosomes.
C. Taylor, University College Dublin, Ireland: Hypoxia, gene expression, and disease.
C.F. Nathan, Weill Medical College of Cornell University, New York: Reactive oxygen and nitrogen intermediates signal and kill specifically.

SESSION 5: Aging and Metabolism
Chairpersons: **P. Downes,** University of Dundee, United Kingdom; **K. Griendling,** Emory University, Atlanta, Georgia

T. Finkel, National Heart, Lung, and Blood Institute/NIH, Bethesda, Maryland: Longevity genes as regulators of mitochondrial metabolism.
B.M.T. Burgering, University of Medical Center, Utrecht, The Netherlands: FoxO transcription factor regulation by oxidative stress: Insights into life span and disease.
M. Giorgio, European Institute of Oncology, Milan, Italy: Regulation of adipogenesis by p66Shc-generated oxidative signal.
P. Hwang, National Heart, Lung, and Blood Institute/NIH, Bethesda, Maryland: p53 as a regulator of aerobic respiration.
F. Mechta-Grigoriou, Institute Curie, Paris, France: AP-1, oxidative stress, and aging.



DOLAN DNA LEARNING CENTER

DOLAN DNA LEARNING CENTER
EXECUTIVE DIRECTOR'S REPORT

Preparing students and families to thrive in the gene age

ADMINISTRATION	INSTRUCTION	BIOMEDIA	TECHNOLOGY DEVELOPMENT
Nancy Daidola	Elna Carrasco	John Connolly	Adrian Arva
Mary Lamont	Jeanette Collette	Eun-Sook Jeong	Greg Chin
Stacy Leotta	David Gundaker	Susan Lauter	Cornel Ghiban
David Micklos	Natalia Hanson	Chun-hua Yang	Uwe Hilgert
Karen Orzel	Laura Johns		Bruce Nash
Carolyn Reid	Amanda McBrien		
	Erin McKechnie		
	Danielle Sixsmith		
	Lauren Weidler		

What does it mean to be human? The philosopher, theologian, sociologist, historian, scientist, and every man or woman on the street have different answers to this question. So, too, does the genome biologist who determines and compares the complete genetic endowments of living entities.

The human genome is composed of 25 kinds of chromosomes—22 autosomes, the sex chromosomes X and Y, and the mitochondrial chromosome. Each chromosome is a package for a single, long, and unbroken strand of the molecule deoxyribonucleic acid (DNA). The exact sequence of A, T, C, and G nucleotides that compose the DNA molecule running through each chromosome is unique to each person. Arrayed along the chromosomes are approximately 30,000 genes—stretches of DNA ranging from several hundred to several million nucleotides—that carry the "genetic code" for making the protein components of living cells. This code determines physiological and psychological "traits" that we inherit from our parents, thus setting the parameters of human life, health, and happiness.

The human genome weighs in at about 3 billion nucleotides, and the genomes of any two people are 99.9% identical. Expressed another way, DNA from two people will on average differ by about one nucleotide per 1000. This does not seem like much, but taken over the entire genome, it comes to 3 million differences. The same sort of reasoning applies to our closest living relative, the chimpanzee, whose genome is 99% similar to ours. This leaves room for about 30 million DNA differences between humans and chimps.

These differences are caused by mutations that accumulate in DNA over time, so the number of mutations is roughly proportional to the amount of time since two groups have diverged from a common ancestor. The majority of these DNA differences between humans and chimps are of little or no consequence. This is because only about 1.5% of the human DNA sequence actually carries the code for making proteins. Thus, by chance, most mutations are likely to occur outside of protein-coding regions. Even protein-coding regions can tolerate a good deal of mutation, for two reasons. First, the genetic code is redundant, such that a number of different nucleotide sequences can specify the identical protein. Second, some physical changes in a protein do not actually alter how it functions.

These gross comparisons can only tell so much. Better to compare individual genes. As one might expect, a number of genes have evolved (changed) rapidly since humans and chimps diverged from a common ancestor about 6 million years ago, including those involved in immunity, reproduction, smell, taste, skin, and hair. Especially intriguing are changes in a gene called *FOXP2*, which may have had a role in language development.

Even so, gene-to-gene comparisons confirm that the vast majority of human and chimp genes are functionally identical. This "conservation of function" extends throughout the evolutionary tree. Beyond

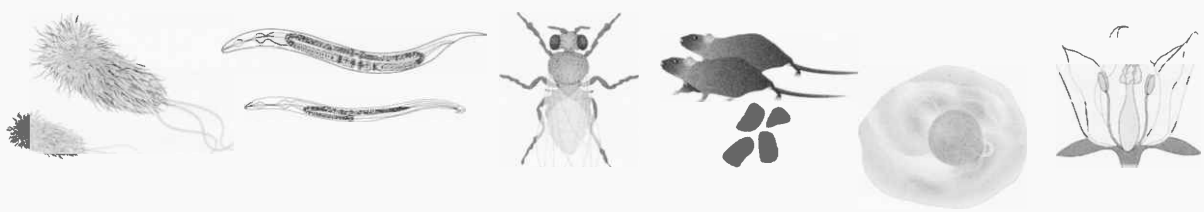
computer comparisons of gene and protein sequence, functional equivalence is convincingly shown by gene-transfer experiments, most commonly when a human gene is inserted into a living mouse to replace its mouse counterpart. In most cases, the human replacement gene functions admirably. This has led researchers to introduce human disease genes to create mouse models that mimic human disorders such as Alzheimer's disease, Huntington's disease, Parkinson's disease, and breast cancer. There are even examples of human genes functioning perfectly well in yeast, the simplest form of advanced cell.

The recent sequencing of the sea urchin genome traced elements of our genealogy even further back in evolutionary time. Surprisingly, the urchin has the most well-developed immune system yet encountered in an animal without a backbone. The urchin shares with us prototypes of genes for key growth factors and receptors that participate in immune and blood system development, including tumor necrosis factor, interleukins, and vascular endothelial growth factor. Going back further still, we share a basic set of genes—including those needed to extract energy from food—with the ancient archaebacteria that inhabit hot springs and deep-ocean vents. These gene comparisons illustrate that evolution has preserved gene "tool sets" that successfully carry out life processes—layering on new gene sets as organisms gained complexity.

Organism	Genes	Genome Size (Nucleotides)	Year Sequenced
Archaebacterium (*Methanococcus maripaludis)*	1,880	1.7 million	2007
Bacterium *(Escherichia coli)*	4,400	4.6 million	1997
Baker's Yeast *(Saccaromyces cerevisiea)*	6,190	12.1 million	1997
Fruit Fly (*Drosophila melanogaster)*	~14,800	180 million	2000
Roundworm *(Caenorhabditis elegans)*	~21,000	100 million	1998
Sea Urchin *(Strongylocentrotus purpuratus)*	~23,300	814 million	2006
House Mouse (*Mus musculus*)	~30,000	2.5 billion	2002
Human (*Homo sapiens*)	~30,000	3.2 billion	2003

As shown in the table, adding genes can add only so much complexity to an organism. Bacteria, single-celled organisms without an organized nucleus, get by with several thousand genes. Yeasts gain an organized nucleus with only about twice as many genes. The fruit fly gains a complicated body plan and external skeleton with only about twice as many genes. Mammals gain an internal skeleton and temperature regulation with only about twice the number of genes as a fruit fly. Here, the gene number levels off, with essentially no difference in gene number between a mouse and a human.

Clearly, humans share many genes with other organisms—especially other mammals. If this is so, how can we achieve "humanness" with essentially the same gene set as a mouse or a chimp? Much of the answer lies in "noncoding" DNA sequences that do not carry the genetic code for making proteins. Much of the noncoding DNA lies in between genes, separating them from one another on the chromosomes. This "intergenic" DNA contains key regulatory sequences—promoters and enhancers—that turn genes on and off in specific tissues and at specific times during development from embryo to adult. Altering the timing and interaction of thousands of genes can produce markedly different mor-



Model organisms (*left to right*) *E. coli*, *C. elegans*, *Drosophila*, mouse, human cell, and *Arabidopsis*.

phological features. For example, the same set of regulatory genes defines the basic body plans of all animals with twofold (bilateral) symmetry. These "master" genes function in organisms as diverse as fruit fly to human, controlling the proper placement of body parts, from wings and antenna to arms and ears.

In the mid-1970s, Rich Roberts, at Cold Spring Harbor Laboratory (CSHL), and Phil Sharp, at the Massachusetts Institute of Technology, discovered that genes themselves are interrupted by noncoding sequences. They shared the Nobel Prize for showing that noncoding sequences—termed "introns"—separate the genes of higher organisms into modular coding "exons." The average human gene contains seven exons that can be joined together in different combinations to specify several different proteins. This "differential splicing" further increases the functional complexity of the genome.

Transposons, the so-called jumping genes discovered by CSHL Nobel laureate Barbara McClintock, are the largest component of noncoding DNA, equal to about half the total mass of the human genome! It could be worse, transposons account for an estimated 85% of the corn (maize) genome. Biologists are split about whether jumping genes serve some purpose in genome evolution or are just extremely effective genetic hitchhikers. Grand scheme or not, the most common human transposon, termed *Alu*, provides a sequence that is used in about 5% of differentially spliced exons.

Introns and transposons account for most of the disparity between genome size and gene number as organisms increase in complexity. Thus, although the fruit fly genome is about 100 times as large as the archaebacterial genome, it contains only about eight times as many genes. About twice as many human genes occupy about 18 times as much space as the fruit fly genome. Comparing extremes, human genes occupy about 100 times the genome space of an equivalent number of archaebacterial genes! Thus, from a genomicist's point of view, being human is a large endowment of genes shared with many organisms through evolutionary time, retasked by regulatory elements, and reshuffled through differential splicing. Gene sets for a basic bilateral body plan and mammalian way of life are further modified by a relatively small set of genes peculiar to our kind. Inherited gene variations are further shuffled during the generation of parental sperm and egg. This shared heritage, attenuated by a unique set of mutations, comes together at the moment of conception to produce a unique individual—one with a brain that is capable of pondering why so much of his or her genome is given over to jumping genes.

Dynamic Gene

The more than 130 billion nucleotides of DNA sequence residing in Genbank and other databases now challenge biology students to come to grips with the complex genomes of higher organisms. The geneticist J.B.S. Haldane once famously said that the universe is not only stranger than we imagine, but may be stranger than we can imagine. He might as well have been talking about the human genome, chock full of noncoding introns and transposons, as well as a new class of RNA genes, that interact in unexpected and still largely unknown ways to regulate gene expression. Thus, in 2006, we continued our work with CSHL researchers to develop interfaces to DNA databases, which open a window on the strange and wonderful world of genome science.

The *Dynamic Gene* Internet site (www.dynamicgene.org) is the educational outreach component of *Gramene*, an online resource for analyzing and comparing genes from plants in the *Gramineae*, or grass, family. This group includes the cereal grains that feed most of the world's people—rice, wheat, corn, barley, sorghum, millet, oats, and rye. The site is being developed in collaboration with CSHL genomicists Doreen Ware and Lincoln Stein, as well as three faculty producers: Debra Burhans (Canisius College, Buffalo, New York), Charlie Gutierrez (John H. Reagan High School, Austin, Texas), and Bob Wheeler (Pine Creek High School, Colorado Springs, Colorado).

The site is designed to let students learn about plant genomes by using bioinformatics to analyze genes in the newly sequenced rice and maize genomes. Many of these genes have only been predicted by computers and have never been closely examined by human beings! The site's name emphasizes the gene both as a dynamic structure that changes through evolutionary time and as a dynamic concept that changes with our increasing knowledge of genome organization. The design for *Dynamic Gene* recalls the "streamlining movement" that influenced design during the middle of the 20th century with ideas borrowed from aviation and automobile design.

Animated tutorials in the first three sections—*Meaning*, *Structure*, and *Evidence*—illustrate (1) how DNA sequences encode biological information, (2) how bioinformatics uncovers sequence patterns that predict the structural components of genes, and (3) how computer-generated gene "models" are annotated with gene features and evidence from biological experiments. The *Annotation* section provides step-by-step instructions on how to analyze a gene model with *Apollo*, research software that was used to annotate the *Drosophila* genome. Once students understand the basics of gene annotation, they go to the *Projects* section to download large DNA sequences from cereal chromosomes. They then pit their logic against the computer that predicted the gene models encountered and upload their new annotations to share with other researchers.

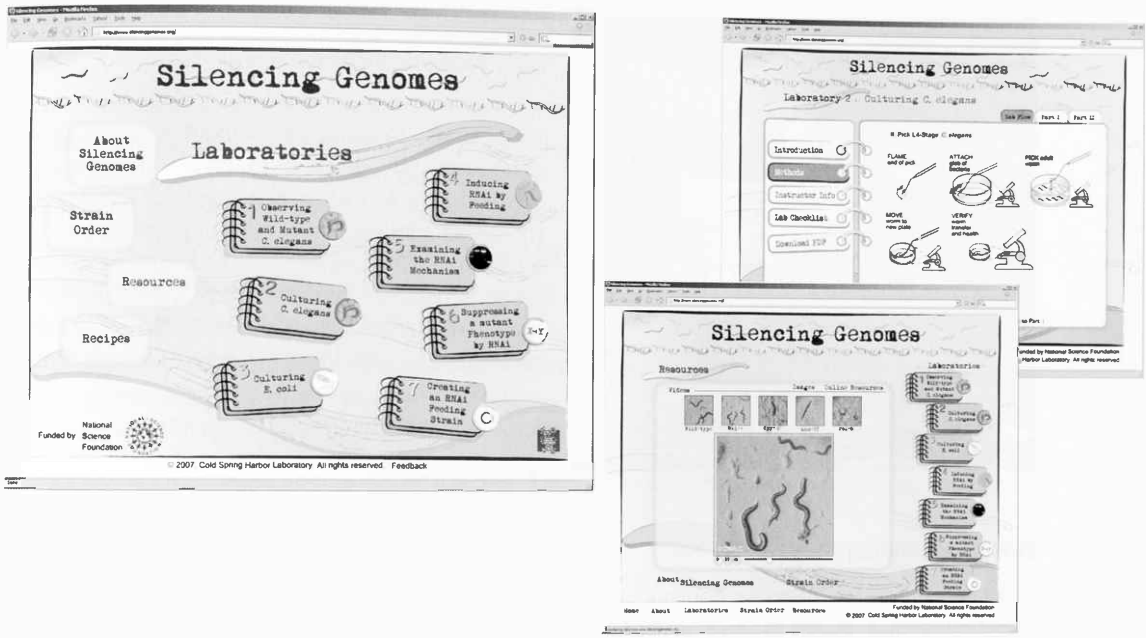
Bringing Nobel-winning Science to Students

With complete genome sequences accumulating at an accelerating rate, ahead lies the massive task of determining the physiological function of thousands of newly identified genes for which little is known beyond their sequences. The 2006 Nobel Prize in Physiology or Medicine recognized the discovery RNA interference (RNAi), a basic mechanism of gene regulation that also provides an important new tool for functional genome analysis. American scientists Andrew Fire and Craig Mello shared the Prize for showing that short, double-stranded RNA (dsRNA) molecules can down-regulate gene expression of a corresponding target gene. By deliberately introducing defined sequences of dsRNA into living organisms, biologists can observe the physiological consequences of "silencing" virtually any gene in *Caenorhabditis elegans*, as well as other plants and animals. MicroRNAs, which use part of the RNAi mechanism, have emerged as a powerful force in large-scale gene regulation and genome organization. CSHL researchers Greg Hannon, Leemor Joshua-Tor, and Scott Lowe have pioneered work on the mechanism of RNAi and its adaptation as a tool for genome exploration.

Despite its power, RNAi is amazingly simple to perform in the roundworm *C. elegans*, an important model system for eukaryotic gene function. Any gene of choice can be "silenced" merely by feeding worms bacteria that express the correct dsRNA. In its simplest form, RNAi requires little more than the ability to grow bacteria and observe *C. elegans* traits with a dissecting microscope. The vast majority of high schools and colleges meet these requirements, making RNAi in *C. elegans* potentially more accessible than other molecular techniques for which specialized equipment is required, such as polymerase chain reaction (PCR) and gel electrophoresis. For these reasons, we have devoted considerable effort to developing the RNAi/*C. elegans* system as *the* vehicle to deliver functional genome analysis into high school and college classes.

Considering the banner year for RNAi in Stockholm, our timing could not have been better as we completed our National Science Foundation project to develop an integrated experiment- and bioinformatics-based curriculum on RNAi in *C. elegans*. The curriculum begins with observation of mutant phenotypes and basic worm "husbandry," and then progresses to simple methods to induce RNAi and to use RNAi to rescue (compensate) a mutant phenotype. A more advanced experiment uses "single-worm PCR" to examine the mechanism of RNAi— comparing the DNA of worms with identical phenotypes induced by either RNAi or a gene mutation. The curriculum culminates with open-ended methods that support student projects. Students can perform RNAi "from scratch" using bioinformatics to develop PCR primers for a target gene, then cloning the amplified product into an RNAi feeding vector, and finally observing the phenotype of treated worms. Students also have free access to the DNALC's collection of RNAi feeding strains, which can be used to conduct a miniscreen to identify genes involved in a particular biological pathway.

An online lab notebook, *Silencing Genomes* (www.silencinggenomes.org), combines lab methods with user-friendly features adapted from the DNALC's popular text *DNA Science*, including flow charts, reagent recipes, and extensive instructor information. Supporting resources include photos and video of *C. elegans* mutants, as well as a simple checkout system to obtain any of 80 *C. elegans* mutants and *Escherichia coli* feeding strains. The Internet site also provides a launch pad for bioinformatics exercises that accompany each experiment. Students use online databases—including *WormBase* and *Pubmed*—to explore the molecular genetics and physiological functions of the genes targeted by RNAi.



NCBI's BLAST and the DNALC *Sequence Server* are used to explore the evolutionary relatedness of genes in worms and humans.

