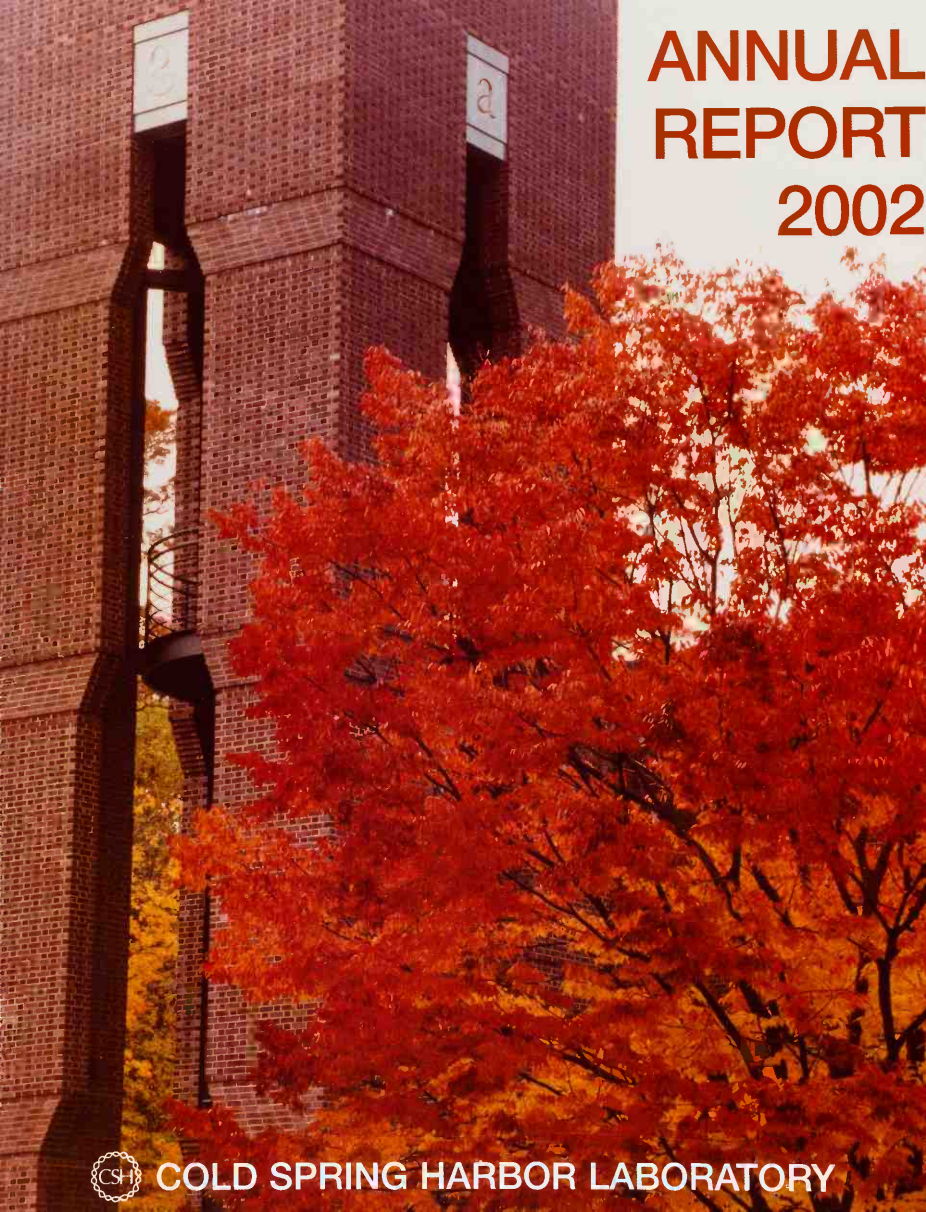

ANNUAL REPORT 2002

COLD SPRING HARBOR LABORATORY

ANNUAL REPORT 2002

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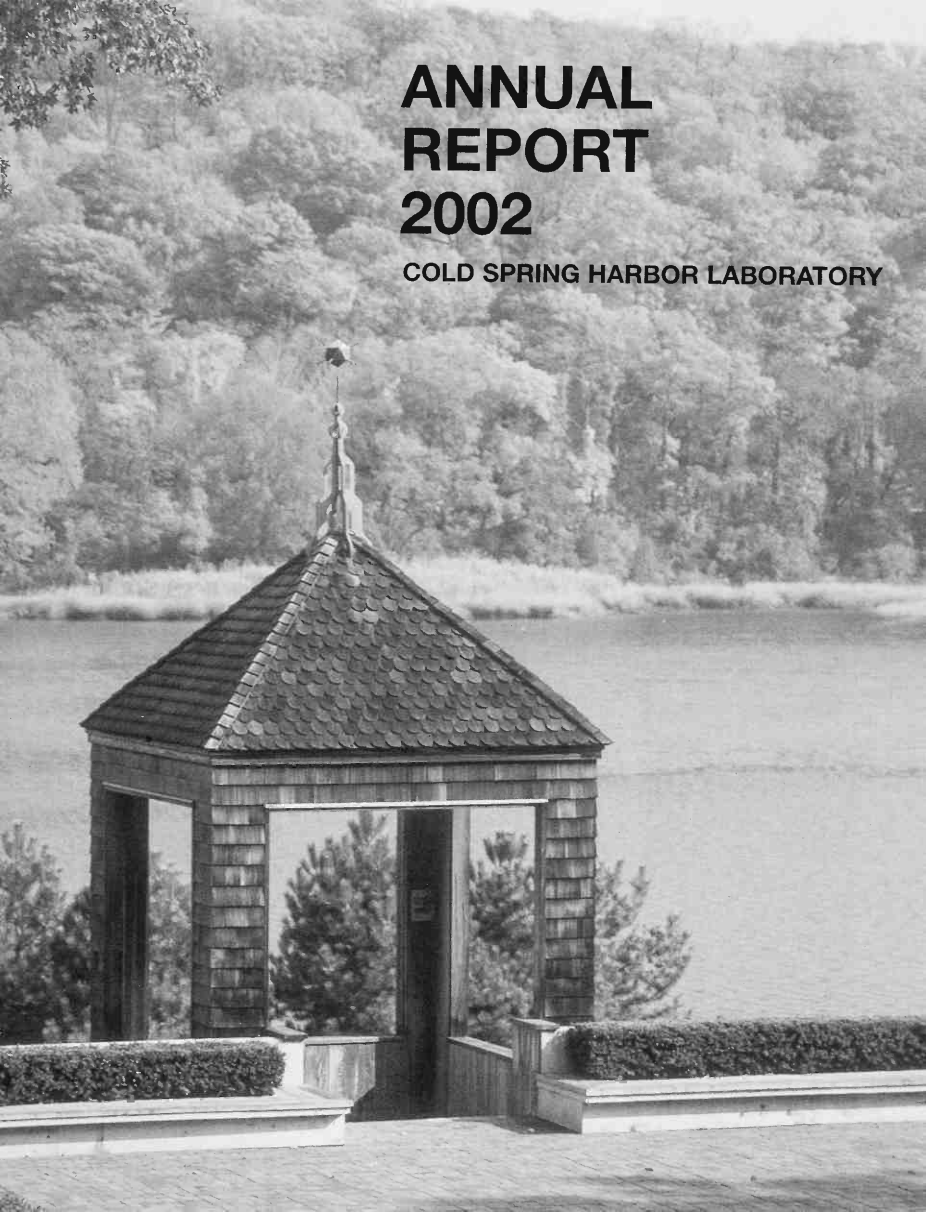

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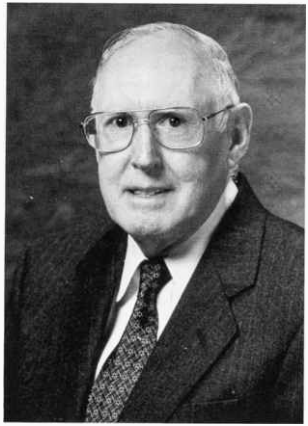


William Maxwell Cowan (1931–2002)

It is with great sadness that the world of biomedical research learned of the death of Dr. William Maxwell Cowan on June 30, 2002. A long-time friend, course lecturer, symposium speaker-organizer, and trustee (1988–1994), Dr. Cowan greatly enhanced our Laboratory through his intelligence, teaching abilities, and the open fairness with which he judged science and its participants.

He was born in Johannesburg, South Africa on September 27, 1931. After receiving Bachelor's degrees from the University of Witwatersrand in 1951 and 1952 (with honors), he attended Oxford University where, between 1956 and 1959, he received B.M., B.Ch., M.A., and D. Phil. degrees. During this early period at Oxford, his obvious skills led to a fellowship at Pembroke College and an almost instant Demonstratorship in Anatomy. Max then completed his clinical training and embarked on an illustrious career in neurobiology. Among his first studies were those that mapped an unusual group of connections in the brain between the thalamus and the corpus striatum. It provided the foundation for a career that included more than 200 published articles.

Max remained at Oxford until 1966, when he moved briefly to the University of Wisconsin as an Associate Professor. In 1968, he joined the Washington University School of Medicine in St. Louis, where he was appointed Professor and Chairman of the Department of Anatomy. There Max was among the first to use electron microscopy to establish patterns of synaptic connections between nerve cells. In doing so, he uncovered an "activity-dependent" mechanism that prunes erroneous or excess connections.

From 1988 to 2000, Max served as Vice President and Chief Scientific Officer of the Howard Hughes Medical Institute. During this interval, the Howard Hughes Medical Institute successfully navigated a period of unprecedented expansion and is today a premier supporter of biomedical research and education in the United States and throughout the world.

He was a Foreign Associate of the U.S. National Academy of Sciences, a Fellow of the Royal Society (London), and member of the American Academy of Arts and Sciences, the American Philosophical Society, and the Society of Neuroscience (President 1977–1978) for whose main journal he served as Editor-in-Chief between 1980 and 1987. Among his prizes were the Karl Spencer Lashley Prize for Neurobiology and the Dana Foundation Distinguished Achievement Award in Health.

Max's association with Cold Spring Harbor Laboratory began when I invited him in 1971 to give a lecture in the Laboratory's newly created summer neurobiology course, "Basic Principles of Neurobiology." Liking the experience, he returned to Cold Spring Harbor the next summer to give five lectures, living with his family in a cabin next to the Page Motel.

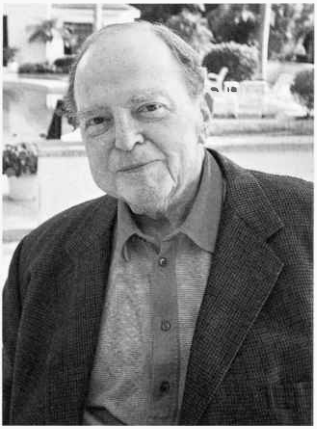
On the basis of these and subsequent summer lectures at Cold Spring Harbor Laboratory, Dr. Cowan was invited to teach a five-week course at The Salk Institute in La Jolla, California. This led him to a series of positions at The Salk Institute and at the University of California, San Diego, between 1980 and 1986, culminating in a vice presidency at The Salk Institute. He returned to Washington University as Provost in 1986. In this position, Max typically enjoyed interacting with the students more than with the faculty, because in discussions with the latter, he would "run out of synonyms for *no*."

As Chief Scientific Officer of the Howard Hughes Medical Institute, he was thought by some recipients of its largesse as a *micro*-manager. In reality, his sense of fairness never let him give out monies that he thought were more for the inflation of egos than for the doing of important science. We frequently had supper together in Bethesda when I was at NIH as Director of its Human Genome effort. Always a realist and in no way a cynic, Max worried about the way the *Decade of the Brain* (1990–1999) had come about. When it was over, he thought no one would remember it. Toward that end, he, Kay Jamison, and I persuaded David Mahoney, the President of the Dana Foundation, to hold a meeting at our Banbury Center in November 1992 to discuss funding for the *Decade of the Brain*. Out of it came the Dana Alliance for Brain Initiatives. Over the last 10 years, it most imaginatively has taken up the task of explaining to the general public how research will lead to the development of new treatments and therapies for brain diseases.

Shortly after his retirement in 2000 from Hughes, Max found himself a victim of prostate cancer and underwent surgery. Wicked complications arose and Max's next months were not easy. Never a complainer, Max's exemplary fortitude was always there when I saw him and his wife Margaret at their home in Rockville. Fortunately, his disease temporarily went into seemingly complete remission allowing him to attend our November 2, 2001 commencement where we bestowed upon him the degree of Doctor of Science honoris causa from our School of Biological Sciences. At the ceremony, he spoke of his fondness for us and his strong support for our research. Six months later, his cancer came back with a vengeance that mercifully did not let him suffer long.

Max is sorely missed by all who had the good fortune to know him.

Jim Watson
President



William L. Matheson (1924–2002)

A long-time resident of Mill Neck, New York, New York City, and Hobe Sound, Florida, Bill Matheson was a gregarious, public-spirited attorney and businessman with a sharp wit and an unfailing sense of humor. Behind his contagious humor was a seriousness of purpose that led him and his wonderful wife of 25 years, Mardi, to become among the most devoted and prolific supporters in the Laboratory's history.

Bill was born in Coeburn, Virginia, and spent his formative years in northern Georgia. He was a graduate of Emory University and the University of Virginia Law School where he earned his LL.B and J.D. degrees. During the war years, he served in the United States Navy from 1942 to 1946. Bill's very successful professional career in New York began as an associate attorney with Patterson, Belknap and Webb, and he was admitted to the New York State Bar in 1951. He was later employed on Wall Street at Werthheim & Company before returning to law practice at Webster, Sheffield and Chrystie. The combination of legal and business skills served Bill well as he went on to become Chairman of Michigan Energy Resources and its predecessor companies, a post he held from 1959 until its merger with UtiliCorp in 1989.

Bill's penchant for hard work did not preclude time for family and leisure activities. He was a lover of the outdoors and of animals. He owned and maintained a cattle farm for many years in Ocala, Florida. Bill had a love for horseback riding and tried his hand at investing in and training racehorses. He was an enthusiastic golfer with an enviable list of memberships at many of the great courses in Florida and on Long Island.

Always generous with his time and resources, Bill had many civic and educational involvements. Among those were the Police Athletic League in New York where he served as a Trustee for 40 years and the Madison Square Boys Club where he was a board member from 1958 to 1976. He also served his alma mater as a member of the University of Virginia Law School Alumni Council.

Most fortunately for this institution, Cold Spring Harbor Laboratory began to capture Bill's imagination during the decade of the 1990s and a wonderful partnership evolved. Increased exposure to the Laboratory and its leadership sparked Bill's innate intellectual curiosity. His interest and support resulted in his election to the Board of Trustees in 1996. Bill was an engaged and active Trustee who served on the Audit Committee. He clearly enjoyed his association with Jim Watson, the scientists, and his colleagues on the board. He grasped the seriousness of the Laboratory's mission, enjoyed learning about the science, and developed a genuine interest in the neuroscience program. Bill understood philanthropy and knew how to employ it to an institution's maximum advantage. His and Mardi's pattern of generosity to Cold Spring Harbor Laboratory has been deliberately planned to give inspiration and support at absolutely critical times in the Laboratory's development. In 1996, *The Marjorie Anderson Matheson and William Lyon Matheson Fund for Neuroscience* was established to provide ongoing support for young investigators in this rapidly growing area of the Laboratory's research. The nature of the neuroscience program was such that many of these young investigators were doing important work not deemed mainstream enough by the government agencies to be publicly fundable. The Matheson Fund provided the necessary shot in the arm.

Having done his part to ensure the success of the neuroscience program, Bill recognized the formation of the Laboratory's innovative Ph.D. program, the Watson School of Biological Sciences, as a seminal event in the institution's history. In 1998, Bill and Mardi announced their intention to establish the *Cold Spring Harbor Laboratory, Watson School of Biological Sciences Founders Endowment* in honor of George A. and Marjorie H. Anderson (Mardi's parents). The importance of this lead gift to the permanent endowment of the new graduate school cannot be overstated. In addition to providing funding for five Anderson Fellowships, it gave all those associated with Cold Spring Harbor Laboratory—Trustees, faculty, and supporters alike—the inspiration and the confidence to proceed with this unprecedented educational mission. The gift provided the cornerstone for former Board Chairman David Luke's successful campaign to endow the school and its talented students.

As Bill's health began to decline in recent years, he and Mardi spent more time at their beautiful home on the inland waterway in Hobe Sound, Florida. Fortunately, distance did not diminish Bill's interest in the Laboratory. He encouraged our visits and kept himself current on our plans and aspirations. He was particularly interested in the plans to expand our research and academic facilities on the western elevation of the main campus. As his body began to succumb to the too familiar ravages of cancer, his mind and his wit remained sharp and, unbeknownst to us, he and Mardi were planning another major gift to the Laboratory during the weeks and days before his death. This gift will give us the distinct pleasure of naming a new cancer research building (one of four new buildings planned for phase one of our upper campus) after Bill and Mardi. Again, the timing and magnitude of this philanthropy instill us with confidence and inspiration as we embark on the next critical stage of the Laboratory's development.

Bill's final days were spent peacefully at his home in Hobe Sound with Mardi at his side. To lose another wonderful friend and Trustee to cancer deeply saddens us. At the same time, it is people like Bill Matheson who inspire us and increase our determination and resolve. As Bill's quality of life declined at the end, Mardi tells us that his sense of humor never left him. We will remember the ever-present twinkle in his eye and will always value his friendship. Bill lived a full life and he did so with a keen sense of humor, dignity, and generosity.

Dill Ayres
Chief Operating Officer

DIRECTOR'S REPORT

Much has been written about the extraordinary events that took place in Cambridge, England, 50 years ago that changed biology forever. The discovery of the double-helical structure of DNA ushered in an immediate future for understanding how genes are inherited, how genetic information is read, and how mutations are fixed in our genome. 2003 will appropriately celebrate the discovery and the stunning developments that have occurred since, not only in biology and medicine, but also in fields unanticipated by Jim Watson and Francis Crick when they proposed the double helix. DNA-based forensics is but one example, having an impact in the law to such an extent that some states are now reviewing whether capital punishment should be continued because of the possibility of irreversibly condemning the innocent.

In all the writings and lore about the double-helix discovery, one of the little discussed points that struck me was the freedom that both Jim and Francis had to pursue what they felt was important, namely, the structure of DNA. Having completed graduate studies in the United States, Jim Watson went to Copenhagen to continue to become a biochemist in the hope that he might understand the gene, but he soon realized that biochemistry was not his forte. Most importantly, after hearing Maurice Wilkins talk about his early structural studies on DNA, Jim had the foresight that understanding DNA structure might help understand the gene and therefore he decided to move to Cambridge, then, as now, a center of the field now known as structural biology. Remarkably, although this decision was strongly supported by his mentors, it was not favored by the U.S. funding agency that paid his stipend in Copenhagen. If Jim had followed the suggestions of the funding agency, he would have moved to Stockholm to work with the cell biologist Torbjörn Caspersson, almost certainly ensuring that he would play no role in figuring out the double helix. Support from Salvador Luria, his Ph.D. research mentor, and his new colleagues at Cambridge enabled him to make this important move and essentially ignore funding considerations. Although supposedly working on protein structure, Jim was intellectually free to think more about DNA than protein. Eventually, Luria's and later Max Delbrück's support facilitated funding for Jim to remain in Cambridge. Thus, it helps to have supporters who can influence funding agencies.

Meeting Francis Crick at Cambridge is now the stuff of legend. Francis was pursuing his thesis work on proteins, but he had the freedom and eventual support from his peers to go after DNA when it was clear that building models was the best way to proceed. Individually, they took a big gamble, particularly Jim Watson because failing meant having nothing to show for a substantial amount of time, talk, and energy. However, Cambridge was an environment where risky projects were the norm. At the same time, Fred Sanger was sequencing the first protein (insulin), and Max Perutz and John Kendrew were determining the first three-dimensional structures of protein.

Fifty years ago at Cold Spring Harbor, Al Hershey and Martha Chase had just demonstrated that DNA was the genetic material in phage, the viruses that infect bacteria. Meanwhile, then-director Milislav Demerec yet again played an important role in the development of Cold Spring Harbor Laboratory as a major research center. Immediately after the

Second World War, the two laboratories at Cold Spring Harbor that were both directed by Demerec proposed an expansion of the inadequate facilities, including the addition of a lecture hall and new laboratory buildings and upgrading the grounds. The necessary funds came in 1950 from the Carnegie Corporation and the Rockefeller Foundation. But the events in Korea (some things never change) prevented starting the project until August 1951. Vannevar Bush, then director of the Office of Scientific Research and Development that was responsible for science during the Second World War, opened the new lecture hall in late May 1953, just in time for the annual Symposium. (In 1945, in response to a request for recommendations from President Roosevelt, Bush issued a now-classic report ["Science: The Endless Frontier"] in which he described the importance of biomedical research "for the War Against Disease, for our National Security, and for the Public Welfare.")

Organized principally by Max Delbrück, it was at the first Symposium in the new Bush Lecture Hall in June of 1953, attended by a record 272 scientists, that Jim Watson presented the double helix to a well-prepared audience. Delbrück had distributed copies of the *Nature* paper prior to Jim's talk. A few weeks earlier, Bush, the engineer turned science administrator, stated about biological research, "If I were a young man I am sure that that would be a field I would plunge into. Every day it becomes more attractive; and it touches the lives of all of us in countless ways." Perhaps he had been tipped off about the lecture that was soon to be presented in the hall that now bears his name. He went on, "It is a privilege of you neighbors on Long Island to extend this opportunity to men of great intellect, who can qualify, and who can thus bear the torch for all of the rest of us, in delving into some of the mysteries of life." It is still a happy circumstance that men and women who work at or visit Cold Spring Harbor receive most welcome support and encouragement from our neighbors.

Bush was correct in noting that the field of biology was then at a pivotal time. The double helix was the seed of a revolution that is still ongoing. Although life is no longer mysterious, surprising discoveries continue, such as the recently discovered RNA interference (RNAi) field that scientists at Cold Spring Harbor have helped understand. These new discoveries come from investigator-initiated research that is generously supported by U.S. taxpayers via grants and very importantly by philanthropic donations that provide the catalyst for new ideas.

The modern scientific enterprise depends on appropriate peer review of grant applications, but there are warning signs that the quality of peer review and the mechanisms for distribution of funds need attention. The number of scientists has greatly increased and thus competition for grants is keener now than it ever has been, even in the era when Congress has most strongly supported National Institutes of Health (NIH) funding increases. But the huge success of molecular biology and the wide availability of techniques provide challenges to the Federal funding mechanism. Due to wide dissemination of kit-based techniques, it is relatively easy for individual scientists to string together a series of standard molecular biology methods and propose in a grant application to tackle what is effectively an incremental problem, most likely a problem that many others are also pursuing. When these grant applications are reviewed, they receive enthusiastic support from a panel of peers because the science can obviously be achieved. Grant applications that propose straightforward research do relatively well compared to grant applications that contain really unique and innovative ideas and experimental strategies with fewer preliminary results. Thus, the process of peer review often stifles innovative science, in much the same way as Jim Watson would have been stifled by taking the advice of his funding agency.

Why does truly innovative science fair poorly in grant applications? One reason is that the panel of scientists who collectively review grant proposals tends to make conservative decisions, even though individual scientists in that group may be very receptive to innovative ideas. Equally important is the sagging quality of peer reviewers. In the past year, I have read many reviews of applications for grants from my colleagues at Cold Spring Harbor and a surprising number defy explanation. In a couple of particularly egregious cases, two of our scientists had separate grant applications in which they proposed research which led them to discover that RNAi-based mechanisms are directly involved in the formation of heterochromatin, the part of the genome that is transcriptionally silent. Incredibly, both of the grant applications not only failed to receive support, but in one case, the review suggested that the whole approach was a waste of time and that the applicant could "improve" his chances of being funded by working on other areas, which the review then went on to suggest. It was obvious to me that the originally proposed science—and more importantly the scientists—were innovative, on the cutting edge, and had a good probability of discovering important new findings. Thus, I found funds from our budget to allow the research to continue. Later, those same two scientists along with another Cold Spring Harbor colleague and others elsewhere were cited by *Science* as having made the #1 "Breakthrough of the Year" in 2002. This Letterman-like top ten list of scientific accomplishments contains what editors at *Science* consider to be the key important advances in all fields of science made over the past year or so.

All scientists can point to such anecdotes, but my reading of many grant reviews over the past couple of years is that these are no longer isolated cases. What can be done to improve peer review? First, it should be stated that investigator-initiated proposals and peer review of them are the foundation of the success of American science, so the system should not radically change. But there are changes that I believe would greatly improve the current situation.

First among these is improving the quality of reviews. Reviewers who remain anonymous to the applicant should nonetheless be held accountable for the comments that they write into grant reviews. One of the principal roles of the chair of the review panel should be to read the reviews of those grant applications that do not get funded and the response to those reviews made by applicants when they resubmit the grant application. If the applicants write a short, limited response to the reviews and only focus on the important issues, this will not be a difficult task for a panel chair. Although the chair will find many of the applicant's responses an overreaction to the panel's comments, the latter that fit into the egregious category would allow the chair to counsel the reviewer on the appropriate way to review applications. If this mechanism were in place, reviewers who spend four years on a review panel would receive some education about the concept of constructive criticism.

A second improvement could be the reintroduction of a limited number of awards that fund truly outstanding scientists and not specific projects, much like the highly successful mechanism of funding science at the Howard Hughes Medical Institute (HHMI). The NIH had for some time an outstanding investigator awards program that allowed those individuals to use the funds to pursue innovative ideas. A modified version of this mechanism within the NIH structure would go a long way toward giving scientists with a proven record of the highest quality of research the confidence that they could remain innovative, without having to receive HHMI support.

The idea of supporting people and not projects should also be extended to younger scientists in the earlier stages of their careers. All too often, they must apply for research fund-

ing for specific projects without the preliminary results that are now so important to obtain an NIH grant. A new mechanism that encourages bright new investigators to be bold in their initial research would greatly enhance NIH support of our best young scientists who are now in danger of being demoralized by the existing peer review process. Cold Spring Harbor Laboratory has for some years now had a highly successful CSHL Fellows program that enables recent Ph.D. graduates to pursue independent research without any financial burden. Most importantly, they have the freedom to do what they think is best. This program has been very productive and all former Fellows are now leaders in their field of research (see www.cshl.edu:80/public/fellows.html).

Finally, there needs to be a reassessment of the "Request for Application" (RFA) mechanism that has grown so prominent within the NIH. Under current practice, staff within the Institutes at NIH can initiate funding in a particular area of research by advertising an RFA and soliciting grant applications in these areas. They often come with designated funds, thereby limiting the pool of funds available for investigator-initiated ideas. The RFA mechanism works very well when staff in an individual Institute organize a meeting of broad thinkers in a particular field to advise on major new research directions that will be beneficial to science. Good examples are large-scale research projects that the National Cancer Institute (NCI) introduced under the leadership of Richard Klausner when he was Director of the NCI. These projects, such as the mouse models for human cancer, the cancer genome anatomy project, and the development of innovative imaging technologies, were introduced after much thought and debate. They also challenged investigators to think of innovative ideas in new areas of potentially high impact to controlling cancer.

Institute staff initiate RFAs within their portfolio of research funding, but unfortunately, my reading of some of them is that they propose obvious, incremental science. For example, some propose the use of RNAi to study a certain aspect of biology. This exciting new genetic technique has raced around the scientific community and there is no need to encourage its use. Those scientists who are thinking well ahead would have already submitted grant applications using the new technology, leaving those who respond to the RFA to be scientists who would have missed the boat if it had not been for an RFA. Such a mechanism, if allowed to proliferate, will encourage only incremental science by those who cannot be innovative on their own. I believe that there needs to be a reassessment of the whole process of issuing RFAs.

In some ways, research has not changed since the days when Jim Watson and Francis Crick proposed the structure that has become an icon for modern medical research. Today, new investigators in the formative stages of their careers still have many of the best ideas. The MRC-funded Cavendish laboratory and its highly successful successor, The Laboratory of Molecular Biology (LMB), were arguably the most productive research centers in the world, mainly because they supported the careers and visions of talented people, not specific research projects. Fred Sanger, who developed both protein sequencing and DNA sequencing over long periods of time and received two Nobel Prizes for the accomplishments, might not have survived if he had been subject to current NIH peer review. When assuming the Directorship in 1968, Jim Watson in many ways modeled Cold Spring Harbor Laboratory after the LMB. Although dependent on NIH grants, he made sure that we had the necessary funds when peer reviewers turned down some applications that were, nonetheless, worth funding. Such support, still common today, is the reason why Cold Spring Harbor Laboratory remains a leader in the fields we choose to pursue.

HIGHLIGHTS OF THE YEAR

Research

Cancer

In October, Masaaki Hamaguchi and Michael Wigler published their discovery of a new tumor suppressor gene that is missing from or inactive in 60% of the breast cancer specimens they examined. The study is significant in part because it focused on sporadic (nonheritable) forms of breast cancer. Sporadic disease accounts for greater than 90% of all breast and other cancers.

Masaaki used a gene discovery method pioneered by Michael (called Representational Difference Analysis or RDA) for detecting the differences between the DNA of normal cells and breast tumors—differences that might contribute to tumor formation. This analysis, combined with other methods, enabled Masaaki to pinpoint a candidate tumor suppressor gene located on human chromosome 8. The gene was dubbed *DBC2*, for *d*eleted in *b*reast *c*ancer. Subsequent analysis confirmed that the *DBC2* gene was either deleted, mutated, or silenced in many of the breast tumors or breast cancer cell lines Masaaki examined.

Masaaki Hamaguchi

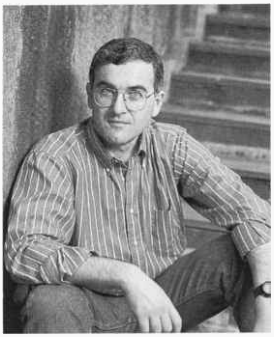
Importantly, restoring *DBC2* gene expression was sufficient to restrain the growth of breast cancer cells that lack *DBC2* expression. When expression of a mutated *DBC2* gene was induced in a breast cancer cell line, the cells continued to grow in an unrestrained manner. However, when expression of a normal *DBC2* gene was induced in the breast cancer cell line, the cells stopped growing. From a clinical standpoint, Mike Wigler thinks it would be difficult to restore a normal *DBC2* gene to cancer cells that lack it, say, by gene therapy. Instead, he believes identifying other proteins activated by the loss of *DBC2* expression in cancer cells, and inhibiting those proteins with new drugs, would be a promising therapeutic strategy.

By using genetically well-defined mouse models of cancer, Scott Lowe and his colleagues continue to reveal important information about the role of the *p53* tumor suppressor in programmed cell death ("apoptosis"), and how the alteration of specific downstream effectors in the *p53* pathway (e.g., Bcl2, Caspase-9) can unleash tumor growth and metastasis. Scott has developed powerful transgenic mouse models of lymphoma in which transplanted cells that are genetically predisposed to forming tumors are also labeled through their expression of a fluorescent protein. In this way, Scott and his colleagues can carry out whole-body fluorescence imaging of the mice to follow tumor formation and progression.

This year, such a study by Scott showed that among the many functions of *p53*, its action through Bcl2 and Caspase-9 to trigger the destruction of precancerous cells is *p53*'s principal tumor-suppressing function. This finding removes some of the complexity previously believed to underlie the role of *p53* in suppressing tumor formation, and significantly improves the theoretical framework necessary for developing rational cancer therapies.

Molecular Biology

By developing a method to correct defects in a complex process called RNA splicing, Adrian Krainer and Senior Fellow Luca Cartegni have moved a step closer to developing treatments for



Adrian Krainer

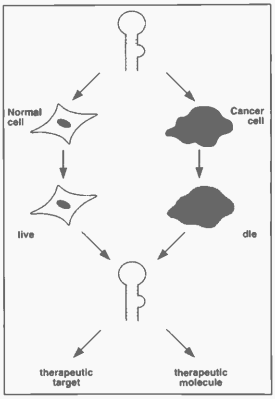
a host of diseases as diverse as breast cancer, muscular dystrophy, and cystic fibrosis.

Mutations in DNA frequently cause errors in RNA splicing. Improperly spliced messenger RNA molecules can lead to the creation of altered proteins that fail to perform their duties properly, often resulting in disease. Many human diseases have been linked to defects in RNA splicing. Adrian's laboratory investigates this molecular editing process, which takes the information coded in genes and ultimately makes it available for building proteins. Adrian and Luca have devised a clever way to correct RNA-splicing defects implicated in breast cancer and spinal muscular atrophy (a neurodegenerative disease). In principle, the technique could correct RNA-splicing defects associated with any gene or disease.

For now, the method has been shown to work under the simplest of conditions—in test tubes with small segments of RNA. The next step is to adapt the technique for use in living cells. Adrian and Luca dubbed the method ESSENCE (for *E*xon-*S*pecific *S*plicing *E*nhancement by small *C*himeric *E*ffectors). They are working to create ESSENCE designer splicing molecules that pass easily into cells and efficiently home in on the desired RNA-splicing targets. If such molecules can be developed, they may ultimately prove useful for treating a great diversity of human diseases.

RNA Interference

In the December 20 issue of the prestigious journal *Science*, the research of Greg Hannon, Shiv Grewal, and Rob Martienssen was recognized along with work from other institutions as the "Breakthrough of the Year" for 2002 among all the sciences. Collectively, the Breakthrough of the Year citation recognized the discovery that through a multifaceted process called RNA interference (RNAi), small RNA molecules can play a surprising variety of key roles in cells: They can inhibit translation of messenger RNA into protein, cause degradation of other messenger RNAs, and even initiate complete silencing of gene expression from the genome. The "Breakthrough of the Year" citation specifically characterized the work of Greg, Shiv, and Rob as "a crucial step" and as "the year's most stunning revelations."



Applications of RNAi

Greg's lab has made a series of important and exciting discoveries concerning the properties and mechanisms of RNAi. Moreover, through detailed examination of the families of proteins involved in RNAi, Greg and his colleagues have uncovered a link between RNAi and the Fragile X syndrome, the most common developmental cause of mental impairment in humans. Finally, Greg's lab has developed methods for using RNAi to uncover genes that are involved in cancer.

Rob, Shiv, and their colleagues have revealed a previously undiscovered biological phenomenon, namely, that RNAi is required to establish specialized subregions within chromosomes in

which genes are kept silent. This finding has profound implications for understanding how chromosome structure is regulated, and how specific active versus silent regions of chromosomes are determined and inherited. In addition, studies at Cold Spring Harbor Laboratory and elsewhere have shown that defects in the establishment or maintenance of silent chromosome architecture in the vicinity of tumor suppressor genes may contribute to cancer.

Neuroscience

Brain Imaging: By literally peering through a window into the brains of living mice, Karel Svoboda and his colleagues have caught a groundbreaking glimpse of adult brain cells forming new connections called synapses. Their study helps overturn a long-held tenet that the structure of the adult brain is largely fixed and sheds light on processes that underlie learning and memory.

Karel's lab has provided clear evidence that the brain can alter its wiring pattern in response to the outside world. Although the neuroscience community had begun to speculate that the architecture of the adult neocortex might be more dynamic than previously believed, definitive evidence was lacking, and neuroscience orthodoxy still held that brain architecture is relatively stable, limiting learning and recovery from injury.

Karel and his colleagues have demonstrated that new synapses are formed in the adult brain in response to new experience. Crucially, Karel has established that synapses form and dissolve in the neocortex continuously, perhaps through a "random sampling" process, and that sensory input stabilizes or makes permanent a subpopulation of particular synapses.

Receptor Trafficking: Evolution is an accidental tinkerer. It uses genes and segments of genes that are lying around in a genome, changes them at random or puts them together in myriad combinations, and auditions the new genes for new roles in the lives of the cell. Successful new genes are passed on efficiently to future generations. Two gene products, Ras and Rap, have well-defined, antagonistic growth-promoting and growth-inhibiting functions in nonneuronal cells which, when altered, can lead to cancer. Demonstrating the happenstance of evolution, this year, Roberto Malinow and Linda Van Aelst have discovered intriguing roles for Ras and Rap in cells of the nervous system.

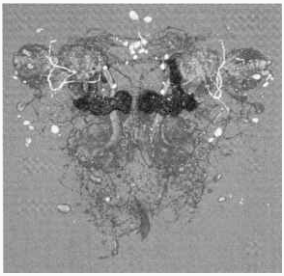
Roberto Malinow

Synaptic plasticity, or the ability of connections between neurons to be shaped by experience, is believed to underlie several important aspects of brain development, learning, and memory. The most widely studied examples of synaptic plasticity are long-term potentiation (LTP), which strengthens the connections between neurons, and long-term depression (LTD), which weakens the connections between neurons.

Linda Van Aelst

Roberto's lab had previously shown that LTP results from the delivery of neurotransmitter receptor proteins called AMPA receptors to synapses. In new work, Roberto, Linda, and their colleagues have shown that Ras mediates this addition of AMPA receptors to synapses during LTP induction, whereas under

conditions that induce LTD, Rap mediates the removal of AMPA receptors from synapses. Moreover, Roberto and Linda established a differential effect of Ras and Rap on different types of AMPA receptors. The resulting dynamics of AMPA receptor replacement at synapses is likely to be a principal mechanism of learning and memory.



Drosophila Brain

Learning and Memory: Tim Tully and Josh Dubnau together have taken two distinct yet complementary approaches to identifying genes that are involved in the formation of long-term memory in the fruit fly *Drosophila melanogaster*. Evolution and experimental evidence dictate that many of these genes are also involved in human memory. By using DNA microarray analysis, Tim, Josh, and their colleagues have identified 42 candidate memory genes. By using a behavioral screen for fruit flies with defective memory, they have identified 60 memory mutants. In his book *Time, Love, Memory*, Pulitzer Prize-winning author Jonathan Weiner called such mutants "Pavlov's Flies" (see below).

The separate approaches described above beautifully converged on an overlapping set of genes, reinforcing the notion that the genes are bona fide memory genes. Additional studies are required to determine the precise neurological functions of all of the candidate memory genes Tim and Josh have identified. Until then, the genes have been given names that correspond to those that Russian psychologist Ivan Pavlov (1849–1936) gave his famed Pavlov's Dogs, e.g., Norka, Laska, Tungus, Chingis Khan, Trezor, John, Jack, etc. (Tim uncovered some 40 such names during a 1992 visit to Pavlov's former residence in St. Petersburg, Russia.) Ultimately, comprehensive knowledge of how these numerous gene products act and interact should provide a solid foundation for understanding the processes of learning and memory.

Awards and Honors

Cold Spring Harbor Laboratory Assistant Professor Z. Josh Huang is one of 18 scientists in the nation named a Scholar in 2002 by the Pew Scholars Program in the Biomedical Sciences. The program, funded by the Pew Charitable Trusts, supports young investigators of outstanding promise in basic or clinical sciences relevant to the advancement of human health.

Z. Josh Huang

Karen Zito, a postdoctoral fellow in Karel Svoboda's lab, is one of 17 researchers named a 2002 Burroughs Wellcome Fund Career Awardee in the Biomedical Sciences. Each award provides a five-year, $500,000 grant to support advanced postdoctoral training as well as the first years of faculty research.

Peter Gillespie, a postdoctoral fellow in Tatsuya Hirano's lab, has received a three-year, $125,000 Postdoctoral Fellowship from the Jane Coffin Childs Memorial Fund for Medical Research. The fund was established in 1937 by the late Miss Alice S. Coffin and Mr. Starling W. Childs as a gift in trust to Yale University to support wide-ranging scientific inquiry into the causes and treatment of cancer.

Two Cold Spring Harbor Laboratory scientists received awards at the 93rd Annual Meeting of the American Association for Cancer Research (AACR) held in San Francisco in April. Scott

Lowe received a Sidney Kimmel Symposium for Cancer Research Scholars Award, and H. Guido Wendel, a postdoc in Scott's lab, was awarded an AACR-Amgen Fellowship in Translational Research. Scott and eight other early career scientists were selected for the Kimmel Symposium Award based on their receipt of highly competitive research grants from one of the following leading funders of cancer research: The Susan G. Komen Breast Cancer Foundation; the Cancer Research Foundation of America; the Damon Runyon Cancer Research Fund; the Leukemia & Lymphoma Society of America; the American Association for Cancer Research; or the Sidney Kimmel Foundation for Cancer Research. Scott is a CSHL Professor and Deputy Director of the CSHL Cancer Center. The AACR-Amgen Fellowship in Translational Research awarded to Guido is intended to foster cancer research throughout the world by scientists currently at the postdoctoral or clinical research fellow level.

DNA from the Beginning, a Web Site created by the multimedia group at the CSHL Dolan DNA Learning Center, is a winner of *Scientific American* magazine's 2002 Science and Technology Awards. *Scientific American's* editors reviewed thousands of sites and selected *DNA from the Beginning* as one of 50 of the most valuable science and technology resources for their readers. Other awards and commendations garnered by *DNA from the Beginning* include "Ten Cool Sites" (Exploratorium, San Francisco), "Best of the Web" (*Popular Science*), "BigChalk Best" (bigchalk.com), "The Webby Awards" (2000 nominee), "Top Rated Site" (MedExplorer), "Hotspot-Keysite" (*New Scientist*), "Selected Member" (Britannica Internet Guide), "Seal of Approval" (*Web Feet: The Internet Traveler's Desk Reference*), and "Digital Dozen" (The Eisenhower National Clearinghouse). *DNA from the Beginning* was made possible by a grant from the Josiah Macy Jr., Foundation.

Thomson-ISI, the Institute for Scientific Information, is an agency that tracks the impact of published research papers, mainly by counting the number of times a particular paper is cited by other scientists in the field. In January 2003, ISI ranked Cold Spring Harbor Laboratory as the number-one-cited institution in Molecular Biology and Genetics Papers for the past decade. This honor and distinction underscores the laboratory's mission to spread the details of our research to other scientists worldwide.

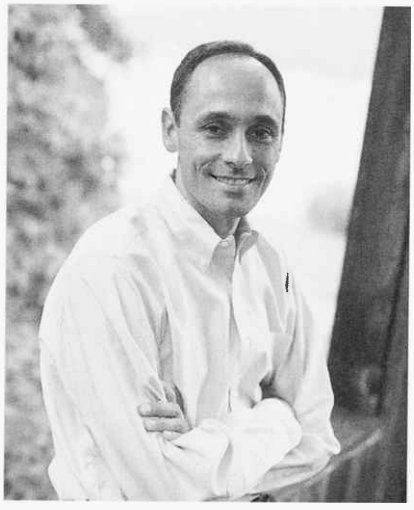
Cold Spring Harbor Laboratory President James D. Watson was again an award-winner, following the 2001 announcement that made him an Honorary Knight of the British Empire. This year, Jim was honored with the Gairdner Foundation Award of Merit for his work and dedication to genomics science, together with Francis S. Collins, Director of the National Human Genome Research Institute. The Gairdner Foundation, based in Toronto, is a nonprofit corporation devoted to the recognition of outstanding achievement in biomedical research worldwide. This year's awards focused on achievements in the field of genomics science.