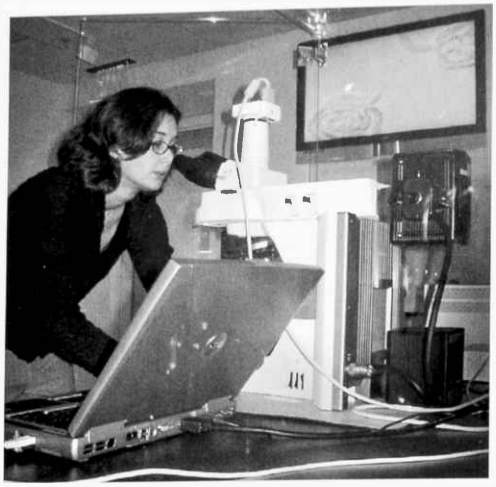
The experiments were initially tested during a spring workshop of our Advisory Panel of 12 lead high school and college faculty from around the United States. Building on their feedback, the curriculum was then field-tested at summer workshops in Oklahoma City, San Francisco, and New York City, which were attended by 53 high school teachers, 6 junior college faculty, and 9 four-year college faculty. In addition, the RNAi curriculum was a major focus of the Amgen *Leadership Symposium* and workshops conducted for the Singapore Ministry of Education and the Novartis Exploratory Clinical Development Group.

To get a quick picture of early classroom implementation, in December, we administered an online survey for faculty who participated in testing summer workshops. The majority (71%) of respondents had already begun to integrate workshop content into their teaching, by using RNAi (51%) or *C. elegans* (41%) as examples while teaching about other topics or by teaching entire units of RNAi (37%) or *C. elegans* (16%). This teaching impacted 1781 students and 58 other teachers. More than a quarter (27%) had done one or more labs—basic *C. elegans* manipulation and mutant analysis (25% each), RNAi by feeding (16%), analyzing mutants by PCR (12%), and generating a targeting vector (4%). This lab instruction impacted 329 students and 17 teachers. The same proportion (27%) had used bioinformatics to analyze *C. elegans* genes or to compare worm to human genes, impacting 410 students and 19 teachers.

Real-time Microscopy Exhibit

The DNALC's museum exhibits are tightly tied to experiments that students conduct in the teaching labs. For several years, an Applied Biosystems 307 DNA sequencer did double duty as exhibit as it sequenced mitochondrial DNA samples isolated by students in the teaching labs and submitted from schools around the United States. As our volume increased to more than 5000 student sequences per year, we shifted this work to a faster capillary machine at the CSHL Genome Center. In 2006, this space was taken over by a new Nikon Eclipse 80i microscope, with fluorescence and differential interference contrast (DIC) optics. A high-resolution digital camera output to a 46-inch LCD display allows us to display striking, live images taken from the microscope.

Real-time exhibits prepared each day give students a glimpse of the world as seen through this research-grade microscope. As a default, live mounts of *C. elegans* mutants, including the bizarre "bag of worms," coordinate with an adjacent exhibit on key model systems used in biological research. At



Educator Lauren Weidler focuses in on *C. elegans* with the Nikon fluorescence microscope and flat-panel display newly installed in the exhibit space.

other times, mounts complement a variety of labs conducted by 5th to 12th grade students. Cells scraped from an instructor's cheek, or stained chromosomes spreads, show the source of student DNA for human PCR. Live *E. coli* or *C. elegans* expressing green fluorescent protein (GFP) vividly illustrate the results of student's own efforts to genetically engineer bacteria with the GFP gene. Mounts of plant and animal cells, as well as *C. elegans* mutants, reinforce student observations of differences in cell structure and variations in phenotype.

DNA Sequencing Service

The DNA Sequencing Service continued to offer students nationwide the opportunity to look at their own DNA sequence and to use it as the basis for understanding human diversity. Every human cell has a "second" genome, found in the cell's energy-generating organelle, the mitochondrion. Mutations are common in part of the mitochondrial chromosome, largely due to exposure to oxygen-free radicals generated as a by-product of energy production. Because of this mutational variability, the noncoding region of the mitochondrial chromosome is a mainstay in studies of human variation and evolution.

The DNALC popularized methods to use mitochondrial mutation analysis in education, focusing on the same region of DNA featured in the National Geographic's Genographic Project and the popular book *The Seven Daughters of Eve*. Using DNALC protocols or ready-to-use kits, students isolate a 440-nucleotide sequence of their mitochondrial genome, and then send their samples by overnight mail to the DNALC. At the DNALC, college interns Alina Duvall (Hofstra University) and Jennifer Aiello (Long Island University) perform dye terminator reactions that label student samples for DNA sequencing. The reactions are then sent to the CSHL Sequencing Shared Resource Facility in Woodbury, where they are sequenced on an Applied Biosystems 3730xl Genetic Analyzer. The finished sequences are uploaded to a student DNA database at the DNALC's BioServers Internet site (www.bioservers.org).

Using bioinformatics tools at the Internet site, students can compare their own mitochondrial DNA with their classmates' to see that people have inherited unique patterns of mutations. In comparing mitochondrial DNA samples from world populations, students can understand the evidence for the human lineage developing in Africa and recently migrating "out of Africa" to populate the rest of the world. Students can also compare ancient DNA samples from Neandertals and Otzi "the ice man" to understand their relationships to modern humans.

In 2006, we sequenced 6400 students' DNA samples submitted from 31 states, Washington, D.C., and Canada. On average, results were posted on the Internet site within 12 days of receipt. This unique educational system—DNA sequencing, student DNA database, and Internet bioinformatics tools—is provided free of charge to high school and college classes. This is made possible by the donation of sequencing reagents by Applied Biosystems of Foster City, California.

Genes to Cognition Online

We continued with key formative work on the new Internet site, *Genes to Cognition (G2C) Online*, which will explore the molecular basis of human thinking and disorders of thinking. During the first part of the year, we worked closely with advisory panelist and education pioneer, Joe Novak, to develop methods to obtain detailed concept maps in three knowledge domains: cognitive disorders, approaches to science, and systems-level biology. These concept maps form the backbone of the *G2C Online* "knowl-

edge network," which guides content development and defines how different content items are related to one another. Thirteen experts from neuroscience and education were interviewed:

- Eric Kandel (Nobel laureate, Columbia University): Basic memory mechanisms.
- Steve Hyman (Harvard University Provost and Harvard University Medical School Professor of Neurobiology): Reward- and fear-based learning.
- Marilyn Albert (Johns Hopkins University): Alzheimer's disease.
- Ray DePaulo and Kay Redfield Jamison (Johns Hopkins University): Bipolar disorder.
- Adam Kaplin (Johns Hopkins University): Depression.
- Alysa Doyle (Harvard University): Attention deficit and hyperactivity disorder.
- Daniel Weinberger (National Institutes of Health): Schizophrenia.
- Seth Grant (Wellcome Trust Sanger Institute): Genomic neuroscience.
- Joy Hirsch (Columbia University): Neuroimaging.
- Mary Colvard, Laura Maitland, and Caren Gough (Faculty Fellows): Biology and psychology education.

In conjunction with concept mapping, we developed an Internet-accessible editing system to meta-tag *G2C* content. The system uses key words, scales, and classifications to define an item's position in the knowledge network. A *Yahoo!* term extractor automatically identifies suggested keywords from any inputted text field. The item's relevance is then rated according to 20 conceptual domains within biological systems, cognitive disorders, and approaches to science. The system also collects a number of basic data fields that we have developed as a collaborator in BiosciEdNet, a National Digital Science Library (NSDL) project headed by the American Association for the Advancement of Science. The NSDL metadata scheme aligns with the other major projects and includes technical specifications, media type, educational uses and context, end-user roles, knowledge taxons, creator information, life cycle, and rights.

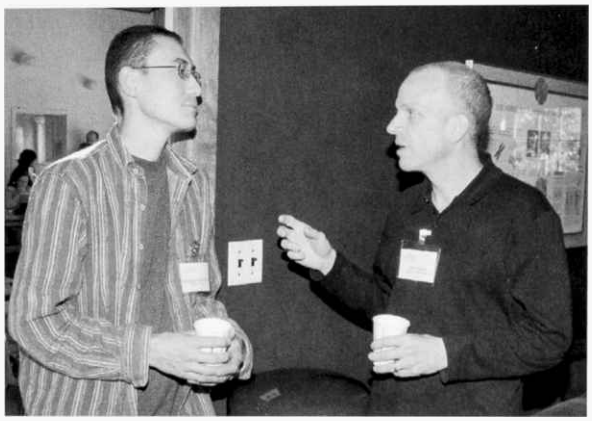
We chose autism as the topic for which to develop prototype content and interactive student activities. Of note is an emotion-recognition game that highlights the difficulty many autistic people have in "reading" facial expressions. This engaging activity is framed within the context of current research on the neural and genetic basis of the disorder, including interviews with Trevor Robbins (Cambridge University), Ron McKay (University of Edinburgh), and Dan Geschwind (University of California, Los Angeles). We also

Genes to Cognition (G2C) Online demonstration screen shots featuring autism.

capitalized on the project's February Advisory Panel meeting at the Wellcome Trust Sanger Institute, videotaping interviews and lab demonstrations with collaborators on the *G2C* research team.

In June, we conducted the first evaluation of the prototype site. High school biology and social science students reviewed two entirely different *G2C Online* designs and navigation systems. We were especially interested in their understanding of the purpose of the site and how to move from topic to topic. The student reactions and misperceptions prompted important changes that should improve how they interact with the site and integrate the "big" ideas we hope to get across. This study is the first of a number of keystone evaluations that will guide the development of *G2C Online* and, ultimately, demonstrate its usefulness in modern biology and psychology education.

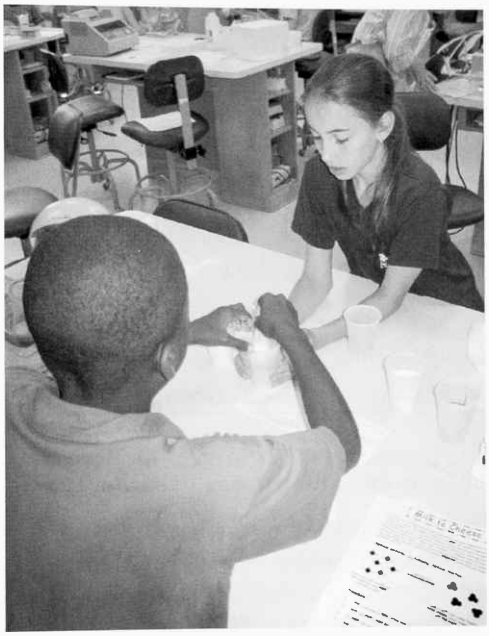
The project also gained insight from a high-level workshop, *New Horizons in Internet Site Development*, held October 22–25 at the CSHL Banbury Center. Cochaired by Ed Rover, President of the Dana Foundation, and Marshall Smith, Director of Education at the Hewlett Foundation, the 2.5-day meeting drew together 30 experts and opinion leaders from diverse fields, including cognitive science, neuroscience, network theory, knowledge management, learning theory, and technology convergence. A common theme throughout the meeting was the growing ability of the Internet to connect people in real-time in "communities" of common interest. Among the presenters were Tim Tully (CSHL), Walter Baer (Annenberg Center for Communication), John Jungck (Beloit College), John Kruper (Cardean Learning Group), John Beisty (SAP America), and Katy Börner (Indiana University). A working paper on sights gained from the meeting was developed with the Center for Children and Technology (CCT), with whom we are collaborating to evaluate site development.



New Horizons in Internet Site Development presenters Simon Buckingham (Open University) and John Kruper (Cardean Learning Group) network during session break. Conference organizer John Connolly and Katy Börner (Indiana University) discuss her talk on mapping science.

Record Number of Real and Virtual Visitors in 2006

Annual visitation topped 40,000 in 2006. This included 29,657 students who conducted experiments with DNALC staff (on field trips to the DNALC or at their own schools) and 824 summer campers who spent a week at the DNALC—both record highs. The DNALC has been the beneficiary of a growing demand for science enrichment, precipitated by new teaching standards and the specter of the impending national science achievement test for 6th graders in 2007. More than 60 school systems participated in middle school programs, and younger students comprised a majority of our clientele for academic year field trips and summer workshops. A typical middle school field trip includes a mix of hands-on labs (such as DNA extraction or bacterial transformation), guided tour of *The Genes We Share* exhibit, and virtual labs (such as *The Mystery of Anastasia* and *Otzi: The Iceman Cometh*). As basic genetics and biotechnology labs have moved increasingly to the middle schools, we have encouraged high school faculty to upgrade their instruction. Thus, more than half of high school field trips allowed students to look at their own DNA and to understand how DNA differences (polymorphisms) are used to understand human evolution.



Middle-school students participate in a *World of Enzymes* summer camp.

A grant from the Porter Foundation allowed us to provide field trip scholarships for 1378 middle school students and 569 high school students from underserved schools from Long Island, Queens, Brooklyn, and Manhattan. This included 222 students from the Gateway Institute for Pre-College Education, which provides intensive enrichment to prepare high school students for success in college. The grant also supported 30 disadvantaged students who attended a five-day workshop on forensic biology conducted in cooperation with John Jay College of Criminal Justice, and four students who attended DNA camps at the DNALC.

Saturday DNA! Programs offered 375 parents and children fun opportunities to learn about the latest developments in the biological sciences. The DNALC's education and scientific divisions presented lively two-hour programs, such as "When Dinosaurs Roamed the Earth," "Jellyfish Genes," and "Mapping Your Way Through DNA." Sessions included hands-on DNA experiments, computer bioinformatics, and microscopic observation.

The DNALC's reach was effectively doubled by the activities of four institutions that license our teaching and Internet technology. The DNA Learning Lab at the Singapore Science Centre led the way conducting labs with 22,188 students and providing outreach activities for an additional 14,799 visitors. The DNA Centre at Singapore's National Institute of Education provided in-depth training for 278 precollege teachers. Vienna Open Lab, located in the new Campus Vienna Biocenter complex began operation in the spring, providing labs for 990 students, training for 13 teachers, and outreach activities for 425 visitors. The South Carolina DNA Learning Center at Clemson University got off to a strong start, reaching 926 students with lab filed trips, 86 students in extended summer camps, and 425 public participants.

Visits to DNALC's family of nine Internet sites rose 14% in 2006 to 7 million. *BioServers*, tools for analyzing student DNA differences, registered the largest increase (43%), followed by *DNA Interactive*, commemorating the 50th anniversary of the discovery of the structure of DNA (31%), and *Image Archive on the American Eugenics Movement*, searchable materials on the misuse of genetics in the early 20th century (16%). In January, we launched *Inside Cancer*, a multimedia resource on the molecular and cellular basis of cancer. During the year, it garnered considerable recognition, including selection as finalist in the Pirelli International Awards (life sciences category), *Science* Magazine *Netwatch* (May 12), *Yahoo* "New and Notable Sites" (May 18), and *Adobe* Site of the Day (August 4).

Internet Site	Average Visit Length (in minutes)	Visits in 2006	% Change Over 2005
Gene Almanac	9:30	2,299,503	8.96
DNA from the Beginning	8:48	1,481,110	9.25
Your Genes, Your Health	8:04	1,115,186	–2.13
DNA Interactive	8:31	1,266,555	31.26
Image Archive on the American Eugenics Movement	12:52	331,388	15.71
BioServers	17:17	228,464	43.48
Genetic Origins	9:26	150,954	7.58
Inside Cancer	8:39	120,342	n.a.
All Sites	**10:23**	**7,002,140**	**13.74%**

Insights into Internet Site Use

Internet educators are increasingly called on to provide data about who uses their sites and what impact they have. To answer this call, in November, we administered an e-mail and online survey that provided a glimpse of how the DNALC's *Inside Cancer* and the *Image Archive on the American Eugenics Movement* sites are used by teachers, students, health professionals, and the general public. Surveys were completed by 1515 *Inside Cancer* and 931 *Eugenics Archive* users. (*Inside Cancer* response percentages are listed first and in italics.)

Consistent with studies by Nielson and the National Center for Education Statistics which show that the United States is well "wired," the vast majority of respondents accessed the site via a broadband connection (*88%*/83%)—with most connecting from home (*47%*, 47%), school (*24%*, 28%), or work (*23%*, 21%). Respondents were enthusiastic about the sites, with the vast majority giving good or excellent ratings on rating overall content (*90%*, 90%), content authority (*87%*, 90%), understandability (*87%*, 87%), videos (*86%*), design (*84%*, 87%), and navigation (*81%*, 81%). Nearly all (*98%*, 98%) said that they likely would visit the sites again and would recommend them to others (*95%*, 93%), including to students (*69%*, 68%), teachers (*63%*, 65%), professionals (*36%*, 39%), cancer patients (*24%*), and friends and family (42%).

The majority of respondents were educators (*55%*, 57%), followed by students (*20%*, 23%) and medical or science professionals (*19%*, 12%). Of educators, most reported readily available computer and Internet resources, including presenter computer with video projector (*74%*, 74%) and broadband access (*61%*, 63%), and student computers with broadband access (*71%*, 74%). Most educators had come to the site looking for lesson materials (*76%*, 74%) or links to assign for student readings (*44%*, 39%), and nearly all (*98%*, 95%) said they were likely to continue to use the site in the future to prepare for class or for student assignments. A number of teachers had already used the sites in their classes (*43%*, 56%) impacting the following number of students.

Classroom Use	Student Exposures	
	Inside Cancer	*Eugenics Archive*
Used site for information for preparing a lesson or lecture	13,383	13,720
Presented site content in lecture or seminar	10,855	10,666
Students used site in class or computer lab	8,610	7,886
Assigned students site as homework or independent study	7,288	8,191
Provided link to site from a community resource	5,607	4,078

Most educators had used the sites with secondary (*64%*, 65%) and college (*22%*, 25%) classes, with fewer in middle school or postgraduate classes (*7% each*, 4% each). Most secondary teachers had used the sites in general biology (*64%*, 66%) or Advanced Placement or honors (*49%*, 45%) classes.

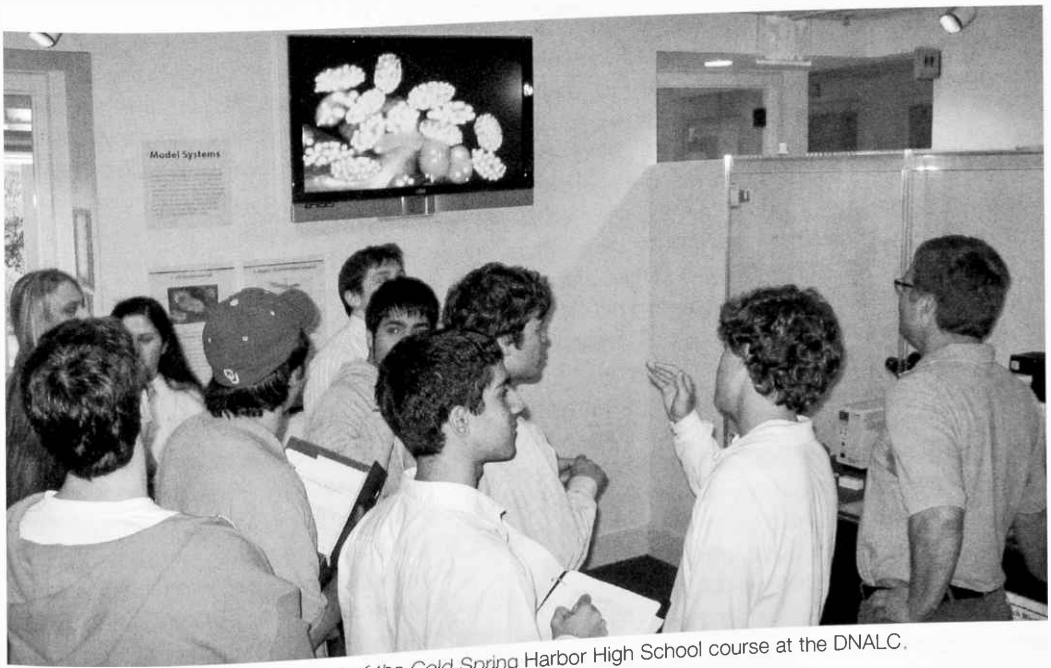
Although relatively few high school teachers had used *Inside Cancer* for health education classes (*6%*), a majority of college faculty had used the site with health and allied health classes (*63%*). These results suggest that there is a strong health context for the site but that we will need to work to develop this constituency at the high school level. Relatively few high school teachers had used the *Eugenics Archive* for nonscience classes (9%). More college faculty had used the site for nonscience classes (32%), including history, philosophy, or psychology—illustrating the potential for cross-disciplinary instruction.

Most students had visited the sites for a school assignment or project (*44%,* 67%) or for general interest (*53%,* 31%) or for a school assignment. Most also thought it likely that they would return to the sites as a reference for homework or a project (*49%*, 65%) or to help them understand course materials (*60%,* 49%).

We were particularly interested in gauging site accuracy by way of the responses of historians and medical, legal, and science professionals. Statistical comparisons revealed no significant differences between professionals' evaluations of the sites compared to other demographic groups. The vast majority of professionals gave good or excellent ratings for rated overall content (*89%,* 87%) and content authority (*86%,* 89%) and said that they would use the site in the future (*95%,* 92%). Most professionals intended to use the *Inside Cancer* site for background information (*68%*), to provide a link to a client or patient (*47%*), or as a source of content to present at a meeting or seminar (*40%*). Professionals thought that they were most likely to use *Eugenics Archive* materials for academic research (61%), to prepare for a meeting (59%), or to present at a meeting or seminar (37%).

Cold Spring Harbor Partnership

Since its founding in 1988, the DNALC's interaction with students had been mainly limited to brief exposures during academic year field trips or summer DNA camps. Although we work intensively with interns, we had for years hoped to implement a "capstone" laboratory course in molecular and genomic biology in conjunction with neighboring Cold Spring Harbor High School (CSHHS). In the spring, we realized this long-held desire when we graduated our first class of 21 high school seniors who participated in the Cold Spring Harbor Partnership. Then, in the fall, we welcomed our second class of Partnership students.



Students observe *C. elegans* as part of the Cold Spring Harbor High School course at the DNALC.

DNALC staff and biology teacher Scott Renart co-teaches the year-long Partnership course on alternating days at CSHHS and the DNALC. The course uses lab experimentation to explore the genetics and genomics of four major biological systems: bacteria, plants, roundworms, and humans. In the bacterial unit, students learn the basics of gene manipulation by making and analyzing recombinant DNA molecules in the bacterium *E. coli*. The plant unit emphasizes concepts in gene structure, including a detailed analysis of newly sequenced genes in rice and an assay for transgenes in genetically modified food. In the human unit, students use their own DNA differences to study genetic variation in human populations and the evolution of sensory receptors. In the final unit, students use the cutting-edge technique of RNAi to study gene function in the nematode *C. elegans*.

The first year's course included two miniprojects in the bacterial and human system. The second year's course places greater emphasis on independent research, providing students opportunities to design and troubleshoot experiments of their own design. This training in critical thinking will prepare the Partnership students for success in the business, legal, and social sciences, as well as the biological sciences.

Teacher Professional Development

Professional development activities reached a record number of high school and college faculty; 281 educators attended 13 2- to 5-day workshops on RNAi, plant genomics, bioinformatics, and genetics. Held in six U.S. states and Singapore, these workshops were sponsored by the National Science Foundation (NSF), National Institutes of Health (NIH), Amgen Foundation, Aspen Science Center, Wachovia Bank, and the Singapore Ministry of Education. An additional 220 educators participated in miniworkshops conducted at professional meetings in collaboration with Carolina Biological Supply Company. Topics at miniworkshops included detecting GM foods by PCR, forensic identification of the Romanov family remains, molecular genetics of bitter taste, bioinformatics, and the American eugenics movement.

In July, our flagship *Leadership Symposium* was reinstated with three-year support from the Amgen Foundation. The program drew 23 high school teachers from 16 American states, Colombia, and Singapore. Originally established in the early 1990s with support from the National Science Foundation (NSF), this three-week long program provides super-order training for teachers who have significant teaching program in genetics or biotechnology. During their stay, *Leadership* participants were immersed in science, living on the main CSHL campus, walking in the footsteps of Nobel Prize winners, and talking informally with high-level participants in the Lab's postgraduate training courses.

The curriculum included experiments in bacteria, plants, roundworms, and humans. Participants had the opportunity to test the DNALC's latest lab methods, in advance their availability at other institutions or from commercial suppliers. These experiments included assaying for mutations in olfactory receptors, analyzing newly discovered genes in rice, and using RNAi to "knock down" genes in the roundworm *C. elegans*. The final week was dedicated to independent study or group projects that included screening supermarket foods for genetic modification, expanding computer-based studies on bioinformatics, constructing web pages, and constructing new reagents for RNAi screens.

With support from the National Science Foundation and the Dana Foundation, ten college and high school faculty took on summer fellowships at Cold Spring Harbor. Six teachers assisted with the development of the new Internet sites *G2C Online* and *Dynamic Gene*; four split time between the DNALC and advanced plant genomics on the main CSHL campus.

The DNALC's professional development programs retained widespread popularity among educators, even while many teacher-training programs experienced difficulty in recruiting faculty participants. As evidence of educator interest, we received more than 240 qualified applications for a total of 152 spaces in six grant-funded summer workshops. Collaborating with historically Black (HBCU) or Hispanic (HACU) institutions enabled us to increase the proportionate attendance of underrepresented minorities; 48% of participants were either Black or Hispanic at workshops conducted at Rust College and North Carolina Agricultural and Technical State University, which compares to 11% at Oklahoma City Community College, City College San Francisco, and New York Institute of Technology. This observation prompted us to increase our collaboration with HBCU and HACU institutions in three proposals

submitted to the NIH and NSF. Ultimately, this initiative will enable us to tackle the ethnographic disparity between groups pursuing careers in science and the general population.

The DNALC has provided the educational outreach components to four NSF-funded research programs led primarily by CSHL faculty. All four educational programs entail development and teacher-training components.

- *Develop a comparative mapping database for cereals*, Lincoln Stein and Doreen Ware (CSHL). We completed a pilot version of the *Dynamic Gene* Internet site, developed a *Dynamic Gene* training curriculum, and disseminated the curriculum in five 1.5-day workshops to 111 high school and college faculty. Teachers were instructed in the use of the educational segments of the *Dynamic Gene* Internet site, as well as in gene annotation using Apollo. Additional workshops will be conducted in 2007 and 2008 for a final total of 16 workshops.
- *Functional analysis of genes in maize*, Marja Timmermans (CSHL) and Mike Scanlon (Cornell University); and *Finishing the sequencing of the rice genome*, Dick McCombie (CSHL). These projects have the objective of expanding the participation of underrepresented minorities in teaching plant genomic biology. Faculty Fellows are selected from minority institutions and spend time in a CSHL research lab as well as at the DNALC, giving them the opportunity to update their research and educational portfolios. In 2006, Dr. Diomede Buzingo (Langston University, Langston, Oklahoma) and Dr. Gokhan Hacisalihoglu (Florida Agricultural & Mechanical University, Tallahassee, Florida) joined us as Faculty Fellows on the maize project. After completing training, the Fellows host a DNALC course at their home institution to introduce local educators to classroom-tailored laboratory and bioinformatics modules. We conducted educator workshops at the home institutions of Dr. Muhammad Mian (Rust College in Holly Springs, Mississippi) and Dr. Mary Smith (North Carolina Agricultural and Technical State University in Greensboro, North Carolina), 2005 Fellows on the rice project. Thirty-eight high school teachers and two-year college faculty participated in these courses. To support local dissemination, each Fellow's institution receives an equipment package, including a thermocycler, centrifuge, UV-lightbox, camera, pipettes and precast gels. Fellows then establish an equipment loan program to provide local educators the physical resources to conduct the plant genomics experiments in their classrooms.
- *Determine the function of protein-coding genes in* Arabidopsis thaliana, David Jackson (CSHL). Although funding for this program expired in 2004, we concluded the educational component of this project with a final workshop in August 2006 at the New York Institute of Technology. Seventeen college educators were introduced to plant genomics laboratory and bioinformatics teaching modules developed during the program.

Watson School of Biological Sciences

Our collaboration with the Watson School of Biological Sciences provides the CSHL graduate students with a unique teaching opportunity. Rather than the traditional training of graduate students as Teaching Assistants, Watson school students complete a spring rotation working with middle and high school students at the DNALC. Over the course of 12 half-day sessions, students work in pairs under the tutelage of seasoned DNALC instructors.

During the first phase of the training, students observe a DNALC instructor deliver a laboratory to a visiting class. Postobservation, the students begin to organize a lesson plan, which integrates their own experience within the context of a specific experiment. The second phase is co-teaching, during which each student is responsible for delivering a specific part of the laboratory. The third phase is independent instruction, during which the students work together to present an entire laboratory—under the observation of the DNALC instructor. During each phase, students receive oral and written critiques aimed at strengthening their presentation and class management skills. After repeating this learning process at the middle school and high school levels, the students are required to independently teach an additional three lessons of their choice.

Some students opt to travel to a local school district to deliver instruction to several middle school classes. Although the CSHL graduate students are well versed in molecular biology, few have ever attempted to teach these concepts to young students. We believe that the skills required to deliver a successful lab experience to precollege students—engagement, organization, and time management—are the same skills needed to communicate with any audience.

Staff and Interns

At summer's end, we said goodbye to high school instructor Jeanette Collette, who took a position at Commack High School to teach Regents biology, forensics, and scientific research. During two years at the DNALC, she proved adept in many areas of biology. She had a key role in developing the *Greenomes* plant curriculum, growing specialized *Arabidopsis* and maize plants, assisting with workshop instruction, and working on the companion Internet site. Through thick and thin, she sang her way through the labs and hallways. We will miss her enthusiasm and sunny personality.

In December, computer programmer Adrian Arva left to pursue a Master's degree in Business Administration at the prestigious Institute for Management Development (IMD) in Lausanne, Switzerland. A native of Romania, in 2001, Adrian responded to a job ad on the DNALC Internet, passed a phone interview with CSHL Professor Lincoln Stein, and was hired site unseen. We could not have been luckier to find such a brilliant programmer during the height of the Internet bubble. His masterful programming, with added insight from his M.D. background, provided the backbone of the DNALC's Internet venture during a time of rapid expansion.

So taken were we with Adrian's performance that we immediately hired another Romanian, Cornel Ghiban, to take his place at year's end. After studying computer science and engineering at Technical "Gheorghe Asachi" University in Iasi, Cornel designed software for financial clients. He also did contract work for the DNALC, developing backend registration, database, and Webserver functions for several of our Internet sites.

During the year, we welcomed Malissa Hewitt back to the instructional staff. Malissa started at the DNALC as a laboratory instructor in 1994, becoming the first full-time employee devoted to middle school instruction. In 1997, she stepped up to manage the middle school program and then left in 1998 to become a full-time mom. Her deep understanding of the history of the DNALC, her strong connections to the local community, and her love of children make Malissa's return especially valuable.

Student interns continued to provide key support for our activities. The sequencing service continued to grow with the help of college interns Alina Duvall (Hofstra University), Jennifer Aiello (C.W. Post), and Alexandra Sloane (Loyola College, Maryland). Joining the program in 2006 were Yusuf Anwar (Syosset), Carissa Maurin (Lynbrook), Seth Schortz (Half Hollow Hills), Andrea Tufano (Syosset), and Brittany Woods (Cold Spring Harbor High School). The new members joined returning interns Matt Giambrone (Walt Whitman), Matthew Levy (Kings Park), Tama Mizuno (Northport), Ronnie Morasse (Plainedge), Nick Wilken (Kings Park), and Janice Yong (Kings Park).

While most interns perform general preparation for lab experiments, Brittany Woods and Carissa Maurin worked with Bruce Nash to develop and maintain *C. elegans* stocks used in our NSF program. Kyle Shybunko, a research student at Ward Melville, worked with Greg Chin to submit a project on human olfactory receptors to the Siemens competition. Yusuf Anwar's research on tissue development at Stony Brook University won him a Special Congressional Recognition Award and placed him as a semifinalist in the Siemens Competition.

Several interns returned from college to assist with summer workshops: Benjamin Blonde (Amherst College), Bryn Donovan (University of Delaware), Kimberly Izzo (Indiana University), and Marie Mizuno (SUNY Binghamton). In August, Joseph Hakoopian (Walt Whitman) left for his freshman year at Cornell University; Margarita Varer (Huntington) left for SUNY Binghamton, and Brittany Woods (Cold Spring Harbor) left for Boston College.

Corporate Advisory Board

Established in 1992, the Corporate Advisory Board (CAB) serves as liaison to the Long Island business community. The CAB conducts a golf tournament and the annual fund campaign to raise unrestricted funds to support the DNALC.

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Edward A. Chernoff, *Motors & Armatures Inc.*

CSHL Trustee Liaison:
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Zock Zamaniyan, Eppendorf North America
Teresa Kemp-Zielenski, United Way of L.I.
Hans Zobel, Festo Corporation

David A. Micklos
Executive Director

2006 Workshops, Meetings, and Collaborations

January 9–11	NSF *Gramene* Faculty Fellowship: Robert Wheeler, Pine Creek High School, Colorado Springs, Colorado
January 11	Site visit and museum tour for the incoming class of the Watson School of Biological Sciences, Cold Spring Harbor Laboratory (CSHL)
January 21	*Saturday DNA!,* "When Dinosaurs Roamed the Earth" and "Teenage Mutant Critters," DNALC
January 30	Site visit by Lisa Blair, Director, Wendi O'Rand, Educator, and Denise Richard, Administrative Assistant, Abernathy Science Education Center, Girard, Kansas
February 1–2	BiosciEdNet (BEN) Collaborators Meeting, Washington, D.C.
February 6–10	*G2C Online* Advisory Meeting, Cambridge, United Kingdom
February 11	CSI Workshop for Children Associated with Hermansky Purdlak Syndrome Foundation Annual Meeting
February 11	*Saturday DNA!,* "2006: A Science Odyssey" and "One Fish, Two Fish, Green Fish, Glofish!," DNALC
February 15	Site visit by Norman Kelker, Senior Vice President, Enzo Life Sciences, Farmingdale, New York
February 17	Annual American Association for the Advancement of Science Meeting, St. Louis, Missouri
February 24–25	Aspen Science Center Teacher Training Workshop, Aspen, Colorado
March 1	Site visit by Rogert Mummert, freelance writer, *New York Times*, New York, New York
March 1	Site visit by Theodore Roosevelt American Inn of Court, St. John's University, School of Law, Nassau County, New York
March 1	Site visit by Sharon Grosser, Executive Director, Roslyn Savings Foundation, Roslyn, New York
March 1	Site visit by Michio Ichimura, Kyowa Hakko Chemical Co., Ltd., Japan
March 2	Site visit by Patrick Foye, President, and Teri Zielenski, Senior Vice President, United Way of Long Island
March 8	Site visit by George Harrison, Harrison Family Foundation, John S. Grace, President, Sterling Grace Capital Management, and Lola N. Grace, Managing Director, Sterling Grace Capital Management, and Vice Chairman CSHL Board of Trustees
March 11	*Saturday DNA!* Seminars, "Wanted: Dead or Alive?" and "Little Fly, Why Did You End Up a Guy?," DNALC
March 15	Site visit by David Skuse, Institute of Child Health, London, United Kingdom
March 19–21	*RNAi* Advisory Board Meeting, DNALC
March 21	West Side School Lecture, "Why All the Fuss about Bird Flu?," Lee Henry, CSHL
March 22	Site visit to William Randolph Hearst Foundation, New York, New York
March 23	Annual *Cancer Biomedical Informatics Grid* (caBIG) Meeting, Bethesda, Maryland
April 4	*Great Moments in DNA Science Honors Student Seminar,* Hollis Cline, CSHL, "Seeing is Believing: Imaging Brain Development in the Intact Animal," CSHL
April 6–8	National Science Teachers Association National Meeting, Anaheim, California
April 11	*Great Moments in DNA Science Honors Student Seminar*, Grigori Enikolopov, CSHL, "Teaching an Old Dog New Tricks? Stem Cells in the Adult Brain," CSHL
April 14	Site visit by Bob Wheeler, Pine Creek High School, Colorado Springs, Colorado; and Brenda Dempsey, George Washington High School, Denver, Colorado
April 18	Site visit by Lillian DeRosa and family, friends of Leo Guthart, owner of Topspin Partners, LP, Roslyn Heights, New York
April 19	Site visit by Lynda Parmely, Program Officer, Horace Hagedorn Foundation, Jericho, New York
April 20	Site visit by Joanne Duhl, Goldman Sachs Foundation, New York, New York
April 20	Site visit by Edward Atwood, Vice President Multimedia Services, Cablevision, Bethpage, New York
April 21	Site visit by Marcia Bishop, Associate Director, and Ronald Sjoerdsma, Fellow, Van Andel Education Institute, Grand Rapids, Michigan
April 21	Site visit to Davidoff Malito & Hutcher LLP, New York, New York
April 22	*Saturday DNA!* Seminars, "Jellyfish Genes" and "Mapping Your Way Through DNA," DNALC
April 24	Site visit by Rosemary Nicholls, Senior Vice President, Teachers Federal Credit Union, Farmingville, New York
April 24	West Side School Lecture, "Why Do We Have Two Copies of Every Gene?," Alea Mills, CSHL
April 26	Site visit to the Harvard Club of New York City for National Institute of Social Sciences Little Think Tank Luncheon, New York
May 1	*Great Moments in DNA Science Honor Student Seminar*, Anthony Leotta, CSHL, "Finding Cancer Genes Using Microarrays," CSHL
May 1	Site visit to New York Hall of Science for Science Task Force Meeting, Queens, New York
May 5	Site visit to New York City Department of Education with Julia Rankin, Director of Science, New York, New York
May 7	Dedication Ceremony for the Marc David Chernoff Bioinformatics Laboratory, DNALC
May 10–11	Site visit to Carolina Biological Supply Company, Burlington, North Carolina