Symposium

The 67th Cold Spring Harbor Symposium on Quantitative Biology was held May 29 through June 3, 2002. This year's Symposium, "The Cardiovascular System," was organized by David Stewart and myself. The Symposium addressed many aspects of cardiovascular science including normal heart and cardiovascular development and function, the failing heart, hypertension, atherosclerosis, vascular biology, angiogenesis, and tumor angiogenesis. The Symposium attracted 256 participants and featured 68 talks and 108 posters. Opening night speakers included Richard Harvey, Mark C. Fishman, Jonathan G. Seidman, and Rakesh K. Jain and the summary was presented by Christine Seidman. Richard Lifton delivered the annual Dorcas Cummings Memorial lecture, "Salt and Blood Pressure: New Insights from Human Genetic Studies" to a mixed audience of scientists and lay friends of the Laboratory.

Laboratory Senior Management Restructured

For more than eight years, Dr. Winship Herr served as the Assistant Director of Cold Spring Harbor Laboratory. During this period, the Laboratory's research program expanded considerably and, with the establishment of the Watson School of Biological Sciences in 1998, has become an accredited Ph.D. degree-granting institution. Winship led the formation of the Watson School and, along with his role as the Laboratory's Assistant Director, has been the inaugural Dean of the School since 1998. The parallel expansion of the Laboratory's research and academic programs has placed ever-increasing responsibilities on the roles of the Assistant Director and Dean. In August, Winship asked to step down as Assistant Director, to devote his efforts to the ever-expanding Watson School and his successful research program, to which I reluctantly agreed. During the past eight years, Winship has done a remarkable job in helping to both expand the Laboratory's research program and develop the highly successful Watson School. I thank him for his dedication and contributions as Assistant Director and am pleased that he will continue to play a senior leadership role at Cold Spring Harbor Laboratory as Dean of the Watson School of Biological Sciences.



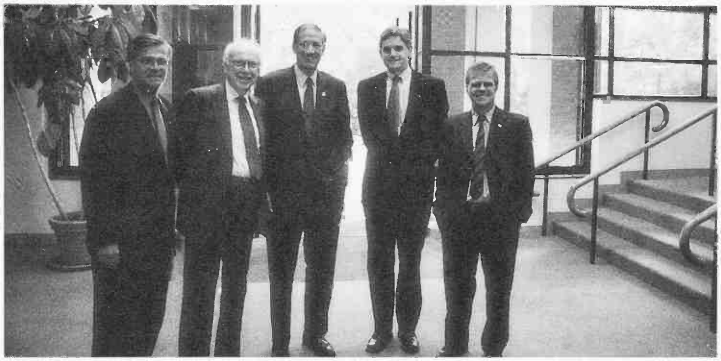
Winship Herr

On August 1, 2002, Dr. Hollis Cline accepted the position of Associate Director for Research at Cold Spring Harbor Laboratory. Holly assumed the position following a reorganization of the research leadership of the Laboratory. As Associate Director for Research, Holly will have an important role in the future research directions of the Laboratory, as well as budget management for the research program and implementing the decisions made by the Laboratory administration. Holly is the Charles Robertson Professor at Cold Spring Harbor Laboratory and has made major contributions to the understanding of brain development and function. She is an outstanding neuroscientist who has already helped to expand the highly successful neuroscience program at the Laboratory. Her broad knowledge of neuroscience, coupled with an interest in cancer research, made her an obvious choice to help lead the Laboratory's research program.

Holly Cline

Governmental Achievements

New York Governor George Pataki, together with Nassau County's Senate Delegation of Dean Skelos, Charles Fuschillo, Kemp Hannon, Michael Balboni, and Carl Marcellino, made a ground-breaking announcement at Cold Spring Harbor Laboratory on Tuesday, September 24, 2002, by creating a biotechnology corridor on Long Island, called "Biotech Island." This new initiative, linking several institutions on Long Island, will create jobs, industry, financial improvements, educational opportunities, and a focus on research that will rival the rest of the nation, including Silicon Valley. With a $48 million investment, and a $20 million investment in Cold Spring Harbor's bioinformatics program, the New York State delegation made Cold Spring



Senator Dean Skelos, Jim Watson, Governor George Pataki, Bruce Stillman, Colin Goddard, CEO, OSI Pharmaceuticals

Harbor Laboratory a cornerstone for this initiative. Due to this effort, Long Island is now poised to become a center for biotechnology in the United States, and Cold Spring Harbor Laboratory has been identified as one of the key players in this exciting transformation.

The Women's Cancer Gene Initiative

In October, a delegation of researchers from Cold Spring Harbor Laboratory traveled to Washington to present to several New York legislators a new concept we call "The Women's Cancer Gene Initiative" for research in breast and ovarian cancer. This new program incorporates the many suc-

Congressman Steve Israel, Bruce Stillman

cesses of our breast cancer research program, combining Greg Hannon's new research developments in RNAi with the exciting cancer gene discovery research in Mike Wigler and Robert Lucito's laboratories, and the mouse models for cancer research in Scott Lowe's laboratory. The initiative was so well-received in Washington that two local representatives—Senator Hillary Rodham Clinton and Congressman Steve Israel—made visits in the following weeks to host press conferences and pledge their support to this exciting program. Representatives Peter King and Carolyn McCarthy also toured our Woodbury Cancer Genome Research Center, and spoke to researchers about their personal support of the program in Congress.

Senator Hillary Rodham Clinton, Scott Lowe

Banbury Center

It is not often that writers at *Sports Illustrated* contact the Banbury Center, but for a few giddy months at the beginning of 2002, Banbury was major news in the sports world. The March meeting on *Genetic Enhancement of Athletic Performance* examined the use of gene therapy in athletics. The World Anti-Doping Agency (WADA) is worried that athletes may use gene therapy to introduce "desirable" performance-promoting genes. The meeting was a fascinating mix of scientists, policy makers, and athletes, including Johann Koss, winner of four Olympic gold medals in speed skating.

Another highly topical meeting was prompted by the heightened awareness of the dangers of biological terrorism. *Microbial Forensics* was devoted to molecular fingerprints that will allow rapid identification of biowarfare agents and that could be used for tracing the source of the agents. The meeting was notable for the mix of scientists from academia and from government agencies—it also set a new record for the number of acronyms appearing in a program.

Robertson Research Fund

The Robertson Research Fund has been the primary in-house support for our scientists for nearly three decades. Since 1973, the Fund has grown from $8 million to more than $95 million. During 2002, Robertson funds supported cancer research in the labs of Gregory Hannon, Eli Hatchwell, Winship Herr, Tatsuya Hirano, Yuri Lazebnik, Bud Mishra, Michael Myers, and William Tansey; neurobiology research in the labs of Grigori Enikolopov, Josh Huang, Roberto Malinow, Jerry Yin, and Yi Zhong; and plant research in the lab of Robert Martienssen. Robertson funds also supported several new investigators, including Shiv Grewal, Alea Mills, Senthil Muthuswamy, Terrence Strick, David Jackson, Marja Timmermans, Carlos Brody, Lee Henry, Zach Mainen, and Tony Zador.

Cold Spring Harbor Laboratory Board of Trustees

Cold Spring Harbor Laboratory's Board of Trustees led the Laboratory through another exciting and eventful year, continuing to shape the direction and vision of this institution.

This year, the Board welcomed the addition of four new Trustees to its ranks: Titia de Lange, Ph.D., a professor of Cell Biology and Genetics at The Rockefeller University; Susan Lindquist, Ph.D., Director of the Whitehead Institute for Biomedical Research; Kristina Perkin Davison, Partner and cofounder of iEurope Capital, LLC; and Amy Goldman, an Agricultural Preservationist and author. Mrs. Evelyn Lauder was also elected to the Board as an Honorary Trustee.

Helen A. Dolan, William L. Matheson, Leon B. Polsky, and Mark Ptashne reached the end of their allotted terms as Trustees and were honored at the Board's November meeting for their outstanding service to the Laboratory. As noted in previous pages, we were saddened to learn in late 2002 of the death of William Matheson.

Helen A. Dolan

William L. Matheson

Mark Ptashne

Leon B. Polsky

Dolan DNA Learning Center

The Dolan DNA Learning Center (DNALC) significantly expanded its educational reach in 2002 by embarking on major agreements to license DNALC "know-how" in the United States and abroad. In the spring, the DNALC collaborated with North Shore–Long Island Jewish Research Institute (NS–LIJ) to open DNALC *West* to better serve students in western Nassau County and

CSHL Trustee Arthur Spiro, Bruce Stillman, Michael Dowling, CEO, NS–LIJ

New York City. This teaching laboratory, located adjacent to the NS–LIJ diagnostic laboratory in Lake Success, is managed and staffed by the DNALC and uses its teaching curricula. In the summer, the DNALC signed a three-year Memorandum of Understanding with the Ministry of Education (MOE) to support expansion of life sciences education in Singapore. The contract provides support for the DNALC and involves consultation and training of teachers. Initiated in the context of a national thrust to make biotechnology the "fourth pillar" of Singapore's future economy, the project revolves around the development of "sister" DNA centers at the Singapore Science Centre and National Institute of Education. In the fall, the DNALC entered into a licensing agreement with the Science EpiCenter of New London, Connecticut to develop a DNA Learning Center there to serve southern Connecticut. These licensing agreements, in addition to sponsored research by Carolina Biological Supply Company, and royalties on teaching kits and CDs, contributed about $335,000 in revenue, or 14% of the 2002 operating income.

In September, the Dolan DNALC opened "The Genes We Share," the third major exhibition in the DNALC's history. The exhibit celebrates the 50th anniversary of the discovery of the DNA structure, which set the stage for the recent efforts to determine the entire DNA sequence of the human genome. As visitors explore the exhibition, they are encouraged to view the human genome as a record of our shared ancestry, an instruction manual for our bodies, and a source of information that can foreshadow a person's future health. Visitors are greeted in the front hall with photomurals that juxtapose New York "peoplescapes" with unique world populations, setting the stage for thinking about human similarities and differences. An interactive area in the main gallery encourages visitors to take stock of traits that make them unique, as well as anatomical, cellular, and molecular features they share with all people.

In another area, an interactive map of the world shows our shared ancestry in Africa, migration paths followed by humans over the past two million years, and environmental factors that account for some of our differences. Visitors then move into a recreation of a Neolithic cave, complete with paintings and the first reconstruction of an adult Neandertal skeleton. Visitors are asked to consider the anatomical, genetic, and behavioral changes that evolved to set humans apart from other primates. A final gallery focuses on the modern "gene age," moving from an eight-foot reconstruction of the original metal DNA model made by Watson and Crick in 1953 to a working DNA sequencer, which operates daily to sequence DNA submitted by student classes from around the United States.

The November completion of *Your Genes, Your Health* (www.ygyh. org) marked the end of a five-year, $1.3 million Web Site project funded by the Josiah Macy, Jr. Foundation. This multimedia guide provides up-to-date, engaging information on 15 common genetic disorders: Fragile X syndrome, Marfan syndrome, hemophilia, cystic fibrosis, Duchenne/ Becker muscular dystrophy, phenylketonuria, Huntington disease, neurofibromatosis, sickle cell disease, hemochromatosis, Tay-Sachs disease, β-thalassemia, Down syndrome, Alzheimer disease, and polycystic kidney disease. Its companion site *DNA from the Beginning* (www. dnaftb. org), an animated primer on genetics and molecular biology, was one of only 50 World Wide Web sites chosen by *Scientific American* for its 2002 SciTech Award (www. scientificamerican.com). The Macy-funded sites received more than 1.6 million visitors in 2002, accounting for 44% of the 3.8 million visitors to the *Gene Almanac* "portal."

CSHL Press

The Cold Spring Harbor Laboratory Press enjoyed another successful year. Twelve new books and two CDs were published and all four research journals grew in impact or circulation. But the most prominent—and ambitious—Press project of the year had true worldwide impact.

John Inglis, Executive Director of CSHL Press, was a member of a team of scientists and educators committed to developing effective, innovative, and sustainable HIV/AIDS prevention strategies for young people in South Africa and other vulnerable communities. Together, they prepared an important book entitled *Staying Alive: Fighting HIV/AIDS* that presents life-saving information in a direct, engaging, and age-appropriate manner to children aged 12 to 16. With funds from the National Institutes of Health, Oxford University, and Cold Spring Harbor Laboratory, 20,000 copies of *Staying Alive* were produced by the CSHL Press and distributed free of charge in three provinces of South Africa in July and August.

Staying Alive was written by Professor Frances Balkwill of Bart's Hospital Medical School, London, and illustrated by Mic Rolph. Balkwill and Rolph have previously collaborated on several highly successful children's books for CSHL Press, including *Enjoy Your Cells, Germ Zappers, Have A Nice DNA,* and *Gene Machines*. To gain a better understanding of the book's audience before creating *Staying Alive*, Balkwill and Rolph first visited schools, squatter camps, and orphanages in the provinces of Gauteng (Johannesburg) and Kwa-Zulu Natal (Durban), South Africa, where they met with students, teachers, medical scientists, and health professionals. The book resulting from these experiences incorporates artwork created by children during workshops conducted by Mic Rolph, as well as Rolph's own illustrations of the biology of HIV and how abstinence, faithfulness, and the use of condoms can reduce the spread of AIDS. With its lively graphics and direct language, *Staying Alive* gives explicit messages about the risks of disease, ways of preventing infection, the building of relationships based on mutual care, and the need for compassion and respect toward people living with HIV.

Staying Alive Book Cover

The available copies of the book are being distributed free of charge to educators and students from repositories in Gauteng, Kwa-Zulu Natal, and the Western Cape. Teachers, academics, and community activists have been recruited to conduct classroom exercises designed to evaluate the book's effectiveness and provide feedback for the development of future editions of *Staying Alive* and other teaching materials. The book-distribution tour included receptions in each province attended by local politicians and educators, as well as meetings with the South African Minister of Education, Dr. Kadar Asmal, the well-known satirist and AIDS educator, Pieter-Dirk Uys, participants in the Memory Box Project at the University of Cape Town, and representatives of Médecins Sans Frontières ("Doctors Without Borders"). During and after the tour, the creators of *Staying Alive* also received feedback from Christian missionaries, traditional healers, and even soccer clubs interested in helping fight the spread of AIDS in South Africa.

After completing this pilot phase of their project, the *Staying Alive* team received substantial funding from the Gates Foundation. With this support, a second edition of the book and related educational materials will be created in several South African languages and distributed on a much larger scale. In addition, a start will be made on addressing a newly identified need—a program that deepens science teachers' knowledge of the immune system and virus biology and increases their effectiveness as communicators of AIDS awareness.

CSHL Library and Archives

In 2002, the CSHL Library made great progress in its efforts to provide information electronically. On-line subscriptions to scientific journals, monographs, and reference materials have

increased significantly. InterLibrary Loan has seen an increased availability of articles in PDF format. This has facilitated the delivery of requested articles in a timely fashion. The Library has acquired several new electronic databases such as BioKnowledge Library from Proteome, FirstSearch that includes BasicBIOSIS and several full text databases, and Faculty of 1000 from BioMed Central. WebCat, the on-line catalog, now includes most of the titles in the Library's collection, including titles in video, electronic, and microfilm formats. The library has continued to participate in the BioInformation Synthesis Collaborative (BISC), which includes the libraries of The Rockefeller University, the American Museum of Natural History, Woods Hole Marine Biological Laboratory, and the New York Botanical Garden, which is developing several projects for interlibrary loan purposes.

The Archives have undergone tremendous growth and development. The James D. Watson Special Collection has been acquired and is beginning to be made available on our Web Site through the use of finding aids. The oral history project to document the Laboratory's history through the eyes of the scientists who worked and visited here has grown to 40 interviews and will ultimately be made available through our laboratory web page. The James D. Watson Photographic Archives are available on-line and the CSHL Symposium photo collection is also near completion. This collection contains a remarkable history of biology in the latter two thirds of the 20th century and into the new millennium. During this past year, a great deal of preparation has been made in planning the traveling exhibition "Seeking the Secret of Life: The DNA Story in New York." This exhibition will mark the 50th anniversary of the discovery of the DNA double helix and opened at the Science, Industry and Business Library of the New York Public Library in February, 2003.

New Major Gifts

The Laboratory was successful in continuing and commencing major campaigns, despite economic challenges in 2002. The campaigns to fund the Watson School of Biological Sciences, the Cancer Genome Research Center, the Women's Cancer Gene Initiative, and the Dolan DNA Learning Center all received substantial gift support. The philanthropic investments of our generous and supportive friends for the work of the educators and researchers at Cold Spring Harbor Laboratory allow us to remain at the forefront of biomedical knowledge. The announcement of New York State's commitment to the Laboratory of $20 million by Governor George Pataki and the Long Island Senate team led by Senator Dean Skelos is a welcome recognition of the accomplishments at Cold Spring Harbor. Through this special commitment and generous private funding, the Laboratory is increasing the pace of research to control cancer and understand neurological diseases.

Watson School of Biological Sciences

Under the leadership of Dean Winship Herr, the Watson School of Biological Sciences continues to go from strength to strength. Not only has Winship introduced a strong graduate program, but the Watson School now effectively oversees all research-based education at the main laboratory campus, including the Undergraduate Research Program, the high school Partners for the Future Program, and Postdoctoral Fellow training.

Led by Honorary Trustee David L. Luke III, the first phase of the Watson School of Biological Sciences Campaign was completed this year, accomplishing an endowment of $32 million as

a firm financial foundation for the Watson School. We are grateful for gifts from the William Stamps Farish Fund of $300,000 and from the Estate of Elisabeth Sloan Livingston of $2,200,000; and we appreciate gifts to build the Watson School's Innovative Education Fund, including the final payment of a challenge gift from an anonymous donor to match gifts of $62,103 from the Seraph Foundation; and of $10,000 from The William H. Donner Foundation; with further gifts to the Fund of $50,000 from The G. Unger Vetelsen Foundation; of $25,000 from Dr. and Mrs. Mark Ptashne; of $10,000 from Mark and Karen Zoller; and of $1,000 from the Packard Foundation; which collectively rounded out a solid campaign to provide funding for graduate student fellowships, lectureships, and courses.

A second phase of fund-raising is being commenced to allow an increase in the enrollment in the Watson School. This will enable the School to help fulfill the growing demand for researchers in the biological sciences. Laboratory Trustee Robert D. Lindsay is leading this phase. A gift of $500,000 from the William Randolph Hearst Foundation has provided the base funding for a fellowship for disadvantaged students and is a welcome start to this important expansion of the School.

The Cancer Genome Research Center

Now in its first full-year in operation, the value of commitments to the Cancer Genome Research Center was evident, with the facilities underpinning many advances in cancer, neuroscience, and plant genomics research. We are grateful for major new contributions to the facility and its programs of $2,500,000 from The Starr Foundation; of $261,354 from the William and Maude Pritchard Charitable Trust; and of $10,000 from Benjamin Development.

Dolan DNA Learning Center

Final gifts were made in support of "The Genes We Share" exhibit, which opened in September. We are grateful for a $20,000 gift from the William Haseltine Foundation, the $50,000 gift from the Richard Lounsbery Foundation, and the $30,000 gift from Dr. Laurie Landeau, all of which helped to make "The Genes We Share" a success. Arrow Electronics also generously gave $30,000.

Private Research Support

Private funding in 2002 advanced important research initiatives in cancer, neuroscience, and other areas. We gratefully acknowledge major gifts in the $100,000 and above category, including for cancer research the Marks Family Foundation for $500,000; The Miracle Foundation for $100,000; The Breast Cancer Research Foundation/Lillian Goldman Trust for $1,000,000; the Louis Morin Charitable Trust for $105,000; The V Foundation for $100,000, and for neuroscience research The McKnight Endowment Fund for Neuroscience for $100,000; and Jeff Hawkins and Janet Strauss for $100,000. We appreciate other support received from The Dana Foundation for $100,000 and from Robert Gardner for $100,000.

Breast Cancer Support

The generous support from local breast cancer groups again made a real difference in 2002, including from the Long Beach Breast Cancer Coalition; the Plainview–Old Bethpage Breast Cancer Coalition; the Elizabeth McFarland Fund; the Babylon Breast Cancer Coalition; and,

long-time supporters, 1 in 9: The Long Island Breast Cancer Action Coalition and Long Islanders Against Breast Cancer (LIABC). As in past years, we are indebted to the many individuals from local breast cancer groups working so hard to make it possible for Cold Spring Harbor Laboratory to continue its breast cancer research.

CSHL Planned Giving Advisory Board

The Cold Spring Harbor Laboratory Planned Giving Advisory Board—drawn from the financial services, accounting, and legal professions—consists of accountants, tax advisors, trust officers, bank officers, investment advisors, investment bankers, and lawyers, representing firms located on Long Island, in New York City, and in Connecticut. The Board was briefed on the Laboratory's activities and accomplishments in 2002. I appreciate the advice members provide in support of the Laboratory's goals.

President's Council

Members of the President's Council were once again invited to another successful one-day meeting at the Laboratory. This year's President's Council meeting explored the topic of "Human Instincts and Evolutionary Psychology." Once again, our presenting scientists were engaging speakers and leaders in the field: famed science writer Dr. Matt Ridley, Dr. Richard Wrangham from Harvard University, Dr. Patricia Wright from Stony Brook University, and Dr. Randolph Nesse from the University of Michigan. The event began with tea at Ballybung, the home of Jim and Liz Watson. Guests were then invited to meet the Cold Spring Harbor Laboratory Fellows, Drs. Lee Henry and Terence Strick, followed by cocktails and then a discussion led by Matt Ridley on "Evolutionary Psychology of Instincts." The lectures continued on Saturday morning with discussions on "The Origins of Human Violence," "Parenting: Insights from Our Primate Cousins," and "Phobias and Depression." All who attended said it was, as always, a worthwhile learning experience.