May 11	Site visit by Landon and Lavinia Clay, Directors, and Jim Carlson, President, Clay Mathematics Institute, Cambridge, Massachusetts
May 13	Site visit by Pamela Omidyar (wife of Pierre Omidyar, Chairman and founder of eBay) and family
May 16	Site visit by Harouna Ba, Senior Research Associate, Center for Children and Technology, New York, New York
May 16	Site visit to North Shore-Long Island Jewish Health System, Feinstein Institute for Medical Research, for talk by Lawrence Scherr, Lake Success, New York
May 18	Site visit by Jason Morris, Assistant Professor of Biology, Fordham University, New York, New York
May 18–19	Site visit to the Life Learning Center, Bologna, Italy
May 20	*Saturday DNA!* Seminars, "Bug Zappers" and "What's a Stem Cell Supposed to Do?," DNALC
May 23	Dedication of Vienna Open Lab, Vienna, Austria
June 5–9	NSF *Frontiers in Genomics: RNA Interference and Genome Annotation* Workshop, with Charlotte Mulvihill, Oklahoma City Community College, Oklahoma City, Oklahoma
June 6	*Cold Spring Harbor Partnership Program* Graduation Ceremony: Tom Dolan, Principal, Scott Renart, science teacher, and 120 students and parents, Cold Spring Harbor High School, New York
June 8	Site visit by Mahlon Hoagland, author, and Ken Gleason, science museum consultant
June 10	*Saturday DNA!* Seminars, "Fruit Fly Island" and "Conquering a Genetic Disease," DNALC
June 12–16	NSF *Plant Molecular Genetics and Genomics* Workshop, with NSF Minority Fellow Muhammad Mian, Holly Springs, Mississippi
June 13	Site visit by Kidgie Williams, diplomats, and family members of United Nations representatives, Hospitality Committee for United Nations Delegations, Inc., New York, New York
June 14	Site visit by Robert Diller, Director of Sales and Marketing, and Zock Zamanian, Brinkmann Instruments, Inc./Eppendorf AG, Westbury, NY
June 19	Site visit by Arthur Rosoff and Henry Bachman, Long Island Museum of Science & Technology, Garden City, New York
June 22	Site visit by Kathryn Silva and Ralph Brave, University of California, Sacramento
June 19–23	NSF *Plant Molecular Genetics and Genomics* Workshop, with NSF Minority Fellow Mary Smith, North Carolina Agricultural and Technical State University, Greensboro
June 26–30	Site visit by Florence Francis, Chief Scientific Officer of the Learning Lab, Singapore Science Center, Singapore
June 26–30	*Fun with DNA* Workshop, DNALC *DNA Science* Workshop, DNALC *World of Enzymes* Workshop, DNALC *Fun with DNA* Workshop, DNALC *West*
June 26–30	NSF *Frontiers in Genomics: RNA Interference and Genome Annotation* Workshop, with Elaine Johnson, Bio-Link, City College, San Francisco, California
June 26–30	*Bioinformatics*–In Silico *Biology* Workshop, California State University, Dominguez Hills: LA Biomed, Torrance, California
June 29	New York City Department of Education Advisory Board on Biotechnology Education, American Museum of Natural History, New York, New York
July 3–7	*Fun with DNA* Workshop, DNALC *Green Genes* Workshop, DNALC *DNA Science* Workshop, DNALC *Green Genes* Workshop, DNALC *West*
July 3–7	Aspen Science Center Workshops: *Fun with DNA; World of Enzymes; PCR & Genomic Biology*, Aspen, Colorado
July 4	Aspen Science Center Board of Trustees Meeting, Aspen, Colorado
July 8–23	USDA *Gramene* Faculty Fellowship: Charlie Gutierrez, John H. Reagen High School, Austin, Texas
July 9–21	NSF Minority Faculty Fellowship: Gokhan Hacisalihoglu, Florida Agricultural & Mechanical University, Tallahassee, Florida
July 9–30	Teacher Training at the DNALC: Lui Shea Nee Shirley, Victoria Junior College; Wee Sock Chin, Hua Yi Secondary School; and Petrina Then Yan Ting, Singapore Science Center, Singapore
July 10	Site visit by Josep Prous, David Prous, and Miriam Bayes, Prous Science, Barcelona, Spain
July 10–14	*Genetic Horizons* Workshop, DNALC *World of Enzymes* Workshop, DNALC *DNA Science* Workshop, DNALC *West*
July 10–28	Amgen *Leadership Symposium*, DNALC
July 17–21	Site visit by Karin Garber MAS, Vienna Open Lab–Dialog Gentechnik, Vienna, Austria
July 17–21	*Fun with DNA*, Workshop, DNALC *Human Genomics* Workshop, DNALC *Fun with DNA*, Workshop, DNALC *West*

July 21	Site visit by Daryl Ogden, Executive Director, Project GRAD of Long Island, Roosevelt, New York, and Teresa Kemp Zielenski, Senior Vice President of Community Impact, and Kristine Donnelly, United Way of Long Island, Deer Park, New York
July 24–28	*World of Enzymes* Workshop, DNALC
	Green Genes Workshop, DNALC
July 24–28	Site visit by *G2C Online* Fellows Caren Gough, Education Consultant, and Laura Maitland, AP Psychology consultant
July 25–26	Site visit by *G2C Online* consultant Joe Novack, Institute of Human and Machine Cognition (IHMC), Pensacola, Florida
July 26–August 5	NSF *Gramene* Faculty Fellowship: Debra Burhans, Canisius College, Buffalo, New York, and Robert Wheeler, Pine Creek High School, Colorado Springs, Colorado
July 26–August 5	Site visit by Suzanne Leeds, member of Cold Spring Harbor High School Improvement Team and DNALC Committee
July 31–August 4	*Fun with DNA* Workshop, DNALC
	Genetic Horizons Workshop, DNALC
	DNA Science Workshop, DNALC
	World of Enzymes Workshop, DNALC *West*
July 31–August 4	*Forensics and Human Genomics* Workshop, with Lawrence Kobilinsky, John Jay College of Criminal Justice, Manhattan, New York
August 1	Site visit by Dee Rawsthorne, John Innes Centre, Norwich, United Kingdom
August 2	Site visit by Lori Bressler, Cold Spring Harbor School Board, Cold Spring Harbor, New York
August 2	Site visit by Ida Cole, former Microsoft executive and philanthropist
August 7–11	*World of Enzymes* Workshop, DNALC
	Green Genes Workshop, DNALC
	DNA Science Workshop, DNALC
	NSF *Plant Molecular Biology, Genomics and Bioinformatics* Workshop, New York Institute of Technology, New York, New York
	Forensics and Human Genomics Workshop, with Lawrence Kobilinsky, John Jay College of Criminal Justice, New York, New York
August 6–19	NSF Minority Faculty Fellow Diomede Buzingo, Langston University, Langston, Oklahoma
August 14–18	*Fun with DNA* Workshop, DNALC
	Genetic Horizons Workshop, DNALC
	DNA Science Workshop, DNALC
	*DNA Science*Workshop, DNALC *West*
August 14–18	NSF *Frontiers in Genomics: RNA Interference and Genome Annotation* Workshop, New York Institute of Technology, New York, New York
August 15	Site visit by Lisa Mars, Vice President and Director for Digital Schoolhouse Foundation, Islandia, and Teresa Kemp Zielenski, Senior Vice President of Community Impact, and Kristine Donnelly, United Way of Long Island, Deer Park, New York
August 16	Site visit by Eric Eversley, Superintendent, Freeport School District, and Teresa Kemp Zielenski, Senior Vice President of Community Impact, and Kristine Donnelly, United Way of Long Island, Deer Park, New York
August 21	Site visit by Ed Rover, President, Burt Mirsky, Vice President, Finance, and Abigal Slovonic, Program Officer, Dana Foundation; and James Watson, Chancellor, CSHL
August 21–25	Site visit by Michael Marshall, Wellcome Trust Sanger Institute, Cambridge, United Kingdom
August 21–25	*Fun with DNA* Workshop, DNALC
	World of Enzymes Workshop, DNALC
	Green Genes Workshop, DNALC
	Human Genomics Workshop, DNALC *West*
August 21–25	New York City Department of Education Educator Workshop, *DNA Investigations*, New York, New York
August 25	Site visit by Fumitaka Akeda, President, and Lee Schuett, Executive Vice President, Nikon Instruments, Melville, New York
August 28–September 1	*Genetic Horizons* Workshop, DNALC
	World of Enzymes Workshop, DNALC
	DNA Science, DNALC
	Plant Genomics Workshop, DNALC *West*
September 7	*Esquire* Magazine photo shoot with James Watson, Chancellor, CSHL
September 8	Site visit by Richard Quest, CNN's London-based anchor and correspondent, London, England
September 20	Site visit by Susan Berland, Town of Huntington Councilwoman, Huntington, New York
September 21	Site visit by Kathy Vandiver, Director of Community Outreach and Education, Center for Environmental Health Sciences, MIT Museum, Cambridge, Massachusetts

September 21	Site visit by Aledandra Manaia, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany
September 21–25	NSF *Gramene* Faculty Fellowship: Robert Wheeler, Pine Creek High School, Colorado Springs, Colorado
September 22–25	NSF *Gramene* Faculty Fellowship: Debra Burhans, Canisius College, Buffalo, New York
September 21	Site visit by Marianne Carolton, Director of Internal Communications, and James Cuniglio, Communications Coordinator, Arrow Electronics, Melville, New York
September 27	Site visit by Edward Atwood, Vice President Multimedia Services, Cablevision, Bethpage, New York
September 28	Site visit to Cold Spring Harbor High School for meeting with Jay Matuk, Principal, and Andy Clouser, Science Department Chairman, Cold Spring Harbor, New York
October 2–4	Novartis Exploratory Clinical Development Group, RNA and Cancer Biology, Banbury Center and DNALC
October 5	Site visit by Daryl Ogden, Robert Troiano, Executive Director, and Kim Arias, Director of Programs, *Project Grad* Long Island, Roosevelt, New York; and Connie Clark, Superintendent, and Robert Root, Assistant Superintendent for Curriculum Instruction and Personnel, Westbury School District, Westbury, New York
October 11–13	National Association of Biology Teachers National Meeting, Albuquerque, New Mexico
October 14	*Saturday DNA!* Seminars, "Genes and Generations" and "Spotlight on Cell Division," DNALC
October 16	Site visit by Eric Krasnoff, President, and Saied Tousi, Senior Vice President of Global Infrastructure, Pall Corporation, East Hills, New York
October 18	Site visit by Lori Bressler, President, Cold Spring Harbor Board of Education; and Judith Wilansky, Cold Spring Harbor Adult Education Program, Cold Spring Harbor, New York
October 22–24	*New Horizons in Internet Site Development* Meeting, CSHL Banbury Center
October 24	Site visit by Bob Malito, Garden City, New York, and Arthur Goldstein, New York, New York, Law Firm Davidoff, Malito & Hutcher
October 25–26	NY Enhancing Collaborative Leadership for Improved Performance in Science Education (ECLIPSE) Leadership Conference, Albany, New York
November 2–4	National Science Teachers Association Regional Meeting, Baltimore, Maryland
November 6	Science Teachers Association of New York State Conference, Ellenville, New York
November 8	Site visit by 30 members of the Princeton Club, New York, New York
November 8	Site visit by Kirk Kordeleski, President and Chief Executive Officer, and Linda Arymn, Vice President Business Development, Bethpage Federal Credit Union, Bethpage, New York
November 8	Site visit to York College, Dr. Linda Barley, Acting Provost, DNA Lab, New York, New York
November 13–23	Singapore Teacher Training, Singapore
November 14	Excellence in Science Award Annual Reception for the American Association of University Women, Huntington Branch, Huntington, New York
November 15	*FIRST (For Inspiration and Recognition of Science and Technology)* Lego Robotics Group, Long Island, New York
November 18	*Saturday DNA!* Seminars, "The Iceman Cometh" and "Heart and Sole: Studying Heart Development in Fish," DNALC
November 22–23	MOE-NIE-STAS International Science Education Conference, Singapore
November 25–December 9	Teacher Training at the DNALC: Rashidah bte Yahya, Marsiling Primary School; Ginger Tay Leng See, Telok Kurau Primary School; Han Tui Kin, Montfort Junior School; and Pok Sat Yoong, St. Hilda's Primary School
November 28	Site visit by Congressman Bud Cramer, Alabama's 5th Congressional District, U.S. House of Representatives, Alabama
November 30	Site Visit by Matthias Haury, European Molecular Biological Laboratory, Heidelberg, Germany
December 7–8	National Science Teachers Association Regional Meeting, Salt Lake City, Utah
December 8	Site visit and field trip by Minnetonka High School students and teachers, Minnetonka, Minnesota
December 8	Site visit by Manny Arias, High School Principal, and Darnel Powell, Middle School Principal, Westbury School District, New York
December 9	*Saturday DNA!* Seminars, "As the Worm Turns" and "Finding Novel Genes with Degenerate PCR," DNALC
December 11	Site visit by Hans Ullrich, freelance writer, United Kingdom
December 15	Site visit by Bettie Steinberg, Chief Scientific Officer, and Kirk Manogue, Director of Technology Transfer, Feinstein Institute for Medical Research, NS-LIJ Health Systems, Lake Success, New York
December 28	Site visit by *G2C Online* Fellows Caren Gough, Education Consultant, and Laura Maitland, Advanced Placement Psychology consultant

Sites of Major Faculty Workshops 1985–2006

Key: *Middle School* High School **College**

ALABAMA	University of Alabama, Tuscaloosa	1987–1990
ALASKA	University of Alaska, Fairbanks	1996
ARIZONA	Tuba City High School	1988
ARKANSAS	Henderson State University, Arkadelphia	1992
CALIFORNIA	**California State University, Fullerton**	**2000**
	Canada College, Redwood City	1997
	City College of San Francisco	2006
	Contra Costa County Office of Education, Pleasant Hill	2002
	Foothill College, Los Altos Hills	**1997**
	Harbor-UCLA Research & Education Institute, Torrance	2003
	Los Angeles Biomedical Research Institute (LA Biomed), Torrance	**2006**
	Laney College, Oakland	1999
	Lutheran University, Thousand Oaks	1999
	Pierce College, Los Angeles	**1998**
	Salk Institute for Biological Studies, La Jolla	2001
	San Francisco State University	**1991**
	San Jose State University	**2005**
	University of California, Davis	1986
	University of California, Northridge	**1993**
COLORADO	Aspen Science Center	2006
	Colorado College, Colorado Springs	1994
	United States Air Force Academy, Colorado Springs	**1995**
	University of Colorado, Denver	1998
CONNECTICUT	Choate Rosemary Hall, Wallingford	1987
FLORIDA	North Miami Beach Senior High School	1991
	University of Western Florida, Pensacola	1991
	Armwood Senior High School, Tampa	1991
	University of Miami School of Medicine	2000
GEORGIA	Fernbank Science Center, Atlanta	1989
	Morehouse College, Atlanta	**1991, 1996**
	Morehouse College, Atlanta	1997
HAWAII	Kamehameha Secondary School, Honolulu	1990
ILLINOIS	Argonne National Laboratory	1986, 1987
	University of Chicago	**1992, 1997**
INDIANA	Butler University, Indianapolis	1987
IDAHO	University of Idaho, Moscow	1994
IOWA	Drake University, Des Moines	1987
KANSAS	University of Kansas, Lawrence	1995
KENTUCKY	Murray State University	1988
	University of Kentucky, Lexington	1992
	Western Kentucky University, Bowling Green	1992
LOUISIANA	Jefferson Parish Public Schools, Harvey	1990
	John McDonogh High School, New Orleans	1993
MAINE	Bates College, Lewiston	1995
	Foundation for Blood Research, Scarborough	2002
MARYLAND	Annapolis Senior High School	1989
	Frederick Cancer Research Center, Frederick	1995
	McDonogh School, Baltimore	1988
	Montgomery County Public Schools	1990–1992
	St. John's College, Annapolis	*1991*
	University of Maryland, School of Medicine, Baltimore	**1999**
	National Center for Biotechnology Information, Bethesda	2002
MASSACHUSETTS	Beverly High School	1986
	Biogen, Cambridge	2002
	Boston University	**1994, 1996**
	CityLab, Boston University School of Medicine	1997
	Dover-Sherborn High School, Dover	1989
	Randolph High School	1988
	Winsor School, Boston	1987
	Whitehead Institute for Biomedical Research, Cambridge	2002
MICHIGAN	Athens High School, Troy	1989
MINNESOTA	University of Minnesota St. Paul, St. Paul	2005
MISSISSIPPI	Mississippi School for Math & Science, Columbus	1990, 1991
	Rust College, Holly Springs	2006

MISSOURI	Stowers Institute for Medical Research, Kansas City	2002
	Washington University, St. Louis	1989
	Washington University, St. Louis	**1997**
NEW HAMPSHIRE	**New Hampshire Community Technical College, Portsmouth**	**1999**
	St. Paul's School, Concord	1986, 1987
NEVADA	University of Nevada, Reno	1992
NEW JERSEY	Coriell Institute for Medical Research, Camden	2003
NEW YORK	Albany High School	1987
	Bronx High School of Science	1987
	Columbia University, New York	**1993**
	Cold Spring Harbor High School	1985, 1987
	Cornell University, Ithaca	**2005**
	DeWitt Middle School, Ithaca	*1991, 1993*
	DNA Learning Center	1988–1995, 2001–04, 2006
	DNA Learning Center	**1990, 1992, 1995, 2000**
	DNA Learning Center	*1990–1992*
	DNA Learning Center *West*	2005
	Fostertown School, Newburgh	*1991*
	Huntington High School	1986
	Irvington High School	1986
	Junior High School 263, Brooklyn	*1991*
	Lindenhurst Junior High School	*1991*
	Mt. Sinai School of Medicine, New York	1997
	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*
	The Rockefeller University, New York	2003
	State University of New York, Purchase	1989
	State University of New York, Stony Brook	1987–1990
	Stuyvesant High School, New York	1998–1999
	Titusville Middle School, Poughkeepsie	*1991, 1993*
	Trudeau Institute, Lake Saranac	2001
	Union College, Schenectady	2004
	U.S. Military Academy, West Point	**1996**
	Wheatley School, Old Westbury	1985
NORTH CAROLINA	CIIT Center for Health Research, Triangle Park	2003
	North Carolina Agricultural & Technical State University, Greensboro	2006
	North Carolina School of Science, Durham	1987
OHIO	Case Western Reserve University, Cleveland	1990
	Cleveland Clinic	1987
	North Westerville High School	1990
OKLAHOMA	**Oklahoma City Community College**	**2000**
	Oklahoma City Community College	2006
	Oklahoma Medical Research Foundation, Oklahoma City	2001
	Oklahoma School of Science and Math, Oklahoma City	1994
OREGON	Kaiser Permanente-Center for Health Research, Portland	2003
PENNSYLVANIA	Duquesne University, Pittsburgh	1988
	Germantown Academy	1988
SOUTH CAROLINA	**Clemson University, Clemson**	**2004**
	Medical University of South Carolina, Charleston	1988
	University of South Carolina, Columbia	**1988**
TEXAS	Austin Community College-Rio Grande Campus	2000
	J.J. Pearce High School, Richardson	1990
	Langham Creek High School, Houston	1991
	Southwest Foundation for Biomedical Research, San Antonio	2002
	Taft High School, San Antonio	1991
	Trinity University, San Antonio	**1994**
	University of Texas, Austin	**1999, 2004**
UTAH	University of Utah, Salt Lake City	1993
	University of Utah, Salt Lake City	**1998, 2000**
VERMONT	University of Vermont, Burlington	1989
VIRGINIA	Eastern Mennonite University, Harrisonburg	1996
	Jefferson School of Science, Alexandria	1987
	Mathematics and Science Center, Richmond	1990
	Mills Godwin Specialty Center, Richmond	1998
	Virginia Polytechnic Institute and State University, Blacksburg	**2005**

WASHINGTON	Fred Hutchinson Cancer Research Center, Seattle	1999, 2001
	University of Washington, Seattle	**1993, 1998**
WASHINGTON, D.C	**Howard University**	**1992, 1996**
WEST VIRGINIA	Bethany College	1989
WISCONSIN	Blood Center of Southeastern Wisconsin, Milwaukee	2003
	Madison Area Technical College	**1999**
	Marquette University, Milwaukee	1986, 1987
	University of Wisconsin, Madison	1988, 1989
	University of Wisconsin, Madison	**2004**
WYOMING	University of Wyoming, Laramie	1991
AUSTRALIA	Walter and Eliza Hall Institute and University of Melbourne	1996
CANADA	Red River Community College, Winnipeg, Manitoba	1989
ITALY	Porto Conte Research and Training Laboratories, Alghero	1993
	International Institute of Genetics and Biophysics, Naples	1996
PANAMA	**University of Panama, Panama City**	**1994**
PUERTO RICO	University of Puerto Rico, Mayaguez	1992
	University of Puerto Rico, Mayaguez	**1992**
	University of Puerto Rico, Rio Piedras	**1993**
	University of Puerto Rico, Rio Piedras	1994
RUSSIA	Shemyakin Institute of Bioorganic Chemistry, Moscow	1991
SINGAPORE	National Institute of Education	2001–2005
SWEDEN	Kristineberg Marine Research Station, Fiskebackskil	1995
	Uppsala University, Uppsala	2000


COLD SPRING HARBOR
LABORATORY PRESS
Cold Spring Harbor
Laboratory Press
Epigenetics

2006 PUBLICATIONS

SERIALS

Genes and Development, Vol. 20, 1–3532 (www.genesdev.org)
Genome Research, Vol. 16, 1–1624 (www.genome.org)
Learning and Memory, Vol. 13, 1–834 (www.learnmem.org)
Protein Science, Vol. 15, 1–2890 (www.proteinscience.org)
RNA, Vol. 12, 1–2236 (www.rnajournal.org)
Cold Spring Harbor Symposia in Quantitative Biology, Vol. 70: Molecular Approaches to Controlling Cancer, Bruce Stillman and David Stewart (eds.) (www.cshl-symposium.org)
Cold Spring Harbor Protocols (www.cshprotoocols.org)

LABORATORY MANUALS

The Condensed Protocols from Molecular Cloning: A Laboratory Manual, Joseph Sambrook and David Russell
Gene Transfer: Delivery and Expression of DNA and RNA: A Laboratory Manual, Theodore Friedmann and John Rossi (eds.)

HANDBOOKS

Binding and Kinetics for Molecular Biologists, James A. Goodrich and Jennifer F. Kugel
Experimental Design for Biologists, David J. Glass
Lab Ref, Volume 2: A Handbook of Recipes, Reagents, and Other Reference Tools for Use at the Bench, Albert Mellick and Linda Rodgers (eds.)

MONOGRAPHS

Genomes, Hillary Sussman and Maria Smit (eds.)
DNA Replication and Human Disease, Melvin DePamphilis (ed.)
Translational Control in Biology and Medicine, Michael Mathews, Nahum Sonenberg, and John Hershey (eds.)
Epigenetics, Danny Reinberg, C. David Allis, and Thomas Jenuwein (eds.)

TEXTBOOKS

From **a** *to* α*: Yeast Mating Type as a Model for Cellular Differentiation*, Hiten Madhani
Recombinant DNA: Genes and Genomes—A Short Course, Third Edition, James D. Watson, Amy A. Caudy, Richard M. Myers, and Jan A. Witkowski (copublished with W.H. Freeman)

GENERAL INTEREST

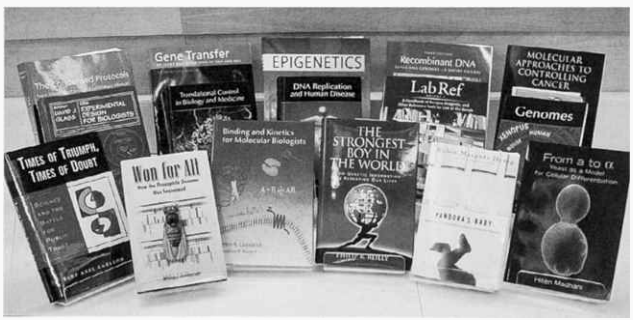
The Strongest Boy in the World: How Genetic Information Is Reshaping Our Lives, Philip R. Reilly
Won for All: How the Drosophila *Genome Was Sequenced*, Michael Ashburner
Pandora's Baby: How the First Test Tube Babies Sparked the Reproductive Revolution, Robin Marantz Henig (paperback edition)
Times of Triumph, Times of Doubt: Science and the Battle for Public Trust, Elof Axel Carlson

OTHER

CSHL Annual Report 2005: Yearbook Edition
CSHL Annual Report 2005
Banbury Center Annual Report 2005
Watson School of Biological Sciences Annual Report 2005

WEB SITES

http://www.dnareplication.cshl.org: Web site to accompany *DNA Replication and Human Disease*
http://kinetics.cshl.edu: Web site to accompany *Binding and Kinetics for Molecular Biologists*
http://rna.cshl.edu: Updated Web site to accompany *RNA World, Third Edition*



A selection of recently published books

The journal publishing program

COLD SPRING HARBOR LABORATORY PRESS EXECUTIVE DIRECTOR'S REPORT

Cold Spring Harbor Laboratory Press is the largest of the five educational divisions of the Laboratory, with offices on the Woodbury campus, marketing operations in San Diego and the United Kingdom, and distributors in Germany, Japan, China, Korea, India, and South America. The Press furthers and financially assists Cold Spring Harbor Laboratory's commitment to the advance and spread of scientific knowledge. Our research journals, books, and manuals provide continuing professional education for working scientists and graduate students, our textbooks support undergraduate teaching, and our other books explore the practice, personalities, and history of science and its influence on medicine, business, and social policy.

In 2006, CSHL Press generated a substantial operating excess, with a preliminary 2006 margin of $400,000 on income of $9.375 million. Its total contribution to the Laboratory, including support for institutional overhead and depreciation, was approximately $1 million.

In a challenging market, institutional journal subscription revenue grew by 6.5% compared with 2005 and showed a continued trend toward online-only subscriptions, as institutional libraries became more comfortable letting go of print copies. All journals increased revenue from regular issues, with *Learning and Memory* showing strongest percentage growth. Advertising sales in regular issues remained steady within our program despite an industry-wide decline. As the commercial momentum of genome science has slowed, interest in RNA interference has picked up, helping to sustain our advertising and sponsorship support. BioSupplyNet, our online product and vendor directory, showed a substantial increase in revenue, and its publication of free laboratory protocols attracted sponsorship from Sigma-Aldrich. The journal program's value was particularly evident in the extensive use made of research articles, which increased by 7.5% in 2006 with downloads of 8.1 million articles.

Genome Research, *RNA*, and most recently *Genes & Development* publish research papers immediately and freely online if a fee is paid for this service by the organization that funded the research. This "hybrid open access" business model accords with the requirements of The Wellcome Trust and other international funding agencies. Currently, a minority of authors choose this option. Free journal access within developing nations expanded with the addition of 37 new countries to the more than 100 lower-income countries covered by the HINARI and AGORA programs.

According to 2005 journal rankings by impact factor, *Genes & Development* was number 2 in developmental biology and number 3 in genetics, and *Genome Research* was number 3 in biotechnology and number 8 in genetics. With the inclusion of *Learning & Memory*, *Protein Science*, published for The Protein Society, and *RNA*, published for The RNA Society, in our program, all five of our journals are within the top 30% of all journals in their disciplines, and *Genes & Development* is ranked among the top 20 of all science journals. Many of the papers published in our journals were highlighted in the world's major news media, including *The New York Times, The Wall Street Journal,* and the BBC News.

The annual Cold Spring Harbor Symposium volume was published online for the third time. Readers within subscribing institutions now have access to Symposium manuscripts many months ahead of print publication and to other features such as video interviews with speakers at the meeting.

Cold Spring Harbor Protocols, an interactive, online source of new and classic research techniques, was launched in June 2006. In the first six months, more than 800 protocols were published. Subscription interest in this online-only serial has been extremely strong and downloads of articles from the database increased by 100% from September to December. The project has made a most promising start.

The book program released 15 new titles, bringing the total number of books in print by the end of year to more than 200; 15,500 book and multimedia orders were handled by our customer service department and over 59,000 units were shipped. The provision of fulfillment and distribution services

in the United States for the books of two other publishers generated commission revenue on an increasing sales income.

The new book titles were a mix of manuals, monographs, reference, and history books. But *Recombinant DNA: Genes and Genomes—A Short Course*, the third edition of a successful textbook originally created by Jim Watson, was also published, a joint project with W.H. Freeman that advanced our expertise with books for students. The best-selling new book in 2006 was *The Strongest Boy in the World: How Genetic Information Is Reshaping Our Lives*, an excellent read by Philip Reilly that was warmly praised in the scientific press. The best-selling backlist titles were two perennials, *At the Bench* by Kathy Barker and *Molecular Cloning: A Laboratory Manual,* by Joe Sambrook and David Russell, both long recognized as uniquely valuable contributions to the molecular biology literature.

Fifteen contracts for new books were signed, including agreements for new manuals in RNA science, single-molecule biology, and proteomics; several monographs; a biography of Francis Crick; and the sixth edition of *Molecular Biology of the Gene,* to be copublished with Benjamin Cummings. Three agreements were signed with foreign publishers for translations of several of our titles into Chinese and Japanese. Two contracts were agreed for foreign reprint rights.

During the year, we began to reshape our sales and marketing departments to make them more responsive to significant financial and technological change in our community. Publications such as ours are vulnerable to reductions in research funding, and in the current climate, cash-strapped scientists are regretfully choosing to turn down resources they would otherwise have cherished. The omnipresence of the Internet in our professional lives is driving all forms of information online, and books are no exception. As the year ended, we embarked on an exploration of the feasibility and economics of publishing all of our research-level books online and were encouraged by positive responses from institutional librarians.

As a specialist publisher, our books are to be found some considerable way down the "Long Tail," the metaphor for non-best-sellers that caught the popular imagination in 2006. Thanks to the distinction of the authors, editors, and contributors we can call on, and the expertise of our staff, Cold Spring Harbor has a strong reputation for the creation of high-quality content that gives our voice some distinction in the cacophony of the Web. That alone, however, will not be sufficient for prosperity or perhaps even survival, without a continuation of the hard processes of innovation, adaptation, and change that characterized much of our work in the past year. I am most grateful to the staff members, particularly the department heads, whose openness to new thinking made this progress possible.

G. Jaitin, L. Schmidt—Press Customer Service

M. Mazzullo—Production editor, Books

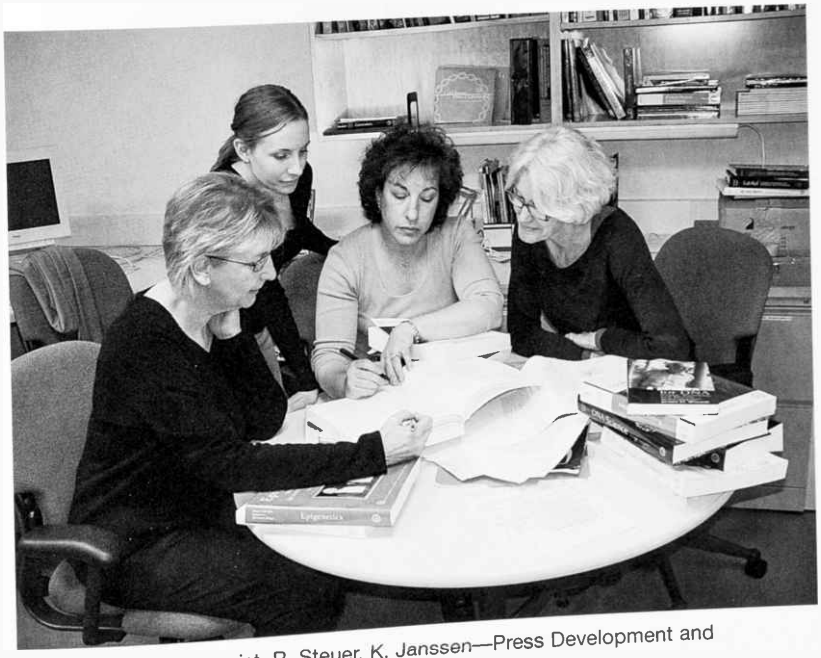
Staff

In 2006 we welcomed new colleagues Jane Carter, Kaaren Hegquist, Lauren Heller, and Kathleen MacDonald and Cher Mattes. We said farewell, thanks, and good luck to Debra Banninger, Richard Cavalieri, Danny deBruin, Katya Gurbeloshvili, and Rita Wallace.

Special thanks are due to the individuals who shouldered supervisory responsibilities within the Press: Jan Argentine, Alex Gann, Nancy Hodson, Geraldine Jaitin, Bill Keen, Wayne Manos, Marcie Siconolfi, Linda Sussman, and Denise Weiss. The Press also owes much to the skill and judgment of the Editors of our successful journals, Terri Grodzicker at *Genes & Development* and Hillary Sussman at *Genome Research*.

A complete listing of staff members of the Press in December 2006 is printed elsewhere in this volume. The Laboratory is fortunate to have the support of such able and hardworking professionals.