Matt Ridley

Gavin Borden Visiting Fellows

The Gavin Borden Lecture series was named in memory of Gavin Borden, a publisher whose *Molecular Biology of the Cell* and other books made a lasting impression on many scientists, both old and new. This year, Sir John Gurdon, former Governor of the Wellcome Trust and former director of the Wellcome/CRC Institute of Cancer and Developmental Biology, delivered the eighth annual lecture titled, "Signal Factor Perception in Development" on March 25, 2002. The annual Gavin Borden Lecture was started by Jim Watson in 1995.

Sir John Gurdon

Building Projects

In addition to continuous up-dates to many of the Laboratory's facilities, Art Brings, the Laboratory's Chief Facilities Officer, and his team completed several major renovation and construction projects.

The first phase of a new project to provide additional student housing at Uplands Farm has begun. The completed project will provide high-quality, on-campus housing for up to 32 students upon completion.

Three rooms in the Carnegie Library were renovated and converted for use as the new Watson Archives, providing much-needed space and an area in which scholars and visitors may view previously inaccessible material.

Several key scientific facilities were completed during the year. Microscopy suites were constructed in the Beckman and Hershey Buildings and several core scientific facilities—Flow Cytometry, 2D Gel, and the Nucleic Acid Facilities—were relocated to newly expanded and renovated facilities.

Many of the Laboratory's administrative departments received upgrades to their offices as well. The Institutional Advancement department moved into renovated offices previously occupied by graduate students. The Nichols Building was virtually rebuilt from the ground up, providing the Accounting, Special Events, Technology Transfer, and Administrative departments with the top-quality facilities they require to efficiently manage the increased demands placed on them by the Laboratory's growth.

A team of craftsmen worked hard to prepare the Dolan DNA Learning Center for the opening of "The Genes We Share" exhibit on September 18. From installing each of the kiosks to wiring the lighting and computers, the team ensured that the exhibit was prepared for the crowds who have already come. The exhibit looked fantastic when it was highlighted in *The New York Times* and on *News 12 Long Island*.

Community Outreach

Traffic problems temporarily slowed down our runners and walkers, but 55 Laboratory employees still participated in the Cigna 5K Run/Walk for 1 in 9: The Long Island Breast Cancer Action Coalition at Eisenhower Park on August 8. All were happy to be there for a group that has been such a tremendous help to the Laboratory research for so many years. Overall, the event raised more than $60,000 for the Laboratory.

Special Events

Public Lectures

After a successful first year, the *Harbor Lecture Series* doubled in size, featuring a total of six lectures this year, and proved it would become an annual favorite at the Laboratory.

May 5 Michael Ruse, the Lucyle T. Werkmeist Professor of Philosophy at Florida State University: "Can a Darwinian be a Christian?—The Relationship between Science and Religion."

May 13 Dr. Chung S. Yang, Professor at the Laboratory for Cancer Research at Rutgers and the Director of the Carcinogenesis and Prevention Program at the Cancer Institute of New Jersey: "Beneficial Health Effects of Tea: Evidence, Myth, and Perspectives."

Bruce Stillman, Christopher Chyba, Edward Scolnick

May 20 Elof Axel Carlson, Distinguished Teaching Professor in the Department of Biochemistry and Cell Biology at Stony Brook University: "Eugenics: When Science and Society Go Astray."

June 14 Steven M. Block, a biophysicist at Stanford University; Christopher Chyba, co-director of Stanford's Center for International Security; and Edward M. Scolnick, CSHL Trustee and President of Merck Research Laboratories, and Executive Vice President for Science and Technology for Merck & Company, Inc.: "Bioterrorism: Protecting Our Country, Protecting Ourselves."

October 1 Dr. Cynthia Kenyon, the Herbert Boyer Distinguished Professor of Biochemistry and Biophysics at the University of California, San Francisco: "Genetics of Aging and Longevity: The Search for the Fountain of Youth."

October 8 Scott Lowe, CSHL Deputy Director of the Cancer Center: "Cancer: Mission Possible."

Other Lectures

As usual, Cold Spring Harbor Laboratory hosted the Huntington Hospital Lecture Series on cardiovascular health and related diseases in both the spring and fall in the Grace Auditorium.

The West Side School Lecture Program was again a hit with local, budding scientists in 4th through 6th grades. This year, five Cold Spring Harbor Laboratory professors—Tony Zador, Leemor Joshua-Tor, Alea Mills, Lee Henry, and Erik Vollbrecht—delivered lectures.

Concerts and Exhibits

The *Music of the Harbor Concert Series* was, as previously, a smashing success. All nine concerts were sold out between Meetings participants, CSHL Association members, and the general public. Co-sponsored by *Newsday*, the concert series featured old favorites such as jazz

guitarist Eric Johnson and off-the-beaten-path performances from people such as multipercussionist Joseph Gramley. The complete concert list included:

April 20	Ju-Young Baek, violinist
April 27	Alexander Fiterstein, clarinetist
May 4	Paavali Jumppanen, pianist
May 18	Courtenay Budd, soprano
September 14	Alpin Hong, pianist
September 21	Joseph Gramley, multipercussionist
September 28	Imani Woods, woodwind quintet
October 5	Eric Johnson, jazz guitarist and his band
October 12	John Kamitsuka, pianist

John Kamitsuka

One of Cold Spring Harbor Laboratory's own, Rachel von Roeschlaub, presented an art exhibit in the Bush Fireplace Room in June. von Roeschlaub is a self-taught artist who paints using a combination of aboriginal pointillism and American Folk Art. Her paintings are finished on modern mediums such as record albums, CDs, and natural paper. She also uses her experience as a technician at Cold Spring Harbor Laboratory to teach biotechnology professional development workshops. Last February, she taught a science workshop for Tibetan monks living in India. Proceeds from this art exhibition went toward promoting science education for the exiled Tibetans living in India.

Former photographer-in-residence, Doug Fogelson, returned to the Laboratory in July for a month-long exhibit of the photographs he took during "FotoLab I" in 2001. "Remember Far Away" was a visual memory and response to Fogelson's time spent at the Laboratory. It expressed sentiment—in some cases, through the use of manipulated images—inspired by photographs of the surroundings at the Cold Spring Harbor Laboratory campus. The exhibit also featured portraits of Dr. James Watson and myself.

Laboratory Employees

Long-term Service

The following employees celebrated milestone anniversaries in 2002.

30 Years	Terri Grodzicker
20 Years	Patricia Bird, Arthur Brings, David Micklos, Clifford Sutkevich
15 Years	Sadie Arana, Patricia Barker, Lisa Bianco, Joan Doxey, James Duffy, Julie Ehrlich, Edward Haab, John Inglis, Barbara Peters, Susan Rose, Stanley Schwarz, Inez Sialiano, Patricia Urena, Jan Witkowski

New Staff

Diane Fagiola has joined the Laboratory as Director of Development. Diane comes to CSHL from a position as Director of Fundraising for the Locust Valley Library.

Gerry Holler has joined the Laboratory as Director of Security. Gerry comes to CSHL from Photo Circuits, where he served as Assistant Director of Security.

Katie Raftery has joined the Laboratory as Director of Human Resources. Katie comes to CSHL from a position as Director of Human Resources for Ericsson Internet Applications.

Long-term Service Employees
First Row: Sadie Arana, Bruce Stillman, Bobbie Peters, Patty Bird, Terri Grodzicker, Inez Sialiano, Julie Ehrlich, Joan Doxey
Second Row: Pat Urena, Stanley Schwarz, Jim Watson, Clift Sutkevich, Lisa Bianco, Jan Witkowski, James Duffy, Pat Barker
Third Row: Dill Ayres, John Inglis, Dave Micklos

Promotions

On January 1, 2002, Lilian Gann was appointed Associate Dean of the Watson School of Biological Sciences. Lilian joined the Laboratory in March 1999 as Assistant Dean of the Watson School. Other promotions this year included Douglas Conklin to Research Investigator; Shivinder Grewal to Associate Professor; Gregory Hannon to Professor; Eli Hatchwell to Investigator; John Healy to Computer Scientist; David Jackson to Associate Professor; and Michael Zhang to Professor.

Departures

Michael Hengartner, Associate Professor, departed this year to take a position as Professor in the Institute of Molecular Biology at the University of Zürich.

Concluding Remarks

The breadth of our highly successful research and education programs is a testament to the people who work here and those who chose to support the Laboratory financially and otherwise. I am most fortunate to work at an institution that savors past accomplishments, yet does not rest on its laurels. We need to move forward, particularly in research that I believe will eventually help to control cancer, and at the same time invest in research on understanding the brain, one of the frontiers of modern biology that has a long way to go before we can comprehend how we learn, think, and behave. But the core of our future success will be securing the necessary funds to allow our scientists to do what they do best and not what grant reviewers think is best.

Bruce Stillman
Director

ADMINISTRATION

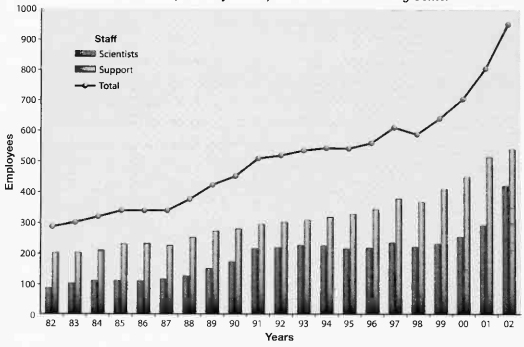
One of the hopes of our neuroscience investigators is to gain a better understanding of the mechanisms of cognitive disorders such as depression and bipolar disease. If a year could have the characteristics of a disease, 2002 might be said to have been bipolar with its manic highs and disappointing lows. On the one hand, the year in science at Cold Spring Harbor Laboratory was as exciting and dynamic as any in recent memory, with great progress and breakthroughs in cancer and other areas of research. The cold shower was provided by the persistently sluggish national economy, uncertainty on the international front, and the dismal performance of U.S. equity markets.

Fortunately, what ultimately determines the success and stability of this institution is the science, and the news on that front is excellent. Our investigators are quickly capitalizing on the vast data provided by the completion of the human genome sequence. Genes implicated in various cancers are being identified at an accelerating pace, and novel techniques developed here for silencing individual gene expression have important implications for drug discovery. The work is being recognized internationally by peer and lay press alike, with *Science* magazine recently naming the research of Drs. Hannon, Grewal, and Martienssen on RNAi the #1 "Breakthrough of the Year." Political leaders know a good thing when they see one, and Cold Spring Harbor Laboratory has become "hot" in this respect. In the months of September and October alone, we had visits and on-campus announcements from leaders as diverse as Governor Pataki, Senator Clinton, Congressman Israel, and the Duke of York. The visit from the Governor was particularly meaningful, as he was here to announce a $20 million grant to the Laboratory from the State of New York to fund the building of a new center for bioinformatics on the main campus. The State leadership has recognized the importance of the biotechnology industry as an engine for economic growth in the 21st century, and they see Cold Spring Harbor Laboratory as a catalyst for the development of this industry in the New York metropolitan region.

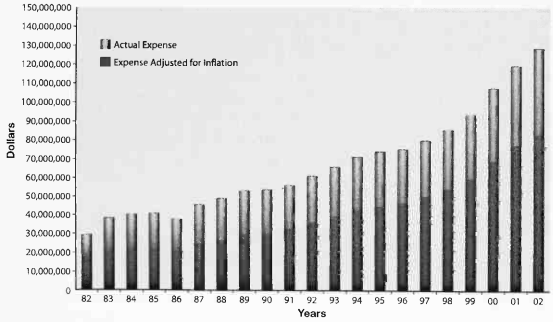
Financially, we planned for a difficult year at the Laboratory in 2002, and we were not proven wrong in our expectations. Fortunately, income from grants and from most of our operating units remained strong so that total revenue grew 4.5% to $79 million. We strive to balance our operating budget after full depreciation expense ($5.1 million in 2002) and have achieved or exceeded this goal for 14 consecutive years. Unfortunately, we realized an operating deficit this year of approximately $565,000 after the utilization of $1.8 million remaining in a reserve account that had been set aside in prior years when we had operating surpluses. To minimize the expected deficit, we instituted cost-control measures at the beginning of the year. These included a hiring freeze and a cap on salary increases, as well as setting the capital spending budget at $500,000 below depreciation expense to preserve cash. Despite the challenging environment, the Laboratory was able to generate positive cash flow of $3 million for the year. There were a number of factors contributing to our budgetary challenges and pressures. One was a $500,000 increase in our depreciation expense resulting from the first full year of operations at the Cancer Genome Research Center at Woodbury and the expanded Dolan DNA Learning Center. Income earned on our cash and short-term investments was down substantially as interest rates plummeted to 40-year lows. While we had a relatively good year in fundraising for major capital projects and research, our two annual funds, which impact the operating budget, were understandably short of their combined goals.

After many years of growth and superior investment performance, the Laboratory's endowment funds suffered for the second consecutive year as U.S. equities continued their dramatic

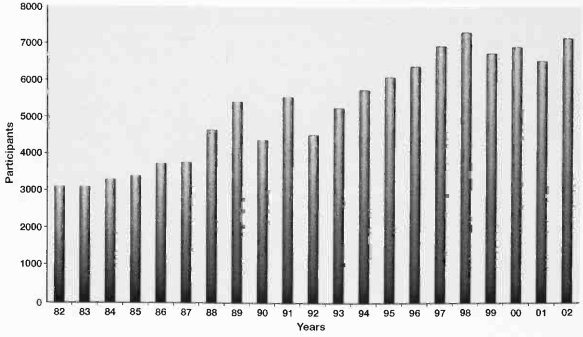
Consists of Full-time and Part-time Technical Support, Core Services, Publications, Meetings, Library, Public Affairs, Building and Grounds, Administrative, Personnel, Banbury Center, and Dolan DNA Learning Center



Operating Expense



Meetings & Course Participants



decline. At year-end 2002, the endowment totaled $187 million, down from $212 million at the previous year end. Because stocks were down and bonds were up, the asset allocation of our managed funds was 47%/53% equities to fixed income investments. Total return on these funds was a negative 9.4%, somewhat below benchmark indices of similarly allocated funds. Both our equity and fixed income managers had disappointing performances in an absolute sense and relative to the benchmarks. While we are certainly not alone in this experience, we are not satisfied, and the Finance & Investment Committee, chaired by Lola Grace, is taking measures to reformulate the investment strategy and procedures. In difficult financial markets, the Laboratory's endowment is well-served by its relatively conservative investment policy of spending a maximum of 4% of the three-year moving average of year-end market value. With two down years in the last three, this discipline now dictates that we budget for a smaller dollar amount of spending from the endowment to support research in 2003.

In years like 2002, we are thankful for the overall financial strength of the Laboratory and pleased that we were able to avoid the layoffs and other more draconian measures taken at less secure academic institutions. While we prepare and budget for another challenging year ahead, we have reason for optimism. Our various operating units—the Cold Spring Harbor Laboratory Press, Meetings and Courses, the Banbury Center, and the Dolan DNA Learning Center—are extremely well managed and make a positive contribution to operations. Our indirect cost rate, the rate at which we are reimbursed for overhead expense by the government granting agencies, has been renegotiated and will increase over the next several years. This sensitive and complicated negotiation with the Department of Health and Human Services was deftly led by our comptroller Lari Russo. The resulting rate increase will have a substantial positive impact on revenue over the next several years. Another bright spot is technology transfer. John Maroney has done a wonderful job of capitalizing on some of the recent research breakthroughs by negotiating licensing and research support agreements with biotechnology and pharmaceutical companies. These arrangements are also expected to contribute very positively to operating revenue in the years ahead.

Last year, this report referred to the Laboratory's master plan and the expansion of facilities on our main campus. The $20 million grant from New York State and other as yet unannounced philanthropy will allow us to proceed with the planning for phase one of this project. We are in the process of seeking the necessary village approvals as we work on engineering and architectural plans for the new research facilities. The plan is ambitious but now within our sights, and we are excited about the prospect of being able to accommodate our world-class scientists with much-needed space and facilities.

Critical to the ongoing health of the Laboratory is a strong base of volunteers from Association Directors to our Board of Trustees. Four extraordinarily influential and generous Trustees had their terms expire in 2002. We thank Helen Dolan, Leon Polsky, Mark Ptashne, and Bill Matheson (see memorial on page viii) for their years of dedicated service. At the same time, we welcome and look forward to working with newly elected Trustees Titia de Lange, Susan Lindquist, Kristina Davison, Amy Goldman, and Honorary Trustee Evelyn Lauder.

Success in any administrative and managerial role is totally dependent on the quality of staff, and it is here that the Laboratory is truly fortunate. From senior department heads to accountants, carpenters, cooks, and custodians, Cold Spring Harbor Laboratory has an extraordinary staff of dedicated people who serve the institution with great pride. Thanks to all.

W. Dillaway Ayres, Jr.
Chief Operating Officer


RESEARCH

Proteins called histones bind to DNA and wrap it into a coiled state called chromatin. Depending on how histone proteins are modified by cellular enzymes, they wrap DNA into either a loose, active structure or a compact, inactive structure called heterochromatin. In collaboration with Rob Martienssen's group (see Plant Development and Genetics), Shiv Grewal's lab has shown how RNA interference (RNAi) targets histone-modifying enzymes to specific regions of DNA, leading to the formation of an inactive, heterochromatin structure. These studies have revealed an entirely unexpected connection between RNAi and DNA structure and have profound implications for understanding many aspects of gene regulation and the formation and stable inheritance of chromosome structure. For this reason, the work of Greg Hannon (see Cancer Genetics), Shiv Grewal, and Rob Martienssen was recognized by *Science* magazine as the "Breakthrough of the Year" among all of the sciences.

Nouria Hernandez and Winship Herr lead groups of researchers that explore the precise biochemical mechanisms by which DNA is transcribed into RNA. Each group has revealed how specific protein-protein interactions control the action of enzymes called RNA polymerases, which produce RNA using the information coded in DNA.

Leemor Joshua-Tor focuses part of her X-ray crystallography work on the events that trigger papillomavirus DNA replication. She is collaborating with Arne Stenlund to unravel the crucial first steps during the replication of papillomavirus DNA. Papillomavirus is associated with virtually all cases of cervical cancer. By studying the biochemical and structural changes that initiate viral DNA replication, Joshua-Tor and Stenlund have revealed key details not only about this cancer-causing virus, but also about the process of DNA replication in general. Moreover, these studies have uncovered specific molecular targets for the development of drugs to prevent or treat cervical cancer.

Rui-Ming Xu focuses on determining the three-dimensional structure of proteins. Xu's lab focuses on proteins that carry out RNA splicing, and on others that affect histone protein modification and thus the structure of DNA. By using X-ray crystallography to determine these protein structures, Xu's lab has provided several clues about how the proteins carry out RNA splicing and chromatin remodeling.

On the way from gene to protein, information passes through two RNA forms. The first is called a pre-messenger RNA (pre-mRNA) transcript, which is a word-for-word translation of a

See previous page for photos of the following scientific staff.

Row 1: Yi-Chun Chen; Bhupinder Pal; Michelle Carmell, Marjorie Brand; Benjamin Boettner; Francois Grenier

Row 2: Hongzhen He; Emily Bernstein, Michael Ronemus, Amy Caudy; Theresa Zutavern; Kimberly Scobie

Row 3: Gianluca Pegoraro; Rebecca Ewald; Eric Allemand, Michelle Hastings; Andrey Revyakin, Terence Strick

Row 4: Nakaya Naoki; Anagha Matapurkar; James Egan; Anitra Auster; Inessa Hakker, Florinda Chaves; Patrice Lassus, Dominik Duelli

Row 5: Vivek Mittal; Sibel Kantarci; Marjorie Brand; Carlos Aizenman

Row 6: Eric Enemark; Sihem Cheloufi; Ramu Umamaheswari, Rulan Shen; Amy Brady

gene's DNA. Pre-mRNA RNA transcripts are then spliced to create mature mRNAs, the templates that cells use to make proteins. In many genetic diseases, gene mutations block RNA splicing. The resulting aberrant mRNAs lead to the production of abnormal proteins that cannot perform their functions properly. Adrian Krainer's lab has developed compounds that correct such RNA-splicing defects. These compounds may ultimately be used as effective therapies for diseases as diverse as breast cancer, muscular dystrophy, and cystic fibrosis.

By identifying all of the 40,000 or so proteins that govern human biology, the completion of the human genome sequence has ushered in a new age of biological discovery. Assigning functions to this myriad of proteins will involve several approaches. The lion's share of this functional characterization may well stem from "proteomics" or the study of how proteins are modified in the complex and dynamic environment of cells and tissues, and how they organize themselves into coherent, highly interactive networks that determine cell function. Michael Myers's lab is focused on identifying the global architecture of these protein networks. In particular, he is exploring the features of protein networks that make them robust (error-tolerant) as well as how such networks are altered in diseases such as cancer. These studies should provide key insights into which network components are promising targets for treating cancer.

Jacek Skowronski's lab focuses on a protein encoded by the genome of the virus that causes AIDS. The protein, called Nef, is an important determinant of HIV virulence and AIDS pathogenesis. By purifying human proteins that specifically bind to Nef, Skowronski's lab has identified several factors that are likely to control crucial events during AIDS pathogenesis. In so doing, they have uncovered potential new targets for therapies to combat this disease.

William Tansey's lab studies a potent transcription factor implicated in cancer. They have found that the factor, a protein called Myc, is regulated by another protein, Skp2, and that the status of these proteins (normal versus abnormal) determines whether cells will grow as they should, or instead become cancerous.

Elena Ejkova

EPIGENETIC CONTROL OF GENE EXPPRESSION

S. Grewal I. Hall G. Shankaranarayana
A. Malikzay G. Xiao
K. Noma

Research in our laboratory is focused on the epigenetic control of higher-order chromatin assembly. The dynamic regulation of higher-order chromosome structure governs diverse cellular processes ranging from stable inheritance of gene expression patterns to other aspects of global chromosome structure essential for preserving genomic integrity. The assembly of heterochromatic structures at centromeres is essential for accurate segregation of chromosomes during cell division, whereas formation of specialized structures at telomeres protects chromosomes from degradation by nucleases and from aberrant chromosomal fusions. Our earlier studies revealed the sequence of molecular events leading to the assembly of heterochromatic structures in the fission yeast *Schizosaccharomyces pombe*. We found that covalent modifications of histone tails by deacetylase and methyltransferase activities act in concert to establish the "histone code" essential for the assembly of heterochromatic structures. Moreover, we showed that distinct site-specific histone H3 methylation patterns dictate the organization of chromosomes into discrete structural and functional domains. Histone H3 methylated at Lys-9 is strictly localized to silent heterochromatic regions, whereas H3 methylated at Lys-4, only a few amino acids away, is specific to the surrounding active euchromatic regions. We have continued to focus on the role of histone modifications and the factors that recognize specific histone modification patterns (such as a chromodomain protein Swi6 that specifically binds histone H3 methylated at Lys-9) in the assembly of higher-order chromatin structures and have made significant progress in understanding the mechanism of higher-order chromatin assembly. More importantly, we provided evidence showing that RNA interference (RNAi), whereby double-stranded RNAs silence cognate genes, has a critical role in targeting of heterochromatin complexes to specific locations in the genome.

ROLE OF RNAi IN HETEROCHROMATIN ASSEMBLY

Our work provided a key connection between heterochromatin assembly and RNAi machinery and suggested that RNAi might specifically target repetitive DNA elements in the genome to promote their silencing and recombinational suppression. The fission yeast genome contains a single homolog for three proteins known to be involved in RNAi, an Argonaute family member (*ago1*), an RNase III-like enzyme similar to Dicer (*dcr1*), and an RNA-dependent RNA polymerase (*rdp1*). In collaboration with R. Martienssen's group here at CSHL, we showed that deletion of *rdp1, dcr1,* or *ago1* results in loss of epigenetic silencing at centromeres. Moreover, we discovered that deletion of each of the RNAi factors results in a loss of Swi6 and heterochromatin-specific histone modification such as H3 Lys-9 methylation and hypoacetylation at centromeres.

We also discovered that specialized repetitive DNA sequences and RNAi mechanisms cooperate to nucleate heterochromatin assembly. Our previous studies showed that a centromere homologous repeat (*cenH*) sequence present at the *mat* locus (see Fig. 1, top panel) has an important role in the initial establishment of heterochromatin throughout the silent mating-type interval. We showed that the *cenH* sequence, which is transcribed at low levels and produces double-stranded transcripts, has the ability to recruit heterochromatin complexes and promote gene silencing at an ectopic site. The capacity of 20-kb *cenH* to confer epigenetic repression on a reporter gene at an ectopic site is strictly dependent on the RNAi machinery, suggesting that an RNAi-mediated process also operates in heterochromatin formation at the mating-type region. We also demonstrated that *cenH* is a heterochromatin nucleation center at the endogenous mating-type region. Unlike flanking sequences within the silent mating-type interval, the *cenH* repeat is a hot spot for H3 Lys-9 methylation (Fig. 1). We found that *cenH* is able to recruit H3 Lys-9 methylation in a Swi6-independent manner, but that the spreading and maintenance of H3 Lys-9 methylation across the rest of the silent mating-type interval require Swi6. This interdependence of Swi6 and H3 Lys-9 methylation suggested an "epigenetic loop" mechanism for inheritance of the heterochromatic

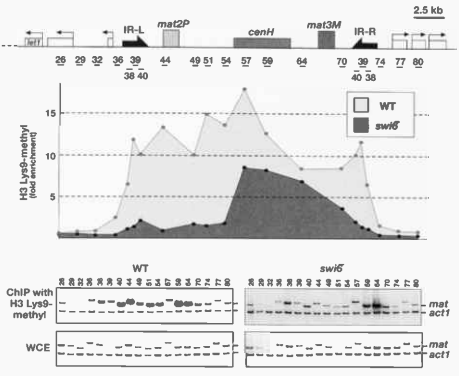


FIGURE 1 Centromere homology (cen*H*) repeat present at the silent mating-type region is a heterochromatin nucleation center. A physical map of the mating-type region with *mat1, mat2,* and *mat3* loci is shown. The IR-L and IR-R inverted repeat heterochromatin boundary elements flanking the silent *mat2-mat3* interval are shown as thick arrows. The cen*H* represents sequences sharing homology with the centromeric repeats. High-resolution mapping of H3 Lys-9 methylation in wild-type and *swi6* mutant cells was carried out by chromatin immunoprecipitation (ChIP) experiments. DNA isolated from immunoprecipitated chromatin (ChIP) or whole-cell crude extracts (WCE) was subjected to multiplex polymerase chain reaction (PCR) to amplify DNA fragments from the *mat* locus as well as *act1* serving as internal control. The distribution of histone H3 methylated at Lys-9 is plotted in alignment with a map of the *mat* locus. Although H3 methylated at Lys-9 is enriched throughout the silent mating-type interval surrounded by boundary elements, this methylation mark in *swi6* mutant cells is mainly restricted to a region encompassing cen*H*. Therefore, the recruitment of H3 Lys-9 methylation to the cen*H* repeat occurs via an Swi6-independent mechanism, but Swi6 is required for spreading and maintenance of H3 Lys-9 methylation across the rest of the silent mating-type region.

state, whereby H3 Lys-9 methylation and Swi6 mutually support their own maintenance in a self-perpetuating manner.

Despite the prominent role that *cenH* has in silencing at the mating-type region and the strict requirement for the RNAi machinery in *cenH*-mediated repression at an ectopic site, the endogenous *mat* region does not display obvious defects when introduced into RNAi mutant backgrounds. This apparent paradox was resolved by the observation that the RNAi machinery is required exclusively for the establishment of heterochromatin at the mating-type region, but not for its subsequent inheritance. This work revealed that the RNAi factors operate to nucleate heterochromatin at the mating-type region, but they are dispensable for the clonal propagation of the pre-assembled heterochromatic state.

Considering the critical importance of higher-order chromatin assembly in gene regulation and maintenance of genomic integrity, and that most eukaryotic genomes are extensively colonized by repetitive elements such as transposons, our discovery of the connection between RNAi and heterochromatin formation will have important implications for understanding the mechanisms governing genome organization and structure. We suggest that transcripts derived from repeat elements such as *cenH* are processed by the RNAi machinery and that the resulting RNA intermediates directly recruit histone-modifying activities to corresponding repeat sequences (Fig. 2). This initial recruitment establishes the "histone code" for binding of heterochromatin proteins such as Swi6. Once bound to chromatin, Swi6 serves as a platform for the recruitment of histone-modifying activities that create additional Swi6-binding sites on adjacent nucleosomes, thus enabling spreading to occur in a stepwise manner. Upon chromosome replication, parental histone H3 and Swi6 are hypothesized to segregate randomly to the daughter chromatids, and Swi6-based activities serve to imprint the parental histone modification pattern onto newly assembled nucleosomes, leading to inheritance of the heterochromatic state.

RNAi AND CHROMOSOME DYNAMICS

Heterochromatin formation at centromeres has an essential role in the proper segregation of chromosomes during cell division. We therefore investigated the role of the RNAi machinery in chromosome dynamics during mitosis and meiosis and found that the RNAi machinery is required for the accurate segregation of chromosomes. Defects in mitotic chromosome segregation are due to loss of cohesin at centromeres. Although the telomeres of RNAi mutants maintain silencing, length, and localization of the heterochromatin protein Swi6, we discovered defects in the prop-

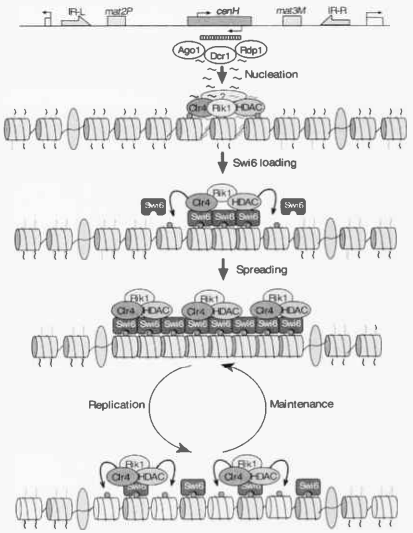


FIGURE 2 Model for heterochromatin formation at the mating-type region of fission yeast. Transcription from *cenH* results in RNAs that yield small heterochromatic RNAs in a process requiring Dcr1, Ago1, and Rdp1. These small RNAs specify the initiation of heterochromatin by interacting with homologous DNA. Histone modification is initiated in a process requiring an HDAC(s), the histone methyltransferase Clr4, the β-propeller domain protein Rik1, and potentially an unidentified protein (*question mark*). Swi6 recognizes deacetylated, H3 Lys-9-methylated histones and binds to chromatin. Chromatin-bound Swi6 recruits HDAC(s) and Clr4 to modify adjacent nucleosomes, which are subsequently bound by additional molecules of Swi6. Such a spreading process is repeated until the flanking IR-L and IR-R boundaries are encountered, or until Swi6 is limiting. Upon chromosome replication, parental histone H3/H4 (darker nucleosomes) are randomly distributed to daughter chromatids, and newly assembled histone H3/H4 (lighter nucleosomes) acquire parental histone modification patterns by the same mechanism that allows spreading in *cis:* Swi6 recruits HDAC(s) and Clr4 to modify adjacent nucleosomes, which become bound by additional molecules of Swi6.

er clustering of telomeres in interphase mitotic cells. Furthermore, a small proportion of RNAi mutant cells display aberrant telomere clustering during meiotic prophase. Our analyses suggest that the RNAi machinery is required for the proper regulation of chromosome architecture during mitosis and meiosis.

HISTONE H3 LYS-4 METHYLATION AND ITS ROLE IN MAINTENANCE OF ACTIVE CHROMATIN STATES

We previously have shown that unlike histone H3 methylated at Lys-9, which is preferentially enriched at heterochromatic loci, the methylation of histone H3 at Lys-4 defines active chromosomal domains. However, in budding yeast, H3 Lys-4 methylation is also necessary for silent chromatin assembly at telomeres and rDNA. It has been suggested that H3 Lys-4 methylation in combination with other histone modifications might perform distinct functions. We tested whether H3 Lys-4 methylation is also required for silencing in fission yeast. We found that deletion of *set1,* which encodes a protein containing a SET domain at the carboxyl terminus, abolishes H3 Lys-4 methylation in fission yeast. Unlike in budding yeast, however, Set1-mediated H3 Lys-4 methylation is not required for heterochromatin assembly at the silent mating-type region and centromeres in fission yeast. We showed that H3 Lys-4 methylation is a stable histone modification present throughout the cell cycle, including mitosis, and that the loss of H3 Lys-4 methylation in *set1*Δ cells is correlated with a decrease in histone H3 acetylation levels, suggesting a mechanistic link between H3 Lys-4 methylation and acetylation of the H3 tail. Our analyses suggest that methylation of H3 Lys-4 primarily acts in the maintenance of transcriptionally poised euchromatic domains and that this modification is dispensable for heterochromatin formation in fission yeast, which instead utilizes H3 Lys-9 methylation.

HISTONE DEACETYLATION AND MAINTENANCE OF GENOMIC INTEGRITY

We previously showed that histone deacetylase Clr6 is essential for viability and that it acts in a partially over-

lapping manner with *clr3* to silence the mating-type region and centromeric repeats. In addition, *clr6* is necessary for genomic integrity, as a conditional mutation in this gene results in severe chromosome loss. Mutations in *clr6* also cause UV light sensitivity, suggesting a defect in general chromatin assembly. To gain insight into the Clr6 function(s), we purified Clr6 and identified its associated proteins. The proteins Pst2, Prw1, and Alp13 copurified with Clr6. Furthermore, all four proteins are predominantly colocalized in the nucleus. These proteins share strong homologies with mammalian components of histone-modifying complexes. Alp13 is a member of the highly conserved MORF4 (mortality factor on chromosome 4)-related gene (MRG) family of chromodomain-containing proteins involved in cellular senescence in humans. The high level of conservation within the MRG proteins among diverse species argues that their molecular function is vital to eukaryotic organisms. Interestingly, deletion of Alp13 or any of the other Clr6-associated factors causes progressive loss of viability, sensitivity to DNA-damaging agents, and cytokinesis defects; this deletion also impairs condensation of chromosomes during mitosis. This is accompanied by hyperacetylation of histone H3 and H4 tails. Therefore, modification of histones by Clr6 HDAC is crucial for fundamental chromosomal functions.

In addition to the studies mentioned above, we also collaborated with R.M. Xu's lab here at CSHL to understand the structural basis of the histone H3 Lys-9 methylation by a SET domain protein Clr4. In another collaborative project (Y. Murakami, Kyoto University), the role of CENP-B proteins in the targeting of H3 Lys-9 methylation and formation of heterochromatin at centromeres in fission yeast was investigated.

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MECHANISMS OF TRANSCRIPTION

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Gene expression is regulated in large part at the level of transcription, and consequently, many diseases result, ultimately, from aberrant transcription. We are interested in deciphering fundamental mechanisms of transcription. For this purpose, we use the human small nuclear RNA (snRNA) genes as a model system. These genes present a number of advantages for such studies. First, some of the snRNA genes are transcribed by RNA polymerase II and some others are transcribed by RNA polymerase III, but all snRNA promoters are very similar to each other. These genes therefore provide a good system to study how a promoter recruits specifically the correct RNA polymerase. Second, the structure of snRNA promoters is well defined, and many of the transcription factors required for transcription of these genes have been identified. Thus, we can address how these transcription factors assemble to form a stable transcription initiation complex, and how the process is regulated.

RNA polymerase II snRNA promoters consist of a proximal sequence element (PSE), which, on its own, is sufficient to direct basal transcription in vitro, and a distal sequence element (DSE), which serves to activate transcription. The RNA polymerase III snRNA promoters contain these same elements as well as a TATA box located downstream from the PSE. In the human snRNA promoters, it is the presence or absence of this TATA box that mainly defines the RNA polymerase specificity of the promoter.

The DSE contains two conserved sequence elements, an octamer sequence that recruits the POU domain transcription factor Oct-1, and another element that recruits the zinc finger protein STAF. In the core promoter, the PSE recruits a complex called $SNAP_c$ that contains five types of subunits, and the TATA box present in the RNA polymerase III snRNA promoters recruits the TATA-box-binding protein (TBP). Transcription from RNA polymerase II snRNA promoters, which do not contain a TATA box, also requires TBP, but in this case, it is not clear how TBP is recruited to the promoter. In addition to these factors, RNA polymerase II transcription from snRNA promoters requires TFIIA, TFIIB, TFIIF, and TFIIE, whereas RNA polymerase III transcription requires the SANT domain protein Bdp1 and the TFIIB-related factor Brf2. One of our main goals is to identify the set of factors required and sufficient to direct RNA polymerase II and III transcription from snRNA core promoters in vitro. We are particularly interested in achieving this with the RNA polymerase III snRNA promoters, because a defined RNA polymerase III transcription system has only been established in yeast, and there only from a simplified core promoter that does not exist in nature.

CHARACTERIZATION OF HUMAN RNA POLYMERASE III

During the last few years, we identified and obtained in recombinant form $SNAP_c$, Bdp1, and Brf2. We also showed that these factors, when combined with material immunoprecipitated with an antibody directed against one of the RNA polymerase III subunits, were capable of directing accurate and efficient RNA polymerase III transcription in vitro. It therefore became a high priority to characterize human RNA polymerase III. Indeed, unlike yeast RNA polymerase III whose 17 subunits had all been cloned, human RNA polymerase III was poorly characterized. cDNAs for five of its subunits, HsRPC4/HsRPC53, HsRPC1/HsRPC155, HsRPC3/RPC62, HsRPC6/RPC39, and HsRPC7/RPC32, had been cloned; however, orthologs of the yeast RNA polymerase III subunits C128 and C37 had not been identified, and for many of the remaining subunits, the available information was limited to database sequences with various degrees of similarity to the yeast subunits. Last year, Ping Hu, with the help of Si Wu, Yuling Sun, Chih-Chi Yuan, Ryuji Kobayashi, and Michael P. Myers, completed a project that started with the generation of cell lines stably expressing a doubly tagged RNA polymerase III subunit. These cells were then used to purify, through a three-step protocol involving an ammonium sulfate precipitation followed by two successive affinity chromatography steps against the two tags, the tagged sub-

unit and associated proteins. The resulting material was fractionated on a gel, and the identity of each visible band was determined by mass spectrometry. This led to a detailed characterization of human RNA polymerase III.

The mass spectrometry analysis confirmed the presence of RPC1, RPC3, RPC4, RPC6, and RPC7 in RNA polymerase III, as well as that of subunits that are common to all three RNA polymerases, namely, RPABC1, 2, 3, and 5. (RPABC4 was not detected, perhaps due to its very small size.) It also gave a number of matches to protein Genbank entries with strong similarity to yeast RNA polymerase III subunits, thus identifying RPC8, RPC9, RPC10, RPAC1, and RPAC2 as orthologs of the *Saccharomyces cerevisiae* C25, C17, C11, AC40, and AC19 subunits. Interestingly, RPC8 and RPC9 display sequence similarity to the RNA polymerase II RPB7 and RPB4 subunits, respectively, and we showed that they associate with one another, paralleling the association of the RNA polymerase II subunits. Thus, RPC8 and RPC9 are paralogs of RPB7 and RPB4. The analysis allowed us to assemble a complete open reading frame encoding the second largest subunit, RPC2, and it led to the identification of an ortholog of the *S. cerevisiae* C37 subunit, which we call RPC5.

RPC5 is an 80-kD protein with limited sequence similarity to the yeast 37-kD C37 subunit. Yet, RPC5 was found to associate with RPC53, paralleling the known association of the yeast C37 and C53 subunits. In addition, we could show that RPC5 is specifically required for transcription from the type-2 VAI and type-3 human U6 promoters, because one of our anti-RPC5 antibodies apparently dissociates the subunit from the rest of the RNA polymerase III enzyme. Depletion of an extract with this antibody debilitated U6 transcription, and transcription could be restored by the addition of recombinant RPC5 produced in *Escherichia coli*. Thus, the subunit itself is essential for promoter-directed transcription. The characterization of human RNA polymerase III opens the way to the reconstitution of U6 transcription with defined factors.

REDUNDANT MECHANISMS FOR ASSEMBLY OF A STABLE U6 TRANSCRIPTION INITIATION COMPLEX

Another focus has been to understand how transcription factors assemble on promoters to form a stable initiation complex. Last year, Beicong Ma completed a project in which she studied how $SNAP_c$ and TBP bind cooperatively to DNA. Our previous studies had shown that both $SNAP_c$ and a subcomplex of $SNAP_c$, which we call mini-$SNAP_c$, bind cooperatively with TBP to the PSE and TATA box on the human U6 promoter. $SNAP_c$ contains five types of subunits: SNAP190, SNAP50, SNAP45, SNAP43, and SNAP19. Mini-$SNAP_c$ consists of just the amino-terminal third of SNAP190, SNAP50, and SNAP43. To determine which region of mini-$SNAP_c$ is required for cooperative binding, Beicong generated complexes smaller than mini-$SNAP_c$ (stm$SNAP_c$s) and tested them for cooperative binding. These experiments identified a 50-amino-acid region from amino acids 34 to 83 of SNAP190 that is required for cooperative binding with TBP in the context of mini-$SNAP_c$. Furthermore, the amino-terminal 90 amino acids of SNAP190 (including amino acids 34 to 83) are sufficient for cooperative binding with TBP when fused to either the SNAP190 RcRd Myb repeats, which are part of the $SNAP_c$ DNA-binding domain, or the heterologous GAL4 DNA-binding domain.

The stm$SNAP_c$s were then tested for their activity in RNA polymerase III transcription from the U6 snRNA promoter. Intriguingly, they were all active, including those missing the first 90 amino acids of SNAP190 and thus incapable of cooperative binding with TBP. These results showed that an stm$SNAP_c$ containing only SNAP190 amino acids 84–505, SNAP43 amino acids 1–268, and SNAP50 (stm$SNAP_c$#8) is still capable of directing basal levels of RNA polymerase III transcription and thus contains all the information required for assembly of the initiation complex . They also showed that stm$SNAP_c$s incapable of recruiting TBP to the DNA were functional, suggesting that there is an alternative way to recruit TBP to the U6 promoter.

Transcription from the U6 promoter requires a protein named Brf2, for TFIIB-related factor 2. Brf2 had been shown to bind cooperatively with TBP, and we therefore asked whether Brf2 could recruit TBP to the U6 promoter in the presence of stm$SNAP_c$s. Indeed, Brf2 could recruit TBP to the U6 promoter even in the presence of stm$SNAP_c$s incapable of binding cooperatively with TBP. This indicates that there are redundant mechanisms to recruit TBP to the U6 transcription initiation complex. There are also redundant mechanisms to recruit $SNAP_c$ to the DNA. Indeed, it can be recruited by cooperative binding not only with TBP, but also with Oct-1. Such redundant mechanisms may ensure efficient formation of the

transcription initiation complex on chromatin templates in vivo. Furthermore, they probably ensure that once the U6 transcription initiation complex is formed, it remains stable for several rounds of transcription.