John R. Inglis
Executive Director



J. Argentine, K. Hegquist, R. Steuer, K. Janssen—Press Development and Production staff


FINANCE

FINANCIAL STATEMENTS

CONSOLIDATED BALANCE SHEET
December 31, 2006
With comparative financial information as of December 31, 2005

		2006	2005
Assets:			
Cash and cash equivalents	$	23,563,414	24,580,708
Accounts receivable		2,995,007	5,735,451
Grants receivable		7,859,380	8,352,217
Contributions receivable, net		43,226,518	47,578,065
Publications inventory		3,831,443	2,979,817
Prepaid expenses and other assets		3,720,386	2,015,019
Investments		308,108,275	256,675,871
Investment in employee residences		5,557,353	5,726,739
Restricted use assets		3,598,745	3,226,538
Deposits with trustee		46,014,949	–
Land, buildings, and equipment, net		130,942,408	115,851,522
Total assets	$	579,417,878	472,721,947
Liabilities and net assets:			
Liabilities:			
Accounts payable and accrued expenses	$	12,450,777	10,435,051
Deferred revenue		3,585,913	4,237,554
Bonds payable		97,200,000	45,200,000
Total liabilities		113,236,690	59,872,605
Net assets:			
Unrestricted		213,562,751	195,431,117
Temporarily restricted		82,791,081	70,289,399
Permanently restricted		169,827,356	147,128,826
Total net assets		466,181,188	412,849,342
Total liabilities and net assets	$	579,417,878	472,721,947

CONSOLIDATED STATEMENT OF ACTIVITIES
Year ended December 31, 2006
With summarized financial information for the year ended December 31, 2005

	Unrestricted	Temporarily Restricted	Permanently Restricted	2006 Total	2005 Total
Revenue and other support:					
Public support (contributions and non-Federal grant awards)	$ 21,344,574	23,169,757	14,917,606	59,431,937	83,882,020
Federal grant awards	33,128,165	–	–	33,128,165	32,067,800
Indirect cost allowances	20,258,404	–	–	20,258,404	19,558,159
Investment return utilized	10,741,551	–	–	10,741,551	9,576,065
Program fees	4,192,163	–	–	4,192,163	3,583,017
Publications sales	9,319,286	–	–	9,319,286	9,751,069
Dining services	3,780,431	–	–	3,780,431	3,349,002
Rooms and apartments	3,043,489	–	–	3,043,489	2,703,382
Royalty and licensing fees	693,345	–	–	693,345	2,873,198
Miscellaneous	931,666	–	–	931,666	652,207
Net assets released from restrictions	10,668,075	(10,668,075)	–	–	–
Total revenue and other support	118,101,149	12,501,682	14,917,606	145,520,437	167,995,919
Expenses:					
Research	62,822,726	–	–	62,822,726	57,766,809
Educational programs	13,565,968	–	–	13,565,968	13,207,352
Publications	9,351,363	–	–	9,351,363	9,432,319
Banbury Center conferences	1,124,415	–	–	1,124,415	1,396,425
Dolan DNA Learning Center programs	1,325,479	–	–	1,325,479	1,231,059
Watson School of Biological Sciences programs	2,973,896	–	–	2,973,896	2,848,109
General and administrative	13,619,460	–	–	13,619,460	13,267,025
Dining services	4,981,661	–	–	4,981,661	4,684,031
Total expenses	109,764,968	–	–	109,764,968	103,833,129
Excess of revenue and other support over expenses	8,336,181	12,501,682	14,917,606	35,755,469	64,162,790
Other changes in net assets:					
Investment return in excess of amount utilized	13,165,561	–	7,780,924	20,946,485	8,264,298
Change in fair value of interest rate swap	(3,370,108)	–	–	(3,370,108)	–
Increase in net assets	18,131,634	12,501,682	22,698,530	53,331,846	72,427,088
Net assets at beginning of year	195,431,117	70,289,399	147,128,826	412,849,342	340,422,254
Net assets at end of year	$ 213,562,751	82,791,081	169,827,356	466,181,188	412,849,342

CONSOLIDATED STATEMENTS OF CASH FLOWS
Year ended December 31, 2006
With comparative financial information for the year ended December 31, 2005

	2006	2005
Cash flows from operating activities:		
Increase in net assets	$ 53,331,846	72,427,088
Adjustments to reconcile increase in net assets to net cash provided by operating activities:		
Change in fair value of interest rate swap	3,370,108	–
Depreciation and amortization	6,032,302	5,852,278
Net appreciation in fair value of investments	(24,403,523)	(13,009,353)
Contributions restricted for long-term investment	(30,918,132)	(47,445,268)
Changes in assets and liabilities:		
Decrease (increase) in accounts receivable	2,740,444	(3,247,129)
Decrease (increase) in grants receivable	492,837	(1,376,762)
Increase in contributions receivable	(31,856)	(125,496)
Increase in publications inventory	(851,626)	(34,718)
Decrease (increase) in prepaid expenses and other assets	535,210	(409,312)
Increase in restricted use assets	(372,207)	(321,615)
(Decrease) increase in accounts payable and accrued expenses	(1,042,172)	900,672
(Decrease) increase in deferred revenue	(651,641)	358,816
Net cash provided by operating activities	8,231,590	13,569,201
Cash flows from investing activities:		
Capital expenditures	(21,123,188)	(10,946,777)
Proceeds from sales and maturities of investments	97,347,685	134,357,645
Purchases of investments	(124,641,857)	(162,172,939)
Net change in investment in employee residences	434,677	(678,172)
Net cash used in investing activities	(47,982,683)	(39,440,243)
Cash flows from financing activities:		
Permanently restricted contributions	14,917,606	12,312,801
Contributions restricted for investment in land, buildings, and equipment	16,000,526	35,132,467
Decrease (increase) in contributions receivable	4,383,403	(34,959,882)
Increase in cash restricted for investment in buildings and equipment	(46,014,949)	–
Cash payments for debt issue costs	(2,240,577)	–
(Decrease) increase in accounts payable relating to capital expenditures	(276,659)	2,031,084
Repayment of notes payable	(35,551)	(34,137)
Redemption of 1993 Suffolk IDA Bond	(3,000,000)	–
Issuance of bonds payable	55,000,000	–
Net cash provided by financing activities	38,733,799	14,482,333
Net decrease in cash and cash equivalents	(1,017,294)	(11,388,709)
Cash and cash equivalents at beginning of year	24,580,708	35,969,417
Cash and cash equivalents at end of year	$ 23,563,414	24,580,708
Supplemental disclosures:		
Interest paid	$ 2,614,630	1,134,372

FINANCIAL SUPPORT OF THE LABORATORY

Cold Spring Harbor Laboratory, Banbury Center, and the Dolan DNA Learning Center receive a substantial portion of their funding through grants from the Federal Government and through grants, capital gifts, and annual contributions from private foundations, corporations, and individuals. The following section summarizes funding that occurred during 2006.

GRANTS January 1–December 31, 2006

COLD SPRING HARBOR LABORATORY

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2006 Funding†*
FEDERAL GRANTS				
NATIONAL INSTITUTES OF HEALTH				
Equipment Support	Dr. Lowe	03/01/06	02/28/07	211,575 *
	Dr. Lowe	06/01/06	05/31/07	647,000 *
Program Project Support	Drs. Hannon/Krainer/Lazebnik/ Lowe/S. Muthuswamy/Myers/ D. Spector/Stenlund/Stillman/Tansey	01/01/02	12/31/06	5,223,402
	Dr. Stillman-CSHL Cancer Center Core	08/01/05	07/31/10	4,208,052
	Drs. Lowe/Hannon/S. Muthuswamy	04/01/04	03/31/09	883,896
	Dr. Huang	09/08/06	08/31/11	481,481 *
Pioneer Award Support	Dr. Cline	09/30/05	07/31/10	827,584
Merit Award Support	Dr. Malinow	05/01/92	04/30/10	658,599
	Dr. Tonks	08/01/91	03/31/06	670,834
Contract Support	Drs. Sebat/Wigler	01/01/06	12/31/06	447,352 *
Research Support	Dr. Brody	07/01/04	04/30/09	201,760
	Dr. Chklovskii	07/08/04	06/30/09	297,930
	Dr. Cline	12/01/04	11/30/09	390,584
	Dr. Dubnau	09/15/04	06/30/09	357,143
	Dr. Hannon	09/01/05	08/31/09	340,021
	Dr. Hirano	07/01/05	03/31/07	347,585
	Dr. Hirano	05/01/96	03/31/07	397,240
	Dr. Huang	07/15/05	04/30/09	382,758
	Dr. Joshua-Tor	02/01/06	01/31/10	303,565 *
	Dr. Joshua-Tor	02/15/02	01/31/07	364,724
	Dr. Joshua-Tor	07/01/05	06/30/09	309,525
	Dr. Krainer	07/01/03	06/30/07	554,951
	Dr. Lowe	07/01/04	06/30/09	498,501
	Drs. Mainen/Brody	09/23/02	08/31/07	379,876
	Dr. Malinow	04/01/95	02/29/08	575,281
	Dr. Martienssen	08/01/03	07/31/07	331,034
	Dr. Mittal	04/01/04	03/31/09	305,379
	Dr. Mitra	03/04/05	02/28/09	510,943
	Dr. S. Muthuswamy	03/01/03	02/28/07	368,274
	Dr. Neuwald	09/01/06	12/31/06	83,799 *
	Drs. Powers/Lowe	12/01/06	11/30/11	383,210 *
	Dr. Sebat	09/30/05	07/31/10	415,341

†Includes direct and indirect costs
*New and competing renewal grants awarded in 2006

	Dr. Skowronski	04/01/98	04/30/08	664,376
	Dr. D. Spector	04/01/90	03/31/07	652,851
	Dr. D. Spector	09/01/04	08/31/08	315,726
	Dr. Stein	09/01/02	11/30/06	400,000 *
	Dr. Stein	09/25/06	06/30/09	418,750 *
	Drs. Stenlund/Hernandez	07/01/04	06/30/07	240,000
	Dr. Stillman	07/01/91	05/31/08	562,712
	Dr. Svoboda	12/01/03	11/30/07	244,965
	Dr. Svoboda	12/01/02	11/30/07	220,136
	Dr. Svoboda	06/01/03	03/31/08	297,482
	Dr. Svoboda	03/01/04	02/28/09	335,171
	Dr. Tansey	05/01/03	04/30/07	322,758
	Dr. Tonks	05/01/97	06/30/07	380,689
	Dr. Tonks	07/21/06	05/31/10	297,313 *
	Dr. Tully	03/01/03	02/29/08	372,414
	Dr. Tully	08/01/03	05/31/07	372,414
	Dr. Van Aelst	05/01/03	04/30/08	331,447
	Dr. Wigler	07/15/98	04/30/07	519,388
	Dr. Zador	01/23/03	12/31/07	451,725
	Dr. Y. Zhong	07/01/03	06/30/08	338,895
Research Subcontracts				
NIH/Affymetrix Consortium Agreement-Supplement	Dr. Stein	08/01/04	05/31/07	96,124 *
NIH/Booz Allen Hamilton caBIG Consortium Agreement	Dr. Stein	03/07/06	12/07/06	157,528 *
NIH/Booz Allen Hamilton caBIG Consortium Agreement	Dr. Stein	03/07/06	11/07/06	150,979 *
NIH/Booz Allen Hamilton caBIG Consortium Agreement	Dr. Stein	03/07/06	12/07/06	151,721 *
NIH/Brookhaven National Laboratory	Dr. Stillman	09/01/06	08/31/11	35,559 *
NIH/Caltech Consortium Agreement	Dr. Stein	09/01/03	08/31/07	757,911
NIH/Caltech Consortium Agreement	Dr. Svoboda	04/04/03	02/29/08	147,818
NIH/Columbia University Consortium Agreement	Dr. Lowe	08/18/06	07/31/11	434,305 *
NIH/Columbia University Consortium Agreement	Drs. Wigler/Sebat	09/01/06	05/31/10	286,030 *
NIH/Georgia Institute of Technology Consortium Agreement	Dr. D. Spector	09/30/06	09/29/11	220,000 *
NIH/Memorial Sloan-Kettering Consortium Agreement	Dr. Mittal	04/01/04	03/31/09	137,480
NIH/Memorial Sloan-Kettering Consortium Agreement	Dr. Van Aelst	07/10/03	06/30/08	338,271
NIH/Oxford University	Dr. Stein	03/01/06	02/28/11	180,874 *
NIH/Rutgers University Consortium Agreement	Dr. Mitra	12/08/04	11/30/08	107,338
NIH/State University at Stony Brook	Dr. McCombie	11/01/05	08/31/06	55,000 *
NIH/Washington University Consortium Agreement	Dr. Stein	09/01/03	08/31/08	56,199
Fellowship Support	Dr. Chicas	04/01/06	03/31/09	49,928 *
	C. Kopec	03/01/04	09/30/06	4,455
	Dr. Gray	03/01/06	12/31/06	29,578 *
	Dr. Slotkin	09/16/06	09/14/09	43,996 *
	Dr. Weimer	07/01/05	09/08/06	12,192
	Dr. Wilbrecht	07/01/05	06/30/08	51,195
	Dr. Yang	03/01/06	02/28/09	50,428 *
Career Development Support	Dr. He	12/01/06	11/30/08	112,383 *

*New and competing renewal grants awarded in 2006

	Dr. Lucito	06/01/02	05/31/07	146,156
Graduate Training Support	Dr. Clark	09/01/06	06/30/07	209,429 *
Course Support	Integrated Data Analysis for High-throughput Biology	09/30/99	04/30/07	57,421
	Immunocytochemistry, In Situ Hybridization, and Live Cell Imaging	07/01/98	08/31/10	99,482
	Programming for Biology	07/07/00	08/31/09	53,561
	C. elegans	08/01/98	08/31/11	80,484
	Computational and Comparative Genomics	06/06/91	08/31/07	48,974
	Cell and Developmental Biology of *Xenopus*	04/01/93	03/31/09	20,624
	Protein Purification and Characterization	01/01/83	03/31/11	80,517
	Molecular Embryology of the Mouse	01/01/83	03/31/11	94,474
	Eukaryotic Gene Expression	01/01/83	03/31/11	100,010
	Mechanisms and Models of Cancer	07/01/06	06/30/07	4,662 *
	Proteomics Course	07/01/03	06/30/11	72,343
	Cell Biology Course	07/01/05	06/30/08	62,696
Meeting Support	Molecular Chaperones and the Heat Shock Response	04/01/04	03/31/09	13,001
	Pharmacogenomics	08/01/04	07/31/09	39,822
	The Biology of Genomes	04/08/05	03/31/08	41,422
	Physiological Genomics and Rat Models	09/30/05	08/31/08	15,000
	Mouse Molecular Genetics	07/12/02	06/30/07	13,352
	Translational Control	07/05/02	06/30/07	6,001
	The Cell Cycle	03/01/06	02/28/09	4,000 *
	PTEN Pathways	03/01/06	02/28/09	15,000 *
	Channels, Receptors, and Synapses	04/01/06	03/31/11	23,265 *
	Germ Cells	04/01/06	03/31/07	6,000 *
	71st Symposium: Regulatory RNAs	05/01/06	04/30/07	15,000 *
	Axon Guidance, Synaptogenesis, and Neural Plasticity	07/01/06	06/30/07	15,000 *
	Molecular Genetics of Aging	07/01/06	06/30/07	20,449 *
	Gene Expression and Signaling in the Immune System	04/19/02	03/31/07	2,001
NATIONAL SCIENCE FOUNDATION				
Multiple Project Award Support	Dr. Hannon	09/01/06	08/31/09	1,078,000 *
	Dr. McCombie	09/01/06	08/31/08	1,891,911 *
	Dr. Stein	12/15/03	11/30/07	2,507,500
	Dr. Jackson	07/01/05	06/30/10	1,173,258
Research Support	Dr. Jackson	03/01/04	02/28/07	145,000
	Dr. Lukowitz	04/01/05	03/31/08	125,970
	Dr. Timmermans	09/01/06	08/31/09	130,000 *
	Dr. Timmermans	09/01/06	08/31/09	159,532 *
	Dr. Van Aelst	08/15/05	07/31/08	133,622
	Dr. Ware	09/01/03	08/31/08	211,634
	Dr. M. Zhang	09/15/03	08/31/08	104,000
Research Subcontract Support				
NSF/Cornell University Consortium Agreement	Dr. Timmermans	01/01/06	08/31/07	697,667 *
NSF/North Carolina State University Consortium Agreement	Dr. Martienssen	10/01/04	09/30/09	204,913
NSF/University of Florida Consortium Agreement	Dr. Martienssen	09/15/05	08/31/07	50,745
NSF/New York University Consortium Agreement	Drs. McCombie/Martienssen	10/01/04	09/30/09	258,164

*New and competing renewal grants awarded in 2006

NSF/Washington Unversity Consortium Agreement	Drs. McCombie/Martienssen/Stein/Ware	11/15/05	10/31/08	1,292,747 *
NSF/University of Arizona Consortium Agreement	Dr. Stein	10/01/03	09/30/07	214,279
NSF/University of Arizona Consortium Agreement	Dr. Stein	10/01/06	09/30/08	152,993 *
NSF/University of Calfornia-Berkeley Consortium Agreement	Dr. Jackson	08/01/06	07/31/11	203,029 *
NSF/University of Wisconsin	Drs. Stein/Ware	01/01/04	12/31/08	208,209
NSF/National Center for Genome Research Consortium Agreement	Dr. Stein	09/01/05	08/31/08	56,132
Fellowship Support	D. Chitwood	09/01/04	08/31/07	40,500
Undergraduate Training Support	Dr. Clark	04/01/05	03/31/08	113,008
Meeting Support	Molecular Genetics of Bacteria and Phages	2006		5,000 *
	Systems Biology: Global Regulation of Gene Expression	2006		12,000 *
Course Support	Advanced Bacterial Genetics	07/15/04	06/30/09	75,557
	Computational Neuroscience: Vision	07/01/06	06/30/07	22,000 *
	Molecular Approaches to Plant Science	07/01/03	06/30/07	77,955
UNITED STATES DEPARTMENT OF AGRICULTURE				
Research Support	Dr. Jackson	09/01/05	08/31/08	117,669
	Dr. McCombie	09/15/04	09/14/09	25,000
	Drs. McCombie/Martienssen	02/01/04	01/31/07	198,479
	Dr. Stein	09/22/03	09/14/08	210,000
	Dr. Timmermans	09/15/06	09/14/09	129,475 *
Research Subcontract Support				
USDA/Oregon State University Consortium Agreement	Dr. Martienssen	12/01/04	11/30/07	17,322
Fellowship Support	Dr. Whipple	09/15/06	08/14/08	61,819
UNITED STATES DEPARTMENT OF THE ARMY				
Research Support	Dr. Hannon	02/01/06	01/31/09	169,500 *
	Dr. Hannon	02/15/06	02/14/09	169,500 *
	Dr. Lazebnik	12/01/06	11/30/09	209,375 *
	Drs. Lucito/Wigler	02/01/05	01/31/07	901,422
	Dr. Y. Zhong	12/15/04	12/14/08	338,733
	Drs. Wigler/Lucito	04/01/04	08/31/07	1,365,000 *
Fellowship Support	D. Khalil	12/01/06	11/30/09	33,300 *
	E. Murchison	02/16/05	02/15/08	30,000
	S. Nunez	02/27/04	02/28/07	30,000
	A. Rosenberg	02/28/05	02/28/08	30,000
	D. Simpson	09/01/06	08/31/09	32,400 *
	Dr. P. Smith	04/01/04	03/31/07	108,000
Research Subcontract Support				
U.S. Army/Johns Hopkins University Consortium Agreement	Dr. Wigler	12/01/05	12/31/06	294,355 *
U.S. Army/New York University Consortium Agreement	Dr. Lucito	09/27/04	09/26/09	147,307