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TRANSCRIPTIONAL REGULATION

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We all originate from a single cell that contains two complete sets of instructions—one from each parent—encoding how we should develop into a human being. Those instructions direct the cells to proliferate and at the appropriate time to differentiate into the many different types of cells that make up an individual. Cells proliferate via repeated cycles of cell growth and division, and differentiate by expressing specific sets of genes that permit them to perform specific functions. Both processes involve intricate patterns of regulation of gene expression, which often result from the regulation of gene transcription: the process by which genes are copied into pre-messenger RNA for subsequent translation into the protein products that effect gene action. In eukaryotes, the genes are packaged into chromatin, and the regulation of gene transcription involves the ability of transcriptional activators and repressors to direct changes in the structure of chromatin and to recruit one of three nuclear RNA polymerases—pol I, pol II, and pol III—to specific promoters.

We study transcriptional regulation in human cells using herpes simplex virus (HSV), a prevalent human pathogen, as a probe. Viruses provide simple regulatory networks in which the cellular transcriptional machinery is manipulated to achieve the goals of virus infection. In a cell infected by HSV, the virus can grow lytically or remain latent for many years. In the lytic cycle, HSV gene expression is initiated by a viral transcription factor called VP16, which is brought by the infecting virion. Before activating transcription, VP16 forms a multiprotein-DNA complex—the VP16-induced complex—on viral immediate-early promoters with two cellular proteins: HCF-1, an abundant chromatin-associated protein that regulates cell proliferation, and Oct-1, a POU-homeodomain transcription factor. Once the VP16-induced complex is assembled, VP16 initiates viral gene transcription, in part, through a potent transcriptional activation domain, which stimulates the cell's basal transcriptional machinery.

Our laboratory focuses on three principal issues: (1) How do transcriptional regulators activate the basal transcriptional machinery? (2) How do transcription factors, as in the VP16-induced complex, modify their transcriptional activity through selective protein-protein and protein-DNA interactions? (3) What are the natural cellular roles of HCF-1 and Oct-1, and how do they influence HSV infection? Our studies in each of these three areas continue to elucidate new mechanisms for transcriptional regulation in human cells. Below is a description of two important discoveries we reported this year that involve (1) the structure and function of TBP and the TFIIB family of basal transcription factors and (2) novel roles for the HCF-1 protein in cell proliferation.

A SHARED SURFACE OF TBP DIRECTS RNA POLYMERASE II AND III TRANSCRIPTION THROUGH ASSOCIATION WITH DIFFERENT TFIIB FAMILY MEMBERS

Xuemei Zhao, in collaboration with Laura Schramm and Nouria Hernandez here at CSHL, showed that a common surface of TBP can direct both pol II and pol III transcription through association with different TFIIB family members. None of the three nuclear RNA polymerases initiates a basal level of specific transcription on its own. Instead, each RNA polymerase is recruited to its respective promoters by different sets of general transcription factors, together forming the basal transcriptional machinery. Among these basal factors, there is one—the TATA-box-binding protein TBP—that is used by all three RNA polymerases. TBP is not known, however, to associate with any of these RNA polymerases directly. Instead, it achieves its diverse transcriptional activities by associating with combinations of promoter- and polymerase-selective basal factors, such as those of the TFIIB family on promoters containing ($TATA^+$) and lacking ($TATA^-$) a TATA box.

Among eukaryotes, TBP contains regions of low and high sequence conservation: The amino-terminal region varies among species in both length and sequence, whereas the carboxy-terminal region, called the TBP_{CORE}, is a highly conserved and structured 180-amino-acid domain, which binds the TATA box. We have analyzed the role of the conserved TBP_{CORE} in transcription from a set of four model pol II and pol III $TATA^+$ and $TATA^-$ promoters. As model promoters, we used (1) the adenovirus major late (AdML) $TATA^+$ and human U1 small nuclear RNA (snRNA) gene $TATA^-$ pol II promoters, and (2) the human U6 snRNA gene $TATA^+$ and adenoviral VAI $TATA^-$ pol III promoters.

On these promoters, TBP associates with different members of the TFIIB family of general transcription factors. The TFIIB family proteins have key roles in recruiting the RNA polymerase machinery to the promoter. TFIIB is involved in pol II transcription from both $TATA^+$ and $TATA^-$ promoters; the TFIIB-related factor Brf1 is involved in pol III transcription from $TATA^-$ promoters and the human-specific Brf2 protein is involved in pol III transcription from a unique set of human $TATA^+$ promoters, called "type 3," which is represented by the U6 snRNA gene promoter. Interestingly, the yeast *Saccharomyces cerevisiae* lacks a homolog of Brf2 and does not contain type-3 pol III promoters, suggesting that Brf2 and type-3 pol III promoters are regulatory elaborations present in human but not yeast cells.

We used these four model pol II and pol III $TATA^+$ and $TATA^-$ promoters for a systematic analysis of the effect of mutations across different surfaces of the human TBP_{CORE} on both transcription from the four model promoters and association with different factors involved in transcription from these promoters. Our analysis demonstrates that human TBP utilizes two modes to achieve its versatile functions. First, it uses a different set of surfaces on the conserved and structured TBP_{CORE} domain to direct transcription from each of the four model promoters; this finding offers an explanation for why the TBP_{CORE} is so highly conserved: Each surface is involved in transcription from some important promoter. Second, unlike yeast TBP, human TBP can use a shared surface to interact with two different TFIIB family members—TFIIB and Brf2—to initiate transcription by different RNA polymerases. Thus, by being able to interact with a more diverse family of TFIIB-like proteins, TBP can activate different types of transcription from diverse contexts while utilizing the same TBP surface.

LOSS OF HCF-1 FUNCTION LEADS TO BOTH CELL PROLIFERATION AND CYTOKINESIS DEFECTS WHICH CAN BE RESCUED BY INACTIVATION OF THE RETINOBLASTOMA PROTEIN FAMILY

Our laboratory continues to place considerable emphasis on understanding the cellular functions of HCF-1. Mammalian HCF-1 is a highly conserved and abundant chromatin-bound protein that has a role in both HSV immediate-early (IE) gene transcription and cell

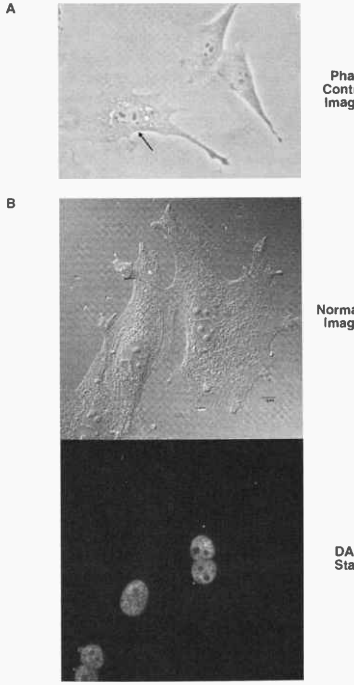

FIGURE 1 Loss of HCF-1 function leads to a conspicuous binucleation phenotype. (*A*) A representative phase-contrast image of arrested tsBN67 cells after 72 hours at nonpermissive temperature. The arrow points to a binucleated cell; two mononucleated cells are also shown. (*B*) Nomarski and DAPI-stained images of a field of arrested tsBN67 cells after 48 hours at nonpermissive temperature; a complete binucleated and mononucleated cell are shown. (Reproduced from Reilly and Herr 2002.)

proliferation. Its role in cell proliferation has been evidenced through the analysis of a temperature-sensitive hamster cell line called tsBN67. When placed at nonpermissive temperature, tsBN67 cells undergo a stable and reversible proliferation arrest after a lag of 36–48 hours. This phenotype results from a single point mutation in HCF-1, which disrupts HCF-1 association with both chromatin and the HSV IE *trans*-activator VP16 at nonpermissive temperature. This year, Patrick Reilly (a graduate student in my lab) reported the isolation and characterization of spontaneous tsBN67 growth-revertant cells that are able to proliferate at nonpermissive temperature. These cells retain the tsBN67 HCF-1 point mutation and grow in the absence of evident HCF-1 chromatin association, demonstrating that complete restoration of tsBN67 HCF-1 functions is not essential for cell proliferation.

Furthermore, microscopic analysis of the cells showed that temperature-arrested tsBN67 cells display a conspicuous multinucleated phenotype, primarily binucleated, in a significant population of the arrested cells, indicating that HCF-1 is necessary not only for cell growth and proliferation, but also for proper cytokinesis. Figure 1 shows examples of this binucleated phenotype by both phase-contrast (A) and Nomarski (B) imaging. As is the tsBN67 cell proliferation defect, the cytokinesis defect is also a result of loss of HCF-1 function, suggesting that HCF-1 has a role in both progression through G_1 and cell exit from mitosis.

Patrick Reilly also showed that the SV40 early region, in particular large T antigen (Tag), and the adenovirus oncoprotein E1A can rescue the tsBN67 cell proliferation defect at nonpermissive temperature. As shown in collaboration with Joanna Wysocka, the SV40 early region rescues the tsBN67 cell proliferation defect without restoring HCF-1 chromatin association, indicating that these oncoproteins bypass a requirement for HCF-1 function. The SV40 early region also rescues the tsBN67 cytokinesis defect, suggesting that the roles of HCF-1 in cell proliferation and cytokinesis are intimately linked. The ability of SV40 T antigen and adenovirus E1A to inactivate the pRb protein family—pRb, p107, and p130—is important for the bypass of HCF-1 function. These results suggest that HCF-1 regulates mammalian cell proliferation and cytokinesis, at least in part, by either directly or indirectly opposing pRb family member function. Our future goals are to elucidate the mechanisms by which HCF-1 mediates its important and diverse roles in cell proliferation.

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STRUCTURAL BIOLOGY OF REGULATION OF DNA REGULATORY MOLECULES AND PROTEOLYSIS

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We study the molecular basis of cell regulatory processes, combining the tools of structural biology and biochemistry, to examine the molecular interactions associated with these processes. X-ray crystallography enables us to obtain the three-dimensional structures of individual proteins and their complexes with other molecules. We use biochemistry and molecular biology to characterize properties that can be correlated with protein structure, and we use information from molecular biology and genetics to study protein function.

Our current efforts center around two distinct themes. The first involves structural studies of protein complexes involved in nucleic acid regulatory processes. The second theme in the lab is the regulation of proteolysis. Here, we are studying caspase activation in apoptosis and a conserved family of oligomeric self-compartmentalizing intracellular proteases, the bleomycin hydrolases, which are involved in drug resistance.

DNA Binding and Assembly of the Papillomavirus Initiator Protein E1

E. Enemark, A. Auster [in collaboration with A. Stenlund, Cold Spring Harbor Laboratory]

Papillomaviruses are a large family of closely related viruses that give rise to warts in their hosts. Infection of the genital tract by the human papillomaviruses (HPV) from this group represents one of the few firmly established links between viral infection and the development of cervical cancer, as HPV DNA is found in practically all cervical carcinomas. The E1 protein belongs to a family of multifunctional viral proteins whose main function is related to viral DNA replication. These proteins bind to the origin of DNA replication, melt the DNA duplex, possess DNA helicase activity, and recruit other cellular replication proteins such as DNA polymerase α and replication protein A (RPA). Most likely, different oligomeric forms of the two proteins are responsible for the different activities, and the sequential assembly of T antigen and E1 complexes ensures an ordered transition between these different activities. Ultimately, E1 forms hexameric ring helicases on each strand which serve as replicative DNA helicases that unwind the DNA in front of the replication fork.

In collaboration with Arne Stenlund's group here at CSHL, we embarked on structural studies to provide high-resolution structural information about E1, its DNA-binding activity, and its assembly on DNA. These would provide general insight into the biochemical events that are involved in viral DNA replication. They can also provide a basis for the development of clinical intervention strategies. Second, the viral DNA replication machinery itself represents an obvious target for antiviral therapy, and detailed information such as high-resolution structures of viral proteins required for replication will greatly facilitate the development and testing of antiviral agents.

The initial binding of an E1 dimer together with E2 serves to recognize the double-stranded origin of replication (*ori*) with high specificity. The subsequent binding of additional E1 molecules leads to *ori* melting, most likely through the positioning of E1 molecules in the proper arrangement necessary for melting of double-stranded DNA (dsDNA). Ultimately, two hexameric rings are formed on the *ori* that could unwind the DNA double helix. Thus, the transition between these complexes represents a transition from a tethering function for E1 to a function that modifies the DNA structure. We have captured structural snapshots of two sequential steps in the assembly process (Fig. 1). Loading of an initial E1 dimer on the origin occurs at adjacent major grooves on one face of the helix. Subsequently, a second dimer is loaded onto another face of the helix. Binding occurs through two DNA-binding modules, a DNA-binding loop (DBL), and a DNA-binding helix (DBH), that bind to the two separate strands of DNA. The majority of contacts are made by the DBL, a long loop that is preorganized for binding. Binding of two individual strands by separate regions of the protein can provide a basis for a transition from double-stranded to single-stranded DNA binding, leading to strand separation, with protein maintaining binding to one strand while dissociating

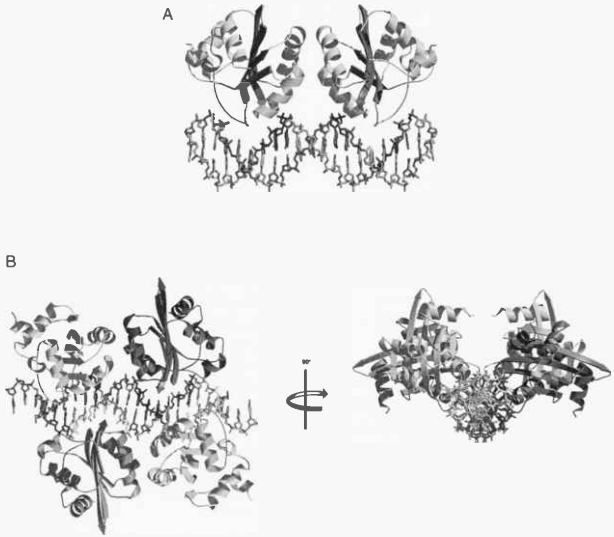

FIGURE 1 Crystal structures of two intermediates in the assembly of E1-DBD subunits on the origin of replication. (*A*) Structure of the E1-DBD dimer bound to sites 2 and 4 determined from the [(E1-$DBD_{159-307}$)$_2$(DNA_9)] crystal structure. (*B*) Structure of the E1-DBD tetramer bound to E1-binding sites 1–4 determined from the [(E1-$DBD_{159-303}$)$_4$(DNA_1)] crystal structure viewed perpendicular and parallel to the DNA helical axis.

from the other. Additional subunit-subunit contacts formed in the assembly of the hexameric ring would then compensate for lost protein-DNA interactions. A more common recognition motif, such as "helix in the groove," in which the same structural element contacts both strands on either side of the groove, would not allow such a facile transition. Since interactions between DNA and the DBH are less extensive than interactions with the DBL, the DBL is a more likely candidate for the binding module that retains binding. Moreover, in the tetramer, the two DBLs of the upstream monomers of each dimer bind to the top strand, whereas the DBLs of the downstream monomers of each dimer bind to the bottom strand. We therefore suggest that one hexameric helicase assembles around the top strand on one side of the *ori* from the proteins at sites 3 and 4, and the other hexameric helicase assembles around the bottom strand on the other side of the *ori* from the proteins at sites 1 and 2. As the hexameric helicases travel in opposite directions around their respective strands, the correct 3′→5′ polarity would occur (Fig. 2).

Bleomycin Hydrolases

P. O'Farrell [in collaboration with F. Gonzalez and S.A. Johnston, University of Texas, Southwestern Medical Center, and S.J. Keding and D.H. Rich, University of Wisconsin, Madison]

Bleomycin hydrolase (BH) is a 300-kD cysteine protease with unusual structural and biological features. It was discovered due to its ability to deactivate the glycopeptide antibiotic, bleomycin, which is used as a therapeutic agent in the treatment of a number of different forms of cancer. The clinical use of bleomycin is limited due to drug resistance and dose-dependent production of pulmonary fibrosis. The endogenous enzyme, BH, is overexpressed in some tumor cells and

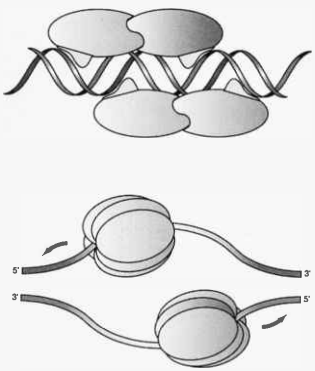

FIGURE 2 Schematic diagram of the proposed role of the DBL in strand selection: A cartoon depicting a model for the assembly of two hexameric helicases around single strands at the *ori*. The two DBLs (*black*) of the upstream monomers of each dimer bind to the top (*black*) strand, and the DBLs of the downstream monomers of each dimer bind to the bottom strand (*white*). One hexameric helicase assembles from the upstream monomers at sites 3 and 4 (*dark gray*) around the top strand and the other hexameric helicase assembles from the downstream monomers at sites 1 and 2 (*light gray*). This arrangement would result in the correct 3′→5′ polarity for the helicases.

is thought to be a major cause of tumor cell resistance to bleomycin therapy. It was shown that in both yeast and mammalian cells, BH is the only enzyme with bleomycin deamidation activity. Interest in its clinical relevance was heightened by a recent report of the genetic linkage of an allelic variant to the nonfamilial form of Alzheimer's disease. BH was also found to bind amyloid precursor protein (APP).

Since mammals rarely encounter bleomycin, bleomycin hydrolysis is probably not the normal function of the enzyme. On the basis of studies with BH knockout mice, it was shown that BH is important for neonatal survival. BH is widely distributed throughout nature. Homologs exist in bacteria, yeast, birds, and mammals, with high sequence identity among the different forms. The yeast form negatively regulates the galactose metabolism system and also binds single-stranded DNA and RNA with high affinity. Nucleic-acid-binding activity was also reported for other members of the family.

We have previously determined the crystal structures of the yeast BH, Gal6, and the human enzyme (hBH). These structures revealed several unique features of these proteases. They both have a hexameric ring-barrel structure with the active sites embedded in a central cavity. The only access to the active sites is through the cavity. BH has little substrate specificity so it is reasonable to assume that this sequestration of the active sites is necessary to prevent indiscriminate proteolysis. This places BH in the class of "self-compartmentalizing proteases" with, for example, the proteasome and the tricorn protease. The carboxyl terminus of BH lies in the active site, in much the same position as that taken by inhibitors complexed with papain. Our previous work on the yeast BH, Gal6, has shown that it acts as a carboxypeptidase on its carboxyl terminus to convert itself to an aminopeptidase and peptide ligase. The carboxyl terminus anchors the substrate into the active site and confers "positional" specificity. This model also serves to explain the unique ability of BH/Gal6 to inactivate bleomycin.

In collaboration with Dan Rich's laboratory at the University of Wisconsin, Madison, we embarked on an iterative study to design specific, tight-binding active site inhibitors of BH activity to increase the efficacy of bleomycin, and perhaps reduce dosage levels in cancer treatment. The emerging link between hBH and Alzheimer's disease is another incentive, since an inhibitor to hBH may be useful in understanding this connection and may also be useful in therapy. We are using our crystal structure to create novel inhibitors by use of computerized structure-generating programs. Using this approach, we can create both peptide-like and nonpeptide inhibitors by a combinatorial process. We identified four compounds in our initial screens that are good inhibitors and have solved two cocrystal structures of hBH with these compounds. These structures are the basis of further drug design.

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RNA SPLICING

A.R. Krainer E. Allemand, L. Cartegni, M. Hastings, R. Karni, L. Manche, H. Okunola, X. Roca, S. Shaw, P. Smith, A. Stella, Q.-S. Zhang, Z. Zhang

CONSTITUTIVE AND ALTERNATIVE PRE-mRNA SPLICING MECHANISMS AND REGULATION

RNA splicing is required for expression of most eukaryotic protein-coding genes. Splice-site selection is a high-fidelity process that requires the recognition of limited and dispersed sequence information present throughout introns and exons. In humans, approximately 60% 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 extracellular signals. 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 relatively small number of genes uncovered by genome-sequencing projects.

Both constitutive and alternative splicing mechanisms involve more than 100 protein components, as well as RNA components that are part of a set 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 especially interested in how the spliceosome correctly identifies and joins the exons on each pre-mRNA, and how certain point mutations in either exon or intron sequences cause aberrant splicing, leading to numerous genetic diseases.

EXON-SKIPPING MUTATIONS IN CANCER AND NEURODEGENERATION

We previously developed, based on our experimental data and in collaboration with Michael Zhang's lab here at CSHL, methods for predicting exonic splicing enhancer (ESE) motifs responsive to particular human SR proteins. A Web-based program, ESEfinder, is now available (http://exon.cshl.edu/ESE) and can be used to calculate ESE scores in any sequence of interest. We applied this type of analysis to the human *SMN1* and *SMN2* genes, which are involved in spinal muscular atrophy (SMA), a common pediatric neurodegenerative disease. SMA patients lack a functional *SMN1* gene but possess at least one copy of the nearly identical *SMN2* gene. It was known that exon 7 in *SMN1* is constitutively included, whereas exon 7 in *SMN2* is skipped 80% of the time, giving rise to a defective protein. Moreover, this difference between the two homologous exons is due to a single, translationally silent point mutation. The low level of functional SMN protein translated from the approximately 20% of *SMN2* pre-mRNA that is spliced correctly is essential for viability, but is not sufficient to prevent the disease. We used mutational analysis, including compensatory mutations, in combination with in vitro splicing, transient transfections, and RNA-protein interaction studies, to show that the single C to T difference at position +6 between *SMN1* and *SMN2* exon 7 maps to an SF2 (splicing factor 2)/ASF (alternative splicing factor)-dependent ESE heptamer motif. The ESE is active in *SMN1* but inactive in *SMN2*, explaining why a constitutive exon in *SMN1* corresponds to a weak alternative exon in *SMN2* (Figs. 1 and 2).

We had previously carried out a similar analysis of a breast cancer nonsense mutation that causes skipping of a constitutive exon in *BRCA1*. More recently, we collaborated with Drs. Fackenthal and Olopade at the University of Chicago to characterize a breast cancer missense mutation in *BRCA2* that likewise inactivates the gene by causing exon skipping, resulting in breast and ovarian cancer susceptibility. In this case, the sequence analysis with ESEfinder showed that the point mutation inactivated three overlapping ESE motifs, leading to the prediction that exon skipping might result, which was then confirmed by analysis of RNA from patient blood cells. These studies can explain how single-base changes in exons allow genes to evolve, e.g., transforming a constitutive exon into an alternative one or vice versa. A further implication is that many mutations traditionally classified as non-

sense, missense, or silent, as well as some polymorphisms, can give rise to exon skipping, such that the mutant or variant site is no longer present in some or all of the mature mRNA. Thus, the effect of the mutation or polymorphism on the protein product is very different from, and usually more drastic than, what would be predicted from analysis of the genomic sequence. Analysis of mRNA splicing can therefore be critical for correct disease diagnosis and assessment of phenotypic risk in individuals with familial predispositions.

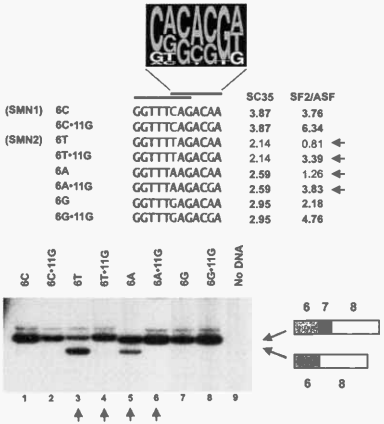

FIGURE 1 An SF2/ASF-dependent ESE motif promotes inclusion of *SMN* exon 7. The SF2/ASF consensus heptamer motif is shown at the top, with the preferred nucleotides shown in *gray*. The size of each letter is proportional to the frequency of that nucleotide at that position in an alignment of 26 ESE sequences. The first 12 nucleotides from eight wild-type or mutant versions of exon 7 are shown, with the mutated positions +6 and +11 shown in *gray*. The *light* and *dark gray* horizontal bars indicate the position of the overlapping SC35 and SF2/ASF motifs, respectively. The effect of the point mutations used in transfection experiments on the calculated SC35 and SF2/ASF motif scores is shown on the right (high scores in boldface; subthreshold scores in lightface). The first and third sequences correspond to *SMN1* and *SMN2*, respectively. The autoradiogram shows a semiquantitative RT-PCR analysis of transiently expressed minigenes. The products corresponding to exon 7 skipping and inclusion are indicated. Two of the minigenes give rise to exon skipping: 6T (*SMN2*) and 6A (*dark gray*). When 6T and 6A are combined with the 11G suppressor mutation to reconstitute an SF2/ASF high-score motif, exon inclusion is restored (*light gray*). Note that the SC35 motif is neither necessary nor sufficient to promote exon inclusion, whereas the SF2/ASF motif is both necessary and sufficient.

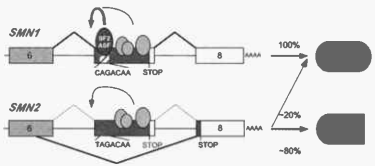

FIGURE 2 Model of SF2/ASF-dependent exon 7 inclusion in *SMN1* and *SMN2*. Binding of SF2/ASF to its cognate heptamer ESE in *SMN1* exon 7 (*top*) promotes exon definition so that exon 7 is constitutively included, allowing for translation of full-length SMN protein. The C6T change in *SMN2* exon 7 (*bottom*) prevents efficient SF2/ASF binding to the corresponding heptamer. Exon 7 is thus mostly skipped, resulting in the production of defective SMNΔ7 protein. Other ESEs in the exon can mediate weak exon inclusion even in the absence of the SF2/ASF motif, probably through binding of other SR or SR-like proteins, including hTra2β1. Partial inclusion of *SMN2* exon 7 generates a small amount of full-length SMN protein, identical to that encoded by the *SMN1* gene, since the C to T change is translationally silent. Exons are represented as boxes and introns as lines. The shaded portion of exon 7 encodes the last 16 amino acids of the SMN protein, which are missing from SMNΔ7. The shaded portion of exon 8 represents the last 4 amino acids of SMNΔ7, which are not present in SMN. Open boxes represent 3′-untranslated regions. The dark oval denotes SF2/ASF and light ovals represent other SR or SR-like proteins. Curved arrows denote promotion of exon definition and chevrons indicate splicing patterns. Line thicknesses are indicative of relative splicing efficiency. The percent values refer to the extent of exon 7 inclusion in vivo. The diagrams of SMN and SMNΔ7 proteins show their different carboxy-terminal domains.

RATIONAL DESIGN OF SYNTHETIC EXON ACTIVATOR MOLECULES

On the basis of our current knowledge about ESEs and SR proteins, we are designing novel synthetic exon activator molecules that can promote inclusion of target exons in a specific manner. The synthetic molecules consist of an antisense moiety covalently attached to a small peptide. The sequence of the antisense moiety is chosen to target a particular exon by Watson-Crick base pairing, whereas the peptide moiety is a minimal splicing activation domain analogous to an SR protein RS domain. So far, we have used a 12-residue peptide-nucleic acid (PNA) backbone for the antisense moiety, and RS domain peptides of 10,

20, or 30 amino acids. We found that compounds targeted to *BRCA1* exon 18 could restore exon inclusion in a mutant that causes skipping of this exon. Likewise, compounds targeted to *SMN2* exon 7 increased the inclusion of this exon. These experiments were done in vitro, and specificity was demonstrated by the fact that the compounds targeted to *BRCA1* did not affect *SMN2* splicing and vice versa. The synthetic RS domains become phosphorylated under splicing conditions. We are presently optimizing several parameters to improve the efficacy of these exon activators, as well as to facilitate their cellular uptake, to determine if they can affect splicing in vivo and to adapt them for therapeutic purposes.

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PROTEOMICS

M. Myers S. Lopez L.L. Schmidt
G. Pegoraro

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 my 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. My laboratory is focused on understanding the global architecture of this network and how this network generates robustness (error tolerance) and adaptablility to the system and how this network is altered to produce and survive complex diseases, such as cancer.

Optimization of Protein Identification

S. Lopez, 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. Importantly, we have found that the method of staining the proteins prior to analysis can greatly affect the outcome of analysis, and we have begun to systematically explore different methods for highly sensitive protein visualization that do not adversely affect downstream MS analysis. We have found that fluorescent stains, such as SYPRO Ruby Red, give the greatest sensitivity without adversely affecting our analysis. However, we have found that negative zinc staining gives the best mix of protein visualization, ease of use, and MS sensitivity.

Monitoring Posttranslational Modifications

L.L. Schmidt, G. Pegoraro [in collaboration with R.-M. Xu, Cold Spring Harbor Laboratory]

One of the challenges in the postgenomic era is to understand how proteins are regulated by posttranslational modifications. These modifications are responsible for controlling the activity of proteins and ultimately determining how a cell responds to its environment. Phosphorylation is considered to be the most common posttranslational modification, and its wide use as a regulatory mechanism has been attributed to its reversible nature. Alterations in protein phosphorylation have been shown to be the hallmark of many pathological conditions including cancer and diabetes. Therefore, efficient identification of phosphorylated proteins, as well as mapping the specific phosphorylation sites, has become one of the primary goals of proteomics. We have been attempting to develop sensitive and robust MS methods for characterizing this important posttranslational modification. Our efforts have centered on developing reagents for the affinity purification of phosphorylated proteins and peptides. Initially, we tried immobilized iron as our affinity reagent, as this has been shown to interact strongly with phosphorylated residues. However, we found that other metals are much more efficient for the affinity purification of phosphorylated residues, gallium being the strongest, followed by copper, iron, and nickel. Using this technology, we estimate that 500 fmoles of phosphorylated peptide is required for characterization. We believe that the major nonphosphorylated contaminants arise from proteins and peptides with acidic regions. To circumvent this, we are developing chemistries designed to methylate, and thereby neutralize, glutamic and aspartic acid residues.

In collaboration with Rui-Ming Xu here at CSHL, we have been developing MS-based assays to analyze histone modifications. It is clear that modification of histone residues has a major role in transcriptional regulation, and histones have been found to be methylated, acetylated, phosphorylated, and even ubiquitinylated. Our interest in developing MS-based assays was because of their speed and sensitivity. We have been focusing on the methylation of the amino terminus of histone H3 and have been able to detect the methylation of lysine 4 and lysine 9 using recombinant proteins. We are able to detect the methylation of either lysine residue using only a few femtomoles of substrate and have found that the methylation of both lysine residues is highly pH-dependent. Significantly, we have also discovered that the recombinant enzymes are capable of using multiple methyl donors.

Analysis of DNA Interacting Proteins

M. Myers

We have developed a technique to analyze the proteins that associate with a specified molecule of DNA in vivo. We have also developed a sequence-specific DNA affinity tag, termed a sequence seeker, which has been designed to purify engineered episomal DNA from mammalian cells. The sequence seeker we have developed targets a sequence (AGCGGATGCGGA), which was picked because it occurs infrequently in the human genome. Using a tagged episome that also contained a transcription unit and an origin of replication, we were able to identify a number of proteins involved in transcription, recombination, and other aspects of chromatin dynamics. Although this technique can be used to analyze origins of replication, promoter sequences, silenced regions, etc., we are planning to adapt this technique to study cellular responses to DNA damage.

"Small World" Proteomics

M. Myers [in collaboration with R. Sachidanandam and J. Faith, 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 which are essential. The overall goal of this study is to understand how these protein networks provide adaptability either to changes in environmental conditions or to genetic alterations, such as loss or duplication of a node (gene) that occurs during tumorigenesis.

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CELL SIGNALING IN HIV PATHOGENESIS

J. Skowronski B. Hill M. Kwofie
A. Janardhan T. Swigut

Our interest lies in understanding the molecular mechanisms underlying the pathogenesis of AIDS and, in particular, understanding the functional consequences of interactions between viral proteins and the cellular regulatory machinery. The focus of our research is to understand the functions of Nef, a regulatory protein of human and simian immunodeficiency viruses (HIV and SIV) that is an important determinant of virulence. A major effort has been directed toward the identification of mechanisms and downstream effectors that mediate the effect of Nef on protein sorting and signal transduction machineries, and our experiments have been focused in two areas. First, we continued to study the mechanisms that mediate the selective down-regulation of cell surface receptors by Nef via the AP-2 clathrin adaptor pathway. We found that this involves cooperative interactions between Nef, clathrin adaptors, and cytoplasmic domains of target cell surface receptors. This will likely provide a general mechanism for selective cargo recruitment to the sites of endocytosis by this small viral protein. Second, we have focused heavily on the isolation and identification of cellular proteins that mediate the effects of Nef on signal transduction and endocytotic machineries. This has led recently to purification and microsequencing of several cellular factors that associate with Nef in T lymphocytes. Among them are factors that control critical events in the regulation of protein sorting, signal transduction, and gene expression. Current studies are aimed at verifying these novel interactions and addressing their relevance to previously known and possibly novel functions of Nef.

NEF INDUCES THE ENDOCYTOSIS OF TCR:CD3-ζ, CD4, AND CD28 VIA THE AP-2 CLATHRIN ADAPTOR PATHWAY

Our previous studies revealed that Nef down-regulates cell surface expression of several receptors, such as CD4, CD28, and the T-cell antigen receptor (TCR:CD3) complex, that mediate signal transduction in T cells and are important for the normal function of antigen-recognition machinery in infected T cells. In the last year, we made significant progress in unraveling the general molecular mechanisms that underlie the endocytosis of cell surface receptors by Nef. Several lines of evidence from our studies support a model whereby Nef induces the endocytosis of target molecules (CD4, CD28, and TCR:CD3) by recruiting them directly to AP-2 clathrin adaptor endocytic pathways. First, using immunofluorescence microscopy, we showed that in cells expressing Nef, CD4 and CD28 (and the CD3-ζ subunit of the TCR:CD3 in cells expressing SIVmac 239 Nef) are translocated to the regions of the plasma membrane coated by AP-2. Second, we presented evidence that Nef directly binds target sequences in the cytoplasmic domain of CD28. Third, we were able to reconstitute the trimeric Nef:CD3-ζ cytoplasmic domain:AP-2 complex in vitro. Finally, we presented evidence that HIV-1 and SIV Nef directly bind the AP-2 clathrin adaptor complex. Together, these data support a general model in which Nef, the AP-2 clathrin adaptor, and the target receptor (cargo) form a trimeric complex that initiates Nef-induced internalization of the target receptor.

SIV NEF POSSESSES AT LEAST TWO STRONG AP-2-BINDING SITES EACH WITH DIFFERENT FUNCTIONAL ROLES

The CD4, TCR:CD3, and CD28 down-regulation functions of Nef are separable by specific mutations. This indicated that each of these functions must involve independent molecular interactions of Nef with target molecules and/or the AP-2 clathrin adaptor. To explain how the selectivity of Nef for endocytosis of these cell surface receptors is generated, we studied in detail the interaction of Nef with AP-2 both in vivo using fluorescence microscopy colocalization assays and in vitro using "pull-down" assays. We mapped the regions and amino acid sequences in the HIV-1 NA7 and SIVmac 239 Nef proteins that mediate the constitutively strong interactions with AP-2 and found that these proteins bind AP-2 by altogether different interaction surfaces. Specifically, we showed that SIV Nef contains two *c*onstitutive high-affinity *A*P-2 *i*nteraction *d*eterminants (CAIDs) located in the amino-terminal region of the molecule. Each CAID can inde-

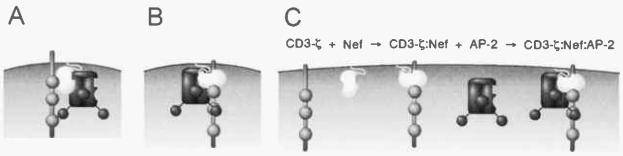

FIGURE 1 Models for the cooperative assembly of heterotrimeric complexes containing SIV Nef, AP-2, and CD3-ζ subunit of the TCR:CD3 as cargo. (*A,B*) "Connector" and "cooperative binding" models for the recruitment of cell surface receptors by Nef to AP-2 clathrin adaptor. (*A*) A "connector" model where Nef contacts AP-2 and the target site in the membrane receptor such as CD4, CD28, or CD3-ζ by independent interactions. Data from the studies on the mechanism of CD3-ζ recruitment to the AP-2 by SIV mac239 Nef provide strong support to an alternative model called a "cooperative binding" model, where Nef and the target receptor bind cooperatively AP-2 (*B*). (*C*) A likely model for the induction of TCR:CD3 endocytosis by Nef. SIV Nef and the CD3-ζ cytoplasmic domain bind with high affinity to form a stable 239.Nef:CD3-ζ complex. Complexed together, 239.Nef and CD3-ζ produce a high-affinity binding site for AP-2. This complex is competent to recruit CD3-ζ to clathrin-coated pits.

pendently target heterologous proteins to AP-2 in vivo and promote association with AP-2 clathrin adaptors in vitro. Notably, we found that the two CAIDs had distinct functional roles. The N-distal CAID associates with the AP-2 clathrin adaptor via a novel interaction surface that lacks dileucine- or tyrosine-based sorting motifs and is required for the induction of CD4 and CD28 endocytosis. In contrast, the N-proximal CAID, which contains tyrosine-based sorting motifs and can contact the μ-subunit of the AP-2, is neither required nor sufficient in vivo for any known function of Nef that involves AP-2.

SELECTIVITY OF SIV NEF FOR TCR:CD3 ENDOCYTOSIS IS GENERATED BY COOPERATIVE BINDING OF THE NEF:CD3-ζ COMPLEX WITH AP-2

Our subsequent studies of the down-regulation of TCR:CD3 cell surface expression by SIV Nef suggested how the selectivity of Nef for cargo is generated. Since previous studies demonstrated that the core domain of Nef binds the cytoplasmic domain of the CD3-ζ subunit of the TCR:CD3 complex with high affinity, we initially thought that Nef may act as a molecular linker to bridge CD3-ζ with AP-2. However, when testing this model, we found that neither of the strong AP-2-binding elements in Nef were required for Nef to induce endocytosis of molecules containing the CD3-ζ cytoplasmic domain, nor to recruit them to AP-2 in vivo. The mechanistic explanation of this observation was provided by subsequent in vitro biochemical studies in which we demonstrated that a recombinant SIV Nef and CD3-ζ in a complex bind AP-2 cooperatively. This cooperative binding is a more likely model to explain the recruitment by Nef of target receptors for endocytosis than a simple model in which Nef acts as a simple "connector" that bridges the target receptor and AP-2 at the cell surface via independent interactions. We think that the cooperative binding of Nef, target receptor, and AP-2 is a general mechanism that confers the specificity of Nef toward selected cargo. We are now testing this possibility through similar biochemical studies of SIV Nef interactions with CD4/CD28 and AP-2. These data have two general implications for the mechanisms that Nef uses to recruit target receptors to AP-2. First, our observation that different surfaces of the Nef molecule mediate the selective endocytosis of CD4/CD28 and the TCR:CD3 complex indicates that for each target receptor molecule, a distinct trimeric complex is formed composed of Nef, the receptor, and AP-2, in which Nef is oriented in a distinct and specific way with respect to AP-2. Second, multiple modular cooperative interactions of Nef and the target receptor with AP-2 determine the selectivity of Nef for its target receptor.

BIOCHEMICAL PURIFICATION AND IDENTIFICATION OF PROTEINS ASSOCIATING WITH NEF IN T LYMPHOCYTES

Previous studies have revealed that Nef acts through associations with large protein complexes. One such

example is the complex(es) that mediates the effects of Nef on the protein-sorting machinery at the plasma membrane and contains Nef and the AP-2 clathrin adaptor described above. Another example is the poorly characterized large complex(es) containing Nef and PAK2 kinase. Some of our goals have been to identify the targets of Nef in such complexes as well as additional downstream effectors of Nef that have escaped detection by previous studies. The unbiased approaches that have been used so far to identify immediate downstream effectors have relied heavily on the ability to reconstruct the relevant interactions in heterologous or in in vitro systems, including the yeast two-hybrid interaction screen (YTH) and chromatography of T-cell extracts on Nef-affinity columns. These approaches have correctly identified a subset of Nef ligands, but have been used exhaustively, and therefore, additional Nef-interacting polypeptides are not likely to be revealed by such assays. Therefore, to bypass the limitations of previously used methods, we developed an immunoaffinity purification protocol to isolate Nef and its associated protein complexes from T-cell lines stably expressing Nef.

With this protocol, we detected a series of polypeptides with apparent molecular masses ranging from 20 kD to more than 250 kD that copurified with HIV-1 and SIV Nef proteins from a model human T-cell line. These additional polypeptides can be divided into three sets: (1) those that copurify with both HIV-1 and SIV Nef, (2) those that associate predominantly or exclusively with HIV-1 Nef, or (3) SIV Nef. That a set of common polypeptides copurifies with both HIV-1 and SIV Nef is strong evidence that they are biologically relevant. The detection of polypeptides that associate with Nef in a strain- or allele-specific manner is also expected, since it is well established that HIV-1 and SIV Nef show different binding affinities to several proteins such as CD3-ζ or the Hck SH3 domain and display different functions as well. We recently increased the scale of the purification of Nef-associated proteins and started to determine their sequences by mass spectroscopic analysis in collaboration with Michael Myers here at the CSHL Protein Chemistry Shared Resource. This led to the identification of several common polypeptides associated with both 7.AH Nef and 239.AH Nef as N-myristoyl transferase-1 (NMT-1, ~60 kD), tubulin (~50 kD), and Filamin (~250 kD). These polypeptides were identified in more than one independent purification/sequencing experiment. Additionally, a component of the 60-kD polypeptide band gave a statistically significant hit to the product of the KIAA0942 gene. KIAA0942 encodes a guanine nucleotide exchange factor (GEF) most closely related to EFA6. EFA6 regulates ARF6, a small G-protein that regulates membrane traffic in the endocytotic pathway, making KIAA0942 a potentially important target of Nef. We are continuing these studies with the goal of identifying critical downstream effectors for the additional Nef functions.

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TRANSCRIPTION AND PROTEOLYSIS

W.P. Tansey E. Ejkova, M.-J. Gething, A. Herbst, S.Y. Kim, E. Kurten, M. Muratani, S. Salghetti, K. Tworkowski

Gene transcription and ubiquitin (Ub)-mediated proteolysis are two processes that have seemingly nothing in common: Transcription is the first step in the life of any protein, and proteolysis the last. Despite the disparate nature of these processes, a growing body of evidence suggests that components of the Ub-proteasome system are intimately involved in the regulation of gene expression. Research in our laboratory is geared at understanding how these two processes intersect, and the consequences of this intersection for cellular growth control.

TRANSCRIPTION FACTOR DESTRUCTION BY THE UBIQUITIN-PROTEASOME PATHWAY

Many transcription factors, particularly those involved in the control of cell growth, are unstable proteins that are destroyed by Ub-mediated proteolysis, a process in which the covalent attachment of Ub to target proteins signals their destruction by the 26S proteasome. Ub-mediated proteolysis is a highly specific process. For a protein to be destroyed by this pathway, it must contain a sequence element—termed a degron—that signals its destruction. Degrons function by interacting with a class of proteins known as Ub-ligases that ultimately catalyze the covalent transfer of Ub to lysine residues somewhere on the target protein. Previous work in our laboratory suggested that the destruction of transcription factors might be linked to their ability to activate transcription, when we discovered that degrons overlap with transcriptional activation domains (TADs) in most unstable transcription factors. We subsequently showed that this overlap is intimate and, in some cases, essential for transcription. For example, we found that activators bearing the prototypical VP16 activation domain cannot activate transcription in the yeast *Saccharomyces cerevisiae* unless they are ubiquitylated. This requirement for ubiquitylation suggests not only that activators engage the Ub-proteasome pathway as part of their *modus operandi*, but also that ubiquitylation functions to "license" transcription factors, by inexorably linking their activity to their destruction by the proteasome. The role of ubiquitylation in activator functioning, together with recent work from other laboratories demonstrating that histone ubiquitylation controls epigenetic gene silencing and that the proteasome itself is required for transcriptional elongation, suggests a multifaceted role for the Ub-proteasome system in transcriptional control that we are anxious to explore.