*New and competing renewal grants awarded in 2006

UNITED STATES DEPARTMENT OF ENERGY				
DOE/Texas A&M Consortium Agreement	Dr. Ware	09/15/06	09/14/08	38,376 *
MISCELLANEOUS SOURCES OF FUNDING				
Equipment Support				
The Berkowitz Family Foundation	Dr. Mittal	07/01/06	06/30/07	25,000 *
F.M. Kirby Foundation, Inc.	Dr. D. Spector	10/01/06	09/30/07	30,000 *
The Seraph Foundation	Dr. D. Spector	10/01/06	09/30/07	37,000 *
Program Project Support				
DART Neurogenomics Project	Drs. Tully/Cline/Dubnau/Henry/ Mitra/M. Zhang/Y. Zhong	10/01/04	09/30/07	2,774,046
Leukemia and Lymphoma Society	Dr. Lowe	10/01/03	09/30/08	1,250,000
Simons Foundation	Drs. Wigler/Sebat	06/01/06	05/31/08	3,775,419
Research Support				
ALS Association	Dr. Enikolopov	09/01/06	08/31/07	118,000 *
American Cancer Society	Dr. Mills	07/01/06	06/30/10	240,000 *
American Cancer Society	Dr. Wigler	01/01/96	12/31/06	10,000 *
American Cancer Society	Dr. Wigler	01/01/06	12/31/10	60,000 *
Anonomous Gift	Dr. Malinow	09/01/06	08/31/08	130,000 *
Anonomous Gift	Dr. Sebat	01/01/06	12/31/07	244,439 *
Berlin Family Foundation	Dr. Stillman	04/01/06	03/31/07	20,000 *
Berlin Family Foundation	Dr. Watson	04/01/06	03/31/07	5,019 *
Brain Tumor Society	Dr. Hannon	11/01/06	10/31/07	100,000 *
Breast Cancer Research Foundation	Dr. Wigler	10/01/06	09/30/07	250,000 *
Breast Cancer Help Inc.	Dr. Wigler	2006		1,553 *
Clear Channel Worldwide (Walk 97.5 FM) Breast Cancer Research Walk	Dr. S. Muthuswamy	04/01/06	03/31/07	4,000 *
CSH Main Street Association	Dr. Powers	07/01/06	06/30/07	4,200 *
Katheryn Wasserman Davis Gift	Dr. Hannon	04/01/06	03/31/07	400,000 *
DeMatteis Family Foundation	Dr. Powers	10/01/04	09/30/06	450,000 *
The Don Monti Memorial Research Foundation	Dr. Lowe	06/01/06	05/31/07	250,000 *
ELJI/SUSB Consortium Agreement	Dr. Huang	01/15/04	01/14/07	70,000
Norman and Dorian Feckl Gift	Dr. Enikolopov	03/16/06	03/14/07	20,000 *
Find A Cure Today (F.A.C.T.)	Dr. S. Muthuswamy	07/01/03	07/31/06	58,167 *
FSMA	Dr. Hastings	01/15/06	01/14/08	219,674 *
GlaxoSmithKline	Dr. Powers	09/20/06	08/19/08	451,524 *
Glen Cove C.A.R.E.S.	Dr. S. Muthuswamy	02/01/06	01/31/07	25,000 *
Robert Goldman Foundation	Dr. Mittal	12/31/06	12/30/07	41,000 *
Lita Annenberg Hazen Foundation	Dr. Powers	11/01/06	10/31/07	250,000 *
Irving Hansen Foundation	Dr. Tansey	08/01/06	07/31/07	20,000 *
Human Frontier of Science Program	Dr. Dubnau	10/01/06	09/30/09	125,000 *
Joan's Legacy: The Joan Scarangello Foundation to Conquer Lung Cancer	Dr. Mu	11/01/05	10/31/07	50,000
Karches Foundation	Dr. Wigler	07/01/05	06/30/10	452,570
Karp Foundation	Dr. Brody	01/01/06	12/31/06	40,000 *
W.M. Keck Foundation	Dr. Mitra	07/01/06	06/30/09	500,000 *
David H. Koch Gift	Dr. Powers	07/01/06	06/30/07	50,000 *
The Forrest and Francis Lattner Foundation in Honor of Andrew Harris	Dr. Sebat	12/16/05	12/16/07	504,800
Lehman Brothers Foundation, Inc.	Dr. Powers	11/01/06	10/31/07	25,000 *
L.I.A.B.C.	Dr. Wigler	2006		93,831 *
Long Island 2-Day Walk	Dr. S. Muthuswamy	08/01/06	07/31/07	26,000 *
Long Island Cruizin' for a Cure	Dr. Powers	2006		19,426 *
March of Dimes	Dr. Huang	06/01/04	05/31/07	98,270
Carol Marcincuk Ovarian Cancer Research Fund	Dr. Lucito	2006		9,001 *

*Includes direct and indirect costs
*New and competing renewal grants awarded in 2006

The Mary LaRuffa Gift	Dr. Stillman	06/01/06	05/31/07	2,727 *
The Miracle Foundation	Dr. Wigler	01/01/06	12/31/06	100,000 *
The Elizabeth McFarland Breast Cancer Fund	Dr. Wigler	06/01/06	05/31/07	45,050 *
Louis Morin Charitable Trust	Drs. Zador/Krainer	12/01/06	11/30/07	75,000 *
NAAR	Dr. Van Aelst	07/01/05	06/30/07	60,000
NARSAD	Dr. Enikolopov	09/15/06	09/14/08	50,000 *
Laura Obadian Smith Initiative	Breast Cancer Research	2006		500 *
Omidyar Foundation	Drs. Powers/Hannon	10/01/06	09/30/07	500,000 *
Philip Morris USA	Dr. Mittal	07/01/05	06/30/06	423,750
Vivian Parisi Gift	Dr. Powers	04/01/06	03/31/07	950 *
The Fannie E. Rippel Foundation	Drs. Lucito/Tonks	12/01/05	11/30/07	154,000
The Seraph Foundation	Dr. Enikolopov	10/01/06	09/30/07	86,000
SMA Foundation	Dr. Krainer	06/01/04	05/31/07	59,333
Linda and Henry Spire Gift	Dr. Tonks	01/01/06	12/31/06	10,000 *
Topspin/Dr. L. Guthhart	Drs. Hannon/Lowe	2006		250,000 *
Town of Islip Breast Cancer Coalition	Dr. Powers	06/01/06	05/31/07	10,250 *
Waldbaums Foundation	Dr. Powers	10/01/06	09/30/07	50,000 *
Waterbor Foundation	Dr. Powers	10/01/06	09/30/07	5,000 *
West Islip Breast Cancer Coalition	Dr. Wigler	11/01/06	10/31/07	10,000 *
Women In Science	Dr. Stillman	2006		33,455 *
Women's Insurance Network of Long Island	Dr. Powers	2006		18,116 *

Fellowship Support

American Foundation For Aging Research	R. Bish	12/01/06	11/30/07	500 *
Arnold and Mabel Beckman Foundation	Dr. Dubnau	09/01/05	08/31/08	88,000
Arnold and Mabel Beckman Foundation	Watson School of Biological Sciences	09/01/05	08/31/09	350,000
Burroughs Wellcome	Dr. Hall	09/01/06	08/31/08	58,000 *
Burroughs Wellcome	Dr. Karpova	09/01/05	08/31/07	58,000
Cashin Family Fund	Watson School of Biological Sciences	09/01/04	08/31/07	40,000
Cody Center/State University at StonyBrook	Dr. Enikolopov	10/01/06	09/30/07	40,000 *
C.J. Martin Fellowship	Dr. Irvine	04/01/06	03/31/10	45,966 *
CSHL Association Fellowship	Dr. Schalch	2006		54,089 *
CSHL Association Fellowship	Dr. Jensen	2006		52,102 *
CSHL Association Fellowship	Dr. Nawy	2006		53,998 *
CSHL Association Fellowship	Dr. Aravin	2006		57,911 *
CSHL Association Fellowship	Dr. Thakar	2006		55,062 *
DAAD (German Academic Exchange Service)	Dr. Bouyer	09/01/06	08/31/07	14,500 *
DFG German Science Foundation	Dr. Bommert	10/01/06	09/30/08	9,810 *
Englehorn Scholarship Program	Watson School of Biological Sciences	09/01/00	12/31/07	89,980
European Molecular Biology Organization (EMBO) Long-term Fellowship	Dr. Schwab	03/01/06	02/28/06	31,371 *
Francis Goelet Fellowship in Biomathematics	Dr. Wigler	10/01/05	09/30/08	70,707
Francis Goelet Foundation	Dr. Stillman	01/01/06	12/31/06	5,000 *
Ford Foundation Predoctoral Fellowship	K. John	09/01/04	08/31/07	22,000
Goldring Fellowship	Dr. Stillman	09/01/06	08/31/07	77,550 *
Teresa Haire Memorial Fund	Watson School of Biological Sciences	04/01/06	03/31/07	2,640 *
Ira Hazan	Dr. Enikolopov	12/22/06	12/21/07	200,000 *
Heat and Stroke Foundation	Dr. Boivin	07/01/06	06/30/08	35,928 *
Helen Hay Whitney Foundation	Dr. He	04/01/04	11/30/06	31,121
Human Frontier Science Program Organization (HFSPO)	Dr. Kasri	09/01/06	08/31/09	38,000 *
Human Frontier Science Program Organization (HFSPO)	Dr. Da Silva	01/01/06	12/31/08	38,000 *
Human Frontier Science Program Organization (HFSPO)	Dr. Aono	04/01/06	03/31/08	38,000 *

*New and competing renewal grants awarded in 2006

Jane Coffin Childs Foundation	Dr. Rivas	07/01/04	06/30/07	45,500
Klingenstein Foundation	Dr. Chklovskii	07/01/04	06/30/07	50,000
Lauri Strauss Leukemia Foundation	Dr. Zuber	05/01/06	04/30/07	15,000 *
Leukemia Research Foundation	Dr. Ceparo	07/01/05	11/11/06	11,250
The Leukemia and Lymphoma Society	Dr. Gangadharan	07/01/05	06/30/08	55,000
The Leukemia and Lymphoma Society	Dr. Krizhanovsky	07/01/06	06/30/09	50,000 *
The Leukemia and Lymphoma Society	Dr. Liu	07/01/05	06/30/08	55,000
The Leukemia and Lymphoma Society	Dr. Wendel	07/01/05	12/31/06	41,250
The Lymphona Research Foundation	Dr. Kurland	07/01/06	06/30/08	50,000 *
Merck Career Development Award	Watson School of Biological Sciences	01/01/05	12/31/07	20,000
McKnight Endowment	Dr. Huang	07/01/04	06/30/07	75,000
NARSAD	Dr. Kuhlman	07/01/06	06/30/08	30,000 *
NARSAD	Dr. Real	07/01/06	06/30/08	30,000 *
NARSAD	Dr. Takahashi	07/01/05	06/30/07	30,000 *
NARSAD	Dr. H. Zhong	07/01/05	08/31/06	5,000
NYS Department of Health, Health Research Science Board, Breast Cancer Research, and Education Program	Dr. S. Chen	01/01/06	12/31/07	60,000 *
The Rita Allen Foundation	Dr. S. Muthuswamy	08/01/04	07/31/07	58,481
The Andrew Seligson Memorial Clinical Fellowship for Cancer Research	Dr. Zender	11/01/06	10/31/07	75,000 *
The Seraph Cancer Research Scholar Award	Watson School of Biological Sciences	10/01/04	09/30/07	45,000
The Judi Shesh Memorial Foundation	Dr. Powers	12/01/06	11/30/07	7,500 *
Swartz Foundation	Center for Neural Mechanisms of Cognition	01/01/06	12/31/06	333,333 *
Swartz Foundation	Dr. Mitra	01/01/06	12/31/06	50,000 *
Swartz Foundation	Drs. Brody/Chow	01/01/06	12/31/06	50,000 *
Swartz Foundation	Drs. Mainen/Felsen	01/01/06	12/31/06	50,000 *
Swartz Foundation	Drs. Chklovskii/Mishchenko	01/01/06	12/31/06	50,000 *
Swartz Foundation	Drs. Mainen/Uchida	01/01/06	12/31/06	50,000 *
Michel David-Weill	CSHL Fellows Program	09/01/06	08/31/09	200,000 *
Training Support				
Cornelius N. Bliss Memorial Fund	Undergraduate Research Program	2006		4,500 *
Hunter College	Undergraduate Research Program	2006		7,000 *
Jephson Educational Trust	Undergraduate Research Program	2006		5,000 *
Steamboat Foundation	Undergraduate Research Program	05/01/06	04/30/07	12,000 *
William Townsend Porter Foundation	Undergraduate Research Program	04/01/06	03/31/07	10,000 *
Course Support				
American Brain Tumor Association	Mechanisms of Neural Differentiation and Brain Tumors	2006		40,000 *
Eppley Foundation	Stem Cells	2006		25,000 *
Eli Lilly	Nuclear Receptors and Disease	2006		5,000 *
Howard Hughes Medical Institute	Cell and Developmental Biology of *Xenopus*	09/01/04	08/31/07	330,000
	Ion Channel Physiology			
	Neurobiology of *Drosophila*			
	Advanced Techniques in Molecular Neuroscience			
	Yeast Genetics and Genomics			
	Imaging Structure and Function in the Nervous System			
	Crystallography			
	Phage Display of Proteins and Peptides			
	Genetics of Complex Human Diseases			
	Computational Neuroscience: Vision			
	Biology of Social Cognition Workshop	2006		17,900 *
Medical Research Council	Novartis Proteomics	2006		141,673 *
Novartis				

*New and competing renewal grants awarded in 2006

Society for Neuroscience	Scholarship Support	2006		17,520 *
Meeting Support				
Cell Signaling Technologies	Translational Control	2006		1,000 *
Lalor Foundation	Germ Cells	2006		5,000 *
March of Dimes	Germ Cells	2006		5,000 *
N.Y. Structural Biology Group	Crystallography Workshop	2006		16,000 *
Swartz Foundation	Neuronal Circuits: From Structure to Function	2006		10,000 *
Swartz Foundation	Neurobiology Students Journal Clubs	2006		15,000 *
Merck & Co., Inc.	Gene Expression and Signaling in the Immune System	2006		5,000 *
Research and Diagnostic Systems, Inc.	Gene Expression and Signaling in the Immune System	2006		2,000 *
BD Biosciences	Gene Expression and Signaling in the Immune System	2006		5,000 *
Abbott Bioresearch Center	Gene Expression and Signaling in the Immune System	2006		5,000 *
Elan Pharmaceuticals	Gene Expression and Signaling in the Immune System	2006		4,000 *
Genetic	Gene Expression and Signaling in the Immune System	2006		2,500 *
Point Therapeutics, Inc.	Gene Expression and Signaling in the Immune System	2006		3,500 *
Cell Signaling Technology, Inc.	Gene Expression and Signaling in the Immune System	2006		1,000 *
Boehringer Ingelheim Pharmaceuticals, Inc.	Gene Expression and Signaling in the Immune System	2006		1,000 *
Society for Developmental Biology	Workshop on Chicken Genomics and Development	2006		3,000 *
Genesis Faraday Partnership, Ltd.	Workshop on Chicken Genomics and Development	2006		2,515 *
Affymetrix	Workshop on Chicken Genomics and Development	2006		980 *
Roslin Institute	Workshop on Chicken Genomics and Development	2006		5,303 *
Library Support				
Josiah Macy, Jr. Foundation		05/01/05	05/31/07	310,857
New York State		07/01/06	06/30/07	4,412 *
Bauman Rare Books	Sidney Brenner Collection	10/01/06	09/30/07	40,000 *
Ridley Rare Books		09/01/02	12/31/06	10,000 *

*New and competing renewal grants awarded in 2006

DOLAN DNA LEARNING CENTER GRANTS

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*2006 Funding*[*]
FEDERAL GRANTS			
AAAS/NSF	BiosciEdNet (BEN) Collaborative Cycle 3	10/05–09/07	$ 60,958
Cornell University/NSF	Functional Analysis of Genes Involved in Meristem Organization and Leaf Initiation	01/06–08/07	33,559
National Science Foundation	Developing and Disseminating New Laboratories on Plant Molecular Genetics and Genomics	02/05–01/07	37,345
National Science Foundation	Developing and Disseminating New Laboratories in RNAi and Functional Genomics	06/05–12/06	153,518
National Science Foundation	VCA: Finishing the Rice Genome	09/04–08/07	68,042
National Science Foundation	VCA: Gramene: A Platform for Comparative Genomics	12/05–11/07	112,411
U.S.D.A.	Systematic Determination of the Gene Set	02/05–01/07	14,611
Virginia Tech/NIH	Partnership for Research and Education in Plants	09/04–08/06	7,694
Washington University/NSF	Sequencing the Maize Genome	11/05–10/07	17,501
NONFEDERAL GRANTS			
Amgen Foundation	Amgen *Leadership Symposium*	04/05–03/08	$ 85,925
Carolina Biological Supply Company	Research support	2006	75,000
Clemson University	License, training, and development	2006	50,000
Dana Foundation	*Genes to Cognition (G2C) Online*: A Network-driven Internet Site on Modern Brain Research	10/05–09/07	235,824
Dialog Gentechnik	License, training, and development	2006	24,950
Goldman Sachs Foundation	Planning grant	2006	50,000
Hewlett Foundation	*Genes to Cognition (G2C) Online*	10/05–10/08	114,451
North Shore–LIJ Health System	DNALC *West* support	2006	50,000
Porter Foundation	Underrepresented Communities Program	2006	30,000

The following schools each awarded a grant of $1000 or more for the ***Curriculum Study*** Program:

Bellmore–Merrick Central High School District	$ 1,250
Bethpage Union Free School District	1,250
Commack Union Free School District	2,500
Elwood Union Free School District	1,250
Fordham Preparatory School	1,250
Friends Academy	1,250
Garden City Union Free School District	1,250
Harborfields Central School District	1,250
Herricks Union Free School District	1,250
Huntington Union Free School District	1,750
Island Trees Union Free School District	1,250
Jericho Union Free School District	1,250
Lawrence Union Free School District	1,250
Levittown Union Free School District	1,250
Locust Valley Central School District	$ 1,250
Long Beach City School District	2,500
Massapequa Union Free School District	2,500
North Shore Hebrew Academy	2,500
Oceanside Union Free School District	1,250
Plainedge Union Free School District	1,250
Plainview–Old Bethpage Central School District	1,250
Portledge School	1,250
Port Washington Union Free School District	1,250
Roslyn Union Free School District	1,250
Sachem Central School District	1,250
South Huntington Union Free School District	2,500
Yeshiva University High School for Girls	1,250

The following schools each awarded a grant of $1000 or more for the ***Genetics as a Model for Whole Learning*** Program:

Bayshore Union Free School District	$ 5,460
Bellmore Union Free School District	3,400
Bellmore–Merrick Central School District	21,000
Brandeis School	1,850
Commack Union Free School District	5,100
East Meadow Union Free School District	1,975
Elwood Union Free School District	5,120
Farmingdale Union Free School District	1,600
Floral Park–Bellerose Union Free School District	5,800
Friends Academy	2,550
Garden City Union Free School District	10,845
Great Neck Union Free School District	23,792
Greenvale School	1,587
Half Hollow Hills Central School District	6,300
Huntington Union Free School District	5,800
Jericho Union Free School District	9,500
Lawrence Union Free School District	$ 7,800
Locust Valley Central School District	8,187
Mamaroneck Union Free School District	6,400
Merrick Union Free School District	1,200
North Bellmore Union Free School District	1,750
Old Westbury School of the Holy Child	4,950
Oyster Bay–East Norwich Central School District	2,267
Plainview–Old Bethpage Central School District	3,469
Port Washington Union Free School District	10,400
Rockville Centre Union Free School District	3,300
St. Dominic Elementary School	3,900
St. Edward the Confessor School	1,820
St. Mary's Elementary School	1,850
Syosset Union Free School District	30,175
Three Village Central School District	3,025
Uniondale Union Free School District	1,200

[*]Includes direct and indirect costs

BANBURY CENTER GRANTS

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*2006 Funding†*
FEDERAL SUPPORT			
Centers for Disease Control and Prevention (CDC)	Chronic Lyme Disease Syndromes: New Avenues for Investigation	2006	$ 7,830*
NIH–National Institute of Allergy and Infectious Diseases	Chronic Lyme Disease Syndromes: New Avenues for Investigation	2006	5,000*
NIH–National Institute of Arthritis and Musculoskeletal and Skin Diseases	Chronic Lyme Disease Syndromes: New Avenues for Investigation	2006	5,000*
NIH–National Institute of Mental Health (through a grant to University of Illinois)	Fragile X Syndrome: Basic Mechanisms and Treatment Implications	2006	47,453*
NIH–National Institute of Neurological Disorders and Stroke	Chronic Lyme Disease Syndromes: New Avenues for Investigation	2006	7,000*
U.S. Food and Drug Administration	Chronic Lyme Disease Syndromes: New Avenues for Investigation	2006	7,000*
NONFEDERAL SUPPORT			
Meeting Support			
Aetos Technologies, Inc.	Chronic Lyme Disease Syndromes: New Avenues for Investigation	2006	10,000*
The ALS Association	Axonal Dynamics and Synaptic Junctions	2006	29,311*
American College of Medical Genetics	The Evolving Role of the Board-certified Medical Geneticist	2006	10,335*
Autism Speaks	A Critical Assessment of Autism Genetics	2006	5,000*
Cure Autism Now	A Critical Assessment of Autism Genetics	2006	5,000*
The Dart Foundation	Design Principles in Biological Systems	2006	50,000*
The Thomas Hartman Foundation for Parkinson's Research	Parkinson's Disease: Insights from Genetics and Toxin Models	2006	25,000
The William and Flora Hewlett Foundation	New Horizons in Internet Site Development	2006	30,200
Nancy Lurie Marks Family Foundation	A Critical Assessment of Autism Genetics	2006	10,000*
McLaughlin Centre for Molecular Medicine	A Critical Assessment of Autism Genetics	2006	5,000*
National Alliance for Autism Research	A Critical Assessment of Autism Genetics	2006	10,000*
Marie Robertson Memorial Fund	The Phenomology of Reconsolidation	2006	20,000
Simons Foundation	A Critical Assessment of Autism Genetics	2006	20,000*
Spinal Muscular Atrophy Foundation	Spinal Muscular Atrophy: From RNA to Synapses	2006	42,267*
The Stanley Foundation	Research on Genetics of Bipolar Disorder: Current Approaches and Future Directions	2006	7,331*
The Swartz Foundation	Computational Approaches to Cortical Function	2006	42,697*
Verto Institute	The Biology of Neuroendocrine Tumors	2006	31,490*
Volkswagen Foundation	The Phenomology of Reconsolidation	2006	19,572*

†Includes direct and indirect costs
*New grants awarded in 2006

DEVELOPMENT

The year 2006 was a very exciting one for all of us at Cold Spring Harbor Laboratory. In addition to the many outstanding research discoveries, the Laboratory officially embarked on a $200 million campaign to raise funds for six new research buildings, to support recruitment of new faculty in cancer and neurobiology, and to build the endowment. To date, the Laboratory has already raised $134 million toward this campaign goal.

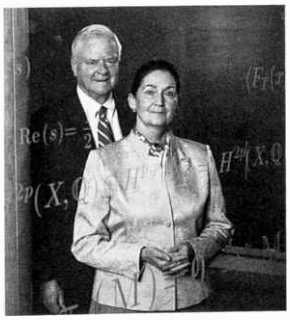
The inaugural Double Helix Medal Dinner was held on November 9, during which Muhammad Ali, Suzanne and Bob Wright, and Phil Sharp were honored for their extraordinary achievements in the field of biomedical research. The evening raised more than $2 million in unrestricted funds for Laboratory research.

This year, as Cold Spring Harbor Laboratory celebrates many successes, we acknowledge all who continue to believe so strongly in the work that we do.

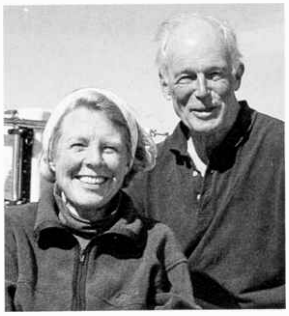
Charles V. Prizzi, Chief Development Officer

Cold Spring Harbor Laboratory is a nonprofit research and educational institution, chartered by the State of New York. ScienceWatch, an independent ratings service, has ranked the Laboratory's molecular biology and genetics program number one during the last decade. Additionally, Charity Navigator, a philanthropic evaluator, has bestowed their highest 4-star rating on the laboratory for 5 consecutive years.

Less than half of the Laboratory's annual revenues are derived from Federal grants and contracts, and thus, we rely heavily on support from the private sector: foundations, corporations, and individuals. Foundations, corporations, and individuals can give to Cold Spring Harbor through a variety of methods:



Landon and Lavinia Clay established the Crick-Clay Endowment for Biomathematics.



A lead gift from Holly and Henry Wendt will enable the Lab to construct a building for neurological disorder research.

Capital and Endowment Campaign Support: Cold Spring Harbor has embarked on a $200 million capital and endowment campaign to speed the translation of genetic discoveries into diagnostic and therapeutic treatments and to expand Lab facilities and staff. Endowment gifts can be directed toward supporting cancer, neuroscience, or educational programs. Capital gifts can be made to name laboratories and conference rooms in one of our six new research buildings.

Annual Fund and Research Support: Donations provide funding for some of the most determined and innovative young researchers in science today. Your gift is an investment in some of the world's most innovative research in cancer, neuroscience, plant biology, and bioinformatics.

Science Education: Donations support programs at the Dolan DNA Learning Center, where the next generation of scientists learn about genetics in an exciting and interactive environment.

Planned and Estate Gifts: Individuals who inform us of their intention to make a gift to Cold Spring Harbor Laboratory from their estate are invited to become members of The Harbor Society. Estate gifts help to ensure that CSHL will continue to pursue its mission for many years to come.

For additional information, please contact the Chief Development Officer, Cold Spring Harbor Laboratory, One Bungtown Road, Cold Spring Harbor, New York 11724. Phone number: 516-367-6865.

President's Council

The Cold Spring Harbor Laboratory President's Council is composed of individuals who gather together for stimulating scientific events related to current CSHL research. Their contributions of $25,000 or more annually support the Cold Spring Harbor Laboratory Fellows, a group of exceptionally talented young scientists who show the capacity for high-level innovative research. Rather than conducting a postdoctoral apprenticeship with a senior scientist, as is conventional, Fellows begin independent research immediately following receipt of their Ph.D.s. This rigorous program promotes outstanding young scientists to advance their careers at a young age so that they can promptly contribute new insights to important biomedical science.

In May, President's Council member and cochair Bill Haseltine hosted a spring reception—A Taste of Cold Spring Harbor Laboratory—for Council members at his home in New York City. At Cold Spring Harbor's campus in October, the 2006 meeting—Cognition and Its Disorders—featured talks by scientists working throughout the entire spectrum of cognitive neuroscience. From exploring the molecular and genetic underpinnings of brain function to developing state-of-the-art tools for clinical studies, these scientists explained how they work to bridge the most promising advances in understanding the brain. The connections they make, such as the neural circuitry that underlies the workings of the brain, may elucidate fundamental aspects of emotion, logic, and decision-making, as well as the biological roots of conditions such as autism, schizophrenia, Alzheimer's, and Parkinson's.

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Karen H. Bechtel
Dr. Gerald Chan
Dr. and Mrs. Lan Bo Chen
Richard Baron Cohen
Michel David-Weill
David B. Ford
Mr. and Mrs. John H. Friedman
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Mr. and Mrs. Charles E. Harris
Dr. William A. Haseltine
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Mr. and Mrs. Bryan H. Lawrence
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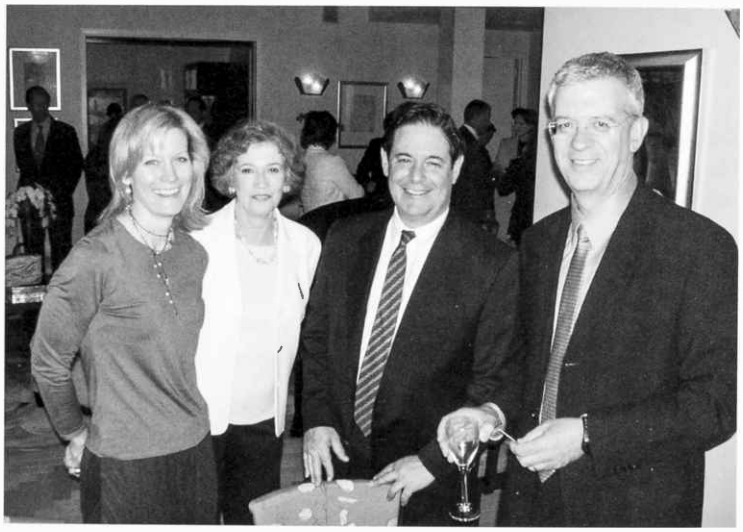
Dr. and Mrs. James M. Stone
Dr. and Mrs. Jerome Swartz
Joan Tilney
Mr. and Mrs. Raymond L.M. Wong

As part of the 2006 President's Council meeting, Mara Haseltine's sculpture "The Waltz of the Polypeptide" was officially donated to the Laboratory and dedicated to her father, President's Council cochair Bill Haseltine.



At the President's Council spring reception "A Taste of Cold Spring Harbor Laboratory" are (*left to right*) Jane Friedman, Beverley Gasner, John Friedman, and Dr. Mark Zoller.

Cold Spring Harbor Laboratory Association (CSHLA)

Members of the Cold Spring Harbor Laboratory Association have supported and encouraged young scientists for many decades. The Laboratory Association annual fund is an important source of unrestricted funding for postdoctoral fellows and new faculty as they set up labs and pursue highly creative projects that are not eligible for government funding. In 2006, for the third year in a row, members contributed well over $1 million in unrestricted funding for the ground-breaking research projects for which our scientists are so well known.

Laboratory Association members are invited throughout the year to lectures on timely social science and health topics ranging from nutrition to bioterrorism. Subscriptions to our popular Saturday evening Young Concert Artist Series are complimentary to members at the Friend level and above. Our quarterly Harbor Transcript newsletter informs Association members about our scientists, students, support staff, and the volunteers who are integral to the Laboratory's important health discoveries. All members are invited to social events at the Laboratory and encouraged to get to know our scientists and their families. They are also encouraged to join our annual Jazz at the Lab benefit committee and to attend our annual Double Helix Medal benefit in New York City.

We are grateful for the nearly 1000 annual members of the Laboratory Association who partner in our mission to lead the world in molecular biological discoveries and to raise awareness of the importance of genetics research to improve the health of people everywhere.

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2006 CSHLA Directors

Dolan DNA Learning Center Corporate Advisory Board

The Corporate Advisory Board (CAB), established in 1992, serves the Dolan DNA Learning Center as a bridge to the corporate community and assists in securing unrestricted funds for annual support. Composed of influential business leaders from large and small companies on Long Island and Manhattan, the CAB is a necessary and vital component to the continued success and mission of the Dolan DNALC.

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Robert Van Nostrand, OSI Pharmaceuticals, Inc.
Zock Zamaniyan, Eppendorf North America
Teresa Kemp-Zielenski, United Way of Long Island
Hans Zobel, Festo Corporation

On June 20 at the 13th annual golf fund-raiser, the newly formed OSI Foundation chose the Dolan DNA Learning Center of Cold Spring Harbor Laboratory to be the recipient of its first-ever award. (Left to right) Dave Micklos, Dolan DNA Learning Center; Dr. James Watson, Chancellor of Cold Spring Harbor Laboratory; Ann McDermott-Kave, Director OSI Pharmaceuticals Foundation; Dr. Colin Goddard, President and CEO OSI Pharmaceuticals; Morgan Browne, Chairman of OSI Pharmaceuticals Foundation.

Honor Roll of Donors

Contributions of $5,000,000+ (lifetime)

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Arnold and Mabel Beckman Foundation
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The Shelby Cullom Davis Foundation*
The DeMatteis Family Foundation*
Mr. and Mrs. Charles F. Dolan–The Dolan Family Foundation
The Lita Annenberg Hazen Foundation
The W.M. Keck Foundation
David H. Koch*

A lead gift from the DeMatteis Family Foundation will provide funding to construct one of the Laboratory's six new research buildings. Pictured are Nancy DeMatteis and Jim Watson at the Cornerstone Dedication Ceremony.

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The Thomas Hartman Foundation for Parkinson's Research
Janet Strauss and Jeff Hawkins*
Jo-Ellen and Ira Hazan
William Randolph Hearst Foundation*
Mr. and Mrs. Martin Gruss–Hope for Depression Research Foundation, Inc.
The Karches Foundation

Three generations of the Monte Family attended an October 30, 2006 dedication of the Tita Monti Cancer Research Laboratory. (Left to right) Anna Travaglia, Arthur Saladino, Caroline Saladino, Richard Monti, and Joseph Monti.

*Gift or pledge to the Hillside Campus Campaign

The inaugural Double Helix Medals Dinner, held on November 9, 2006, at the Mandarin Oriental, honored Muhammad Ali, Bob and Suzanne Wright, and Dr. Phillip Sharp. Pictured from left are Double Helix Medal recipient Muhammad Ali with Monsignor Thomas J. Hartman.

Mr. and Mrs. John Klingenstein–The Esther A. & Joseph Klingenstein Fund, Inc.
Mr. and Mrs. Townsend J. Knight
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The Forrest and Frances Lattner Foundation
Mr. and Mrs. Gerard Leeds*
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Find A Cure Today (F.A.C.T.)
Goldman Sachs Foundation
The Joseph G. Goldring Foundation
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Long Islanders Against Breast Cancer (L.I.A.B.C.)
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Edith and Alan Seligson–The Seligson Foundation
Dr. and Mrs. Charles L. Stone, Jr.–The Stone Foundation*
Waldbaum Foundation

Contributions of $25,000+

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Karen H. Bechtel
The Louis Berkowitz Family Foundation
Berlin Family Foundation
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Dr. Gerald Chan–The Morningside Foundation
Dr. and Mrs. Lan Bo Chen
Richard Baron Cohen
Mr. and Mrs. John H. Friedman
David B. Ford
Brandon Fradd
Glen Cove C.A.R.E.S.

Double Helix Medal recipients Suzanne and Bob Wright with Meredith Vieira (*center*).

Jacob Goldfield
Robert I. Goldman Foundation
Mr. and Mrs. Charles E. Harris II
Harrison's Heart Foundation
William A. Haseltine, Ph.D.
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Cold Spring Harbor Laboratory President Bruce Stillman (*center*) with Bruce Kovner (*left*) and David Koch at the Double Helix Medals Dinner.

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On October 16, 2006, the Cornerstone Dedication for Cold Spring Harbor's Hillside Research Campus was held. Pictured along with Jim Watson (*left*) are Mardi Matheson, Peter Quick, Tom Quick, Nancy DeMatteis, Joan Axinn, Bruce Stillman, and Jennifer Axinn-Weiss.

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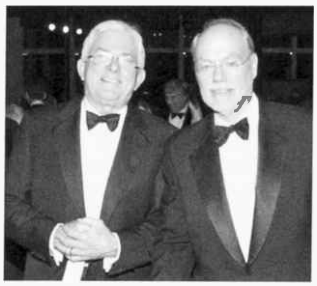

Double Helix Medals Dinner emcee Phil Dohahue (*left*) and Medal recipient Dr. Phillip Sharp.

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Cold Spring Harbor Laboratory Association President, Joe Donohue (*left*) with Linda Valenti, Pien Bosch, and Nick Valenti at Jazz at the Lab.

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Cold Spring Harbor Laboratory Chairman Eduardo Mestre (*left*) with Bridget and John Macaskill at Jazz at the Lab.

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The 8th Annual Jazz at the Lab was held on April 22, 2006. Pictured are Charles Dolan (*left*) and Bob Vizza.

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Cold Spring Harbor Laboratory Trustee Ed Travaglianti (*left*) with Dev Maulik, Paul Amoruso, Artie Saladino, and Joe Faria at Jazz at the Lab.

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