THE UBIQUITIN-LIGASE SKP2 REGULATES MYC STABILITY AND ACTIVITY

As a paradigm for transcription factor destruction, we study Myc. Myc is a basic helix-loop-helix transcription factor that features prominently in the regulation of cell proliferation and in cancer. Myc is also a highly unstable protein that is destroyed by Ub-mediated proteolysis. The link between Myc and mammalian cell growth control provides an excellent opportunity for us to understand the consequences and significance of transcription factor regulation by the ubiquitin system.

To understand how the Ub-mediated proteolysis of Myc regulates its activity, we sought to identify a Ub-ligase that targets Myc for destruction. We established a heterologous yeast assay to measure Myc turnover and used this assay to search for human proteins that could direct appropriate, activation domain-directed, destruction of Myc in vivo. This analysis identified a single Ub-ligase, Skp2, that was capable of directing Myc turnover in the yeast setting. Subsequent experiments revealed that Skp2 can also regulate Myc ubiquitylation and stability in human cells; Skp2 associates with the Myc activation domain and can promote Myc ubiquitylation in vivo, and dominant-negative forms of Skp2—or Skp2 knockdown via silencing RNAs (siRNAs)—stabilize the Myc protein. The implication of Skp2 as a Ub-ligase for Myc is intriguing because Skp2 is itself an oncoprotein with potent growth-promoting properties. A

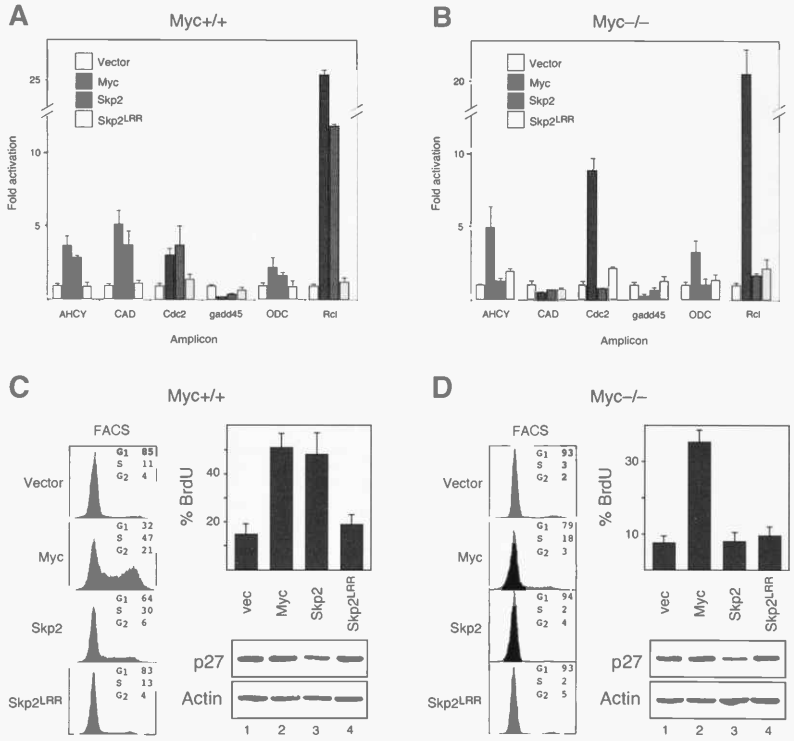

FIGURE 1 Skp2 activates Myc target genes and S-phase entry in a Myc-dependent manner. (*A*) Activation of Myc target genes in wild-type Rat1 cells. Rat1 cells expressing the indicated ER-Myc and ER-Skp2 proteins were driven to quiescence by serum deprivation and density-arrest. ER-fusion proteins were then induced by addition of 4-hydroxytamoxifen and cellular RNA-collected. Transcript levels from six Myc target genes ("amplicons") were determined by quantitative reverse transcription, real-time PCR (Q-PCR). Signals are normalized to actin. (*B*) Activation of Myc target genes in Myc-null Rat1 cells. The experiment was performed as in *A*, except that *myc*-null Rat1 cells were used. (*C*) Skp2 down-regulates p27 and induces S phase in wild-type Rat1 cells. ER-Myc and ER-Skp2 fusion proteins were activated in quiescent Rat1 cells as described in *A*. S-phase entry was measured by FACS or BrdU staining, as indicated. Steady-state p27 and actin (control) levels were determined by immunoblotting. (*D*) Skp2 down-regulates p27 but does not induce S phase in Myc-null Rat1 cells. The experiment was performed as in *C*, except that *myc*-null Rat1 cells were used.

number of targets of Skp2 have been identified, including the cyclin-dependent kinase (CDK) inhibitor p27 and the human replication protein Orc1 (which we showed in collaboration with Bruce Stillman's laboratory here at CSHL). Conventional views would suggest that Skp2 promotes cell growth by down-regulating p27, which in turn leads to liberation of active cyclin-CDK complexes, and progression through S phase. In light of the activator licensing model, however, we hypothesized that Skp2 might function, in part, as a transcriptional co-activator for the Myc protein, coupling Myc's activity to its destruction by the proteasome.

To test this model, we engineered Rat1 fibroblasts to express an inducible form of Skp2, in which we fused Skp2 to the hormone-binding domain of the estrogen receptor (ER). We made these cells quiescent by serum deprivation, activated ER fusions, and analyzed transcript levels from six Myc target genes. This analysis (Fig. 1A) revealed that expression of AHCY, CAD, Cdc2, ODC, and Rcl was stimulated by activation of full-length Skp2 (third bar from left), to a level comparable to that seen by Myc (second bar from left). In contrast, a mutant of Skp2 lacking Ub-ligase function ($Skp2^{LRR}$) failed to activate Myc target gene expression (fourth bar from left), indicating that the ubiquitylating activity of Skp2 is required for this transcriptional effect. To determine whether the ability of Skp2 to activate Myc target genes is dependent on Myc, we performed an analogous experiment in Rat1 fibroblasts in which both copies of the *myc* gene were deleted by homologous recombination. In these cells, Skp2 failed to activate the expression of any genes tested (Fig. 1B), although these genes responded robustly to activation of ectopic ER-Myc. These data reveal that the Ub-ligase Skp2 stimulates Myc target genes in a Myc-dependent manner, consistent with the notion that Skp2 is an upstream activator of the Myc protein.

To test the significance of the Skp2-Myc interaction, we asked whether the ability of Skp2 to signal S-phase entry in Rat1 cells is dependent on Myc. In wild-type, quiescent, Rat1 cells, ER-Skp2 down-regulated p27 levels, and stimulated S-phase entry, as judged from both FACS analysis and bromodeoxyuracil (BrdU) incorporation (Fig. 1C). In similarly treated *myc*-null cells, however, ER-Skp2 down-regulated p27 as expected (Fig. 1D), but the cells failed to enter S phase. These results thus demonstrate that down-regulation of p27 by Skp2 is not sufficient to permit S-phase entry and reveal that Myc is an essential downstream effector of the Skp2 protein. The finding that Skp2 activates Myc's transcriptional activities, and is dependent on Myc for some its functions, suggests that the control of activator function by ubiquitylation is important in the control of cell proliferation in normal and cancer cells.

MYC BOX III IS A HIGHLY CONSERVED ELEMENT IMPORTANT FOR MYC DESTRUCTION AND FUNCTION

The connection between the activity of Myc and its destruction provides the opportunity for us to learn new aspects of how the Myc protein functions. For example, during our analysis of Myc turnover, we identified a second element—located within the interior of the Myc protein—that is important for its destruction. At the core of this region is a highly conserved, but little studied, sequence element called Myc box III (MbIII), which is highly conserved among the c-, N-, and S-Myc family members. Despite its conservation, however, very little is known about how MbIII contributes to Myc activity. We had previously shown that MbIII—together with the two other conserved Myc boxes (MbI and MbII, located within the Myc TAD)—is important for Myc destruction. We have now asked whether MbIII is also important for Myc function.

For this purpose, we generated an inducible (ER-fusion) form of Myc in which we deleted the MbIII element (ΔMbIII). For comparison, we also generated Myc mutants with deletions in MbI (ΔMbI) and MbII (ΔMbII). We then measured the activity of these mutants in various assays of Myc function, including the ability to induce S phase in quiescent fibroblasts, transcriptional regulation, and cellular transformation. We found that deletion of MbIII did not affect the ability of Myc to induce S-phase entry, demonstrating that the ΔMbIII protein was correctly folded and localized. Interestingly, however, we did find that deletion of MbIII severely attenuated the ability of Myc to transform Rat1a fibroblasts (Fig. 2A). Moreover, analysis of the transcriptional capabilities of the ΔMbIII mutant revealed that transcriptional activation by Myc was reduced, but not eliminated, by deletion of MbIII, whereas transcriptional repression of the *gadd45*, *Gas1*, and *p15* genes was completely blocked (Fig. 2B). Thus, MbIII has an important role in cellular transformation by Myc, presumably through its contribution to Myc's transcriptional activities. The charac-

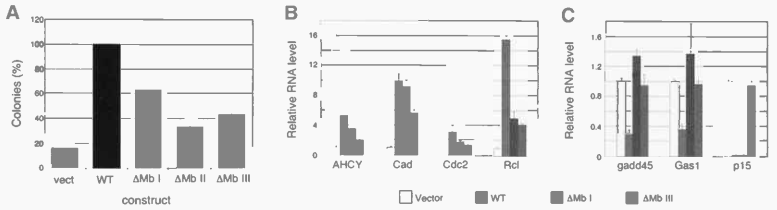

FIGURE 2 MbIII is important for cellular transformation and transcriptional regulation by Myc. (*A*) MbIII is important for transformation of Rat1a cells to anchorage-independent cell growth. Rat1a cells, expressing the indicated ER-Myc fusion proteins, were treated with 4-hydroxytamoxifen and assayed for colony formation in soft agar. (*B*) MbIII is important for activation of transcription by Myc. Quiescent Rat1 cells, expressing the indicated ER-Myc fusion proteins, were treated with 4-hydroxytamoxifen and RNA-collected, and transcript levels from Myc target genes were determined by Q-PCR. (*C*) MbIII is essential for repression of transcription by Myc. The experiment was performed as in *B*, except Myc-repressed genes were analyzed.

terization of MbIII is exciting because it reveals a previously unknown aspect of Myc function and because it provides us with a new opportunity to delve deeper into the biology of the Myc protein.

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STRUCTURAL BIOLOGY

R.-M. Xu Y. Huang J. Min
H.-C. Hsu Z. Li
H. Shi

We continue to focus on structural studies of proteins involved in mRNA processing and proteins that are important for higher-order chromatin structure. During 2002, we determined the three-dimensional structures of (1) the *Drosophila* Mago nashi–Y14 complex, two proteins that are part of the exon-exon junction complex (EJC) produced by pre-mRNA splicing; (2) human UAP56, a DECD RNA helicase/ATPase, which is a splicing factor and an EJC component; (3) the catalytic domain of histone lysine methyltransferase Clr4, which is a Su(var)39-like SET domain protein that methylates Lys-9 of histone H3; and (4) the catalytic domain of human Dot1, hDOT1L, which is an evolutionarily conserved non-SET domain histone lysine methyltransferase methylating Lys-79 of histone H3 located in the globular domain of the nucleosome core particle. These projects are described in more detail below.

PRE-mRNA SPLICING

Splicing of pre-mRNA is essential for generating mature mRNA and is also important for subsequent mRNA export and quality control. The splicing history is imprinted on spliced mRNA through the deposition of a splicing-dependent multiprotein complex, the exon junction complex (EJC), at approximately 20 nucleotides upstream of exon-exon junctions. The EJC is a dynamic structure containing proteins functioning in the nuclear export and nonsense-mediated decay of spliced mRNAs. To date, at least eight EJC proteins have been identified, including Y14, Mago, DEK, RNPS1, SRm160, Upf3, UAP56, and REF/Aly. These proteins have distinct and sometimes multiple functions in various aspects of mRNA metabolism such as splicing, nuclear export, and mRNA quality control.

Mago nashi (Mago) and Y14 are core components of the EJC and they form a stable heterodimer that strongly associates with spliced mRNA. We have solved a 1.85-Å resolution structure of the *Drosophila* Mago–Y14 complex. Surprisingly, the structure shows that the canonical RNA-binding surface of the Y14 RNA recognition motif (RRM) is involved in extensive protein-protein interactions with Mago. The structure also reveals that Mago has a protein fold related to the structure of the peptide-binding domain of the class I major histocompatibility complex (MHC) protein. These findings provide important insights into the molecular mechanisms of RNA binding and protein-protein interactions of the evolutionarily conserved EJC components.

We have also determined the crystal structure of another EJC protein, UAP56, which is also a splicing factor containing a DECD ATPase/helicase sequence motif. We are currently pursuing in-depth characterizations of UAP56 based on the structural information.

HISTONE METHYLTRANSFERASES

Posttranslational modifications of core histones have important roles in the establishment and maintenance of higher-order chromatin structures. The unstructured tails of core histones are subjected to various forms of covalent modifications. Histone methylation has emerged as a major form of histone modification in recent years. In particular, a large family of SET domain-containing histone methyltransferases (HMTases) has been identified. SET domain proteins methylate various amino-terminal lysine residues of histones H3 and H4, and these modifications have been associated with diverse biological processes ranging from transcriptional regulation to the faithful transmission of chromosomes during cell division.

Clr4, A SET Domain Histone Lysine Methyltransferase. The SET domain-containing Clr4 protein, a close relative of Su(var)3-9 proteins in higher eukaryotes, specifically methylates Lys-9 of histone H3 and is essential for silencing in *Schizosaccharomyces pombe*. In collaboration with Shiv Grewal here at CSHL and Xiaodong Cheng at Emory University, we have solved a 2.3-Å resolution structure of the catalytic domain of Clr4 (Fig. 1). The structure reveals a distinct overall fold rich in β-strands, an

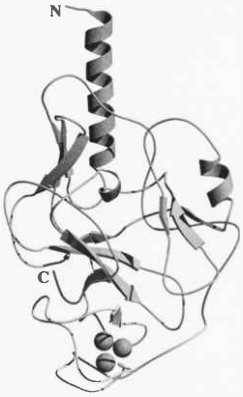

FIGURE 1 Crystal structure of the catalytic domain of Clr4.

active site consisting of an *S*-adenosyl-L-methionine (SAM)-binding pocket, and a connected groove that can accommodate the binding of the histone H3 tail. The pre-SET motif contains a triangular zinc cluster coordinated by nine cysteines distant from the active site, while the post-SET region is largely flexible but proximal to the active site. The structure provides important insights into the architecture of SET domain histone methyltransferases and establishes a paradigm for further characterization of the Clr4 family of epigenetic regulators.

Human DOT1L, a Non-SET Domain Nucleosomal Histone Lysine Methyltransferase. A recent addition to the histone modification repertoire is histone H3 Lys-79 methylation. In contrast to all other known methylation sites, Lys-79 occupies a position in the ordered core domain of histone H3 and resides in a short turn connecting the first and second helices of the conserved histone fold. Dot1 is an evolutionarily conserved protein that was originally identified in *Saccharomyces cerevisiae* as a *d*isruptor *of t*elomeric silencing. It also functions at the pachytene checkpoint during the meiotic cell cycle. Dot1 proteins do not contain a SET domain, but they possess certain characteristic SAM-binding motifs found in classical SAM-dependent methyltransferases. Surprisingly, yeast Dot1 and its human counterpart hDOT1L exhibit intrinsic HMTase activity toward Lys-79 of histone H3. This unexpected finding identifies the Dot1 family of proteins as the first non-SET domain containing histone lysine methyltransferases.

In collaboration with Yi Zhang at the University of North Carolina, Chapel Hill, we have solved a 2.5-Å resolution structure of the catalytic domain of hDOT1L complexed with SAM (Fig. 2). The structure reveals a unique organization of a mainly α-helical amino-terminal domain and a central open α/β structure. It also reveals an active site consisting of a SAM-binding pocket and a potential lysine-binding channel. Structure-guided biochemical analyses show that a flexible, positively charged region at the carboxyl terminus of the catalytic domain is critical for nucleosome binding and enzymatic activity. The structural basis for nucleosome specificity of hDOT1L is

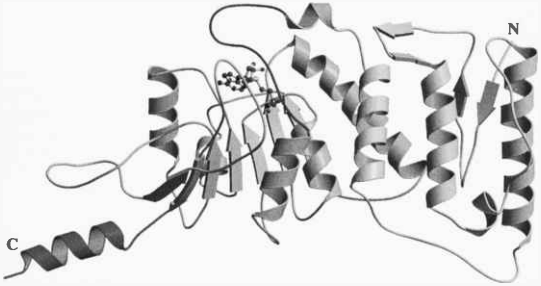

FIGURE 2 Crystal structure of the catalytic domain of hDOT1L.

revealed from molecular modeling of nucleosome-hDOT1L interactions. These analyses provide mechanistic insights into the catalytic mechanism and nucleosomal specificity of Dot1 proteins.

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Hang Shi

R.-M. Xu

Zhizhong Li

CANCER: GENETICS

Cancer is caused by gene mutations. Mutations come in many forms: a change in the DNA sequence of a gene, the movement of a chromosome segment to an abnormal location, the complete or partial removal of a gene (deletion), or the addition of more than one copy of a gene (amplification). Michael Wigler and Rob Lucito have developed a method for scanning the entire human genome to detect deletions and amplifications. By pinpointing areas where amplifications or deletions have occurred in DNA isolated from tumor biopsies, the researchers can identify genes implicated in cancer. Work by Masaaki Hamaguchi, Wigler, and Lucito has identified several such genes in breast and ovarian cancer. These genes are prime targets for the development of new cancer therapies.

Greg Hannon and his colleagues have pioneered the study of a fascinating process called RNA interference (RNAi) as well as the application of RNAi to cancer research. RNAi leads to the silencing of gene expression, in part through the binding of small RNA molecules corresponding to particular messenger RNAs and the subsequent enzymatic destruction of the messenger RNAs (which blocks protein production). Hannon and his colleagues have discovered several of the molecules and mechanisms that carry out RNAi. Recently, they showed that they can use RNAi to permanently silence the expression of virtually any gene in cultured cells, in living animals, or in organs reconstituted from stem cells (the latter in collaboration with Michael Hemann of Scott Lowe's group). These findings have opened the door for them to use RNAi to discover new targets for cancer therapy, or to use RNAi itself as a therapy.

Approximately 1–2% of newborns have symptoms of sporadic genetic disease, or genetic disease that neither parent suffered from but rather arises de novo as a result of a genetic change in a parent's sperm or egg cell DNA. Eli Hatchwell's lab is developing methods for detecting subtle changes in DNA that cause such sporadic human genetic disease. In one study, the researchers have managed to detect a tiny deletion in human chromosome 8, which they suspect is associated with Kabuki syndrome (characterized by learning defects, short stature, and very high rates of congenital heart disease). The methods developed by Hatchwell's group should be useful for many applications, including cancer research.

Yuri Lazebnik is exploring ways to use the programmed cell death machinery to improve the efficiency and selectivity with which cancer treatments kill tumor cells. Paradoxically, although the hallmark of oncogenes is that they are associated with causing cancer, oncogenes also sensitize cancer cells to the lethal effects of chemotherapy drugs by inducing programmed cell death. This observation means that oncogenes might provide a way to induce the programmed cell death machinery specifically in cancer cells. During the last year, Lazebnik's group has shown that chemotherapy activates an enzyme called caspase-2, which in turn activates other caspases, leading to cell death. These findings reveal crucial information about how programmed cell death works, how it is influenced by oncogenes, and how it might be used against cancer.

Alea Mills has developed a "chromosome engineering" system for creating mouse models of human disease. This approach allows her lab to generate mice with precise chromosome rearrangements that mimic alterations found in human diseases such as cancer. The Mills lab is also studying the role of a particular gene called *p63* in stem cell maintenance, cancer, and aging.

Through a process called angiogenesis, the *Id1* and *Id3* genes are involved in forming blood vessels that supply growing tumors with the nutrients they need. Therefore, *Id* genes and their targets are attractive candidates for anti-angiogenic cancer therapies designed to kill tumors by cutting off their blood supply. Vivek Mittal's lab is using DNA microarray technology, RNA interference, and mouse tumor models to identify *Id* targets. They recently identified four such targets and have validated them as candidates for anti-angiogenic cancer therapies. Mittal's group has also created a method for rapidly identifying the pieces of double-stranded RNA that are most effective at silencing any particular gene through RNA interference.

TUMOR SUPPRESSOR GENES INVOLVED IN BREAST CANCER DEVELOPMENT

M. Hamaguchi M. Kanis, N. Kobayashi, J. Meth, D. Roberts, R. von Roeschlaub, R. Shudo, C. von Klitzing

Our lab is interested in the discovery and characterization of cancer-related genes, especially tumor suppressor genes that are ablated in cancer cells. The recent history of tumor suppressor gene investigation has been revolutionary. The identification of these genes and subsequent studies into their biological functions have provided vast new insights into the development of cancer. A great deal is now known about the relationship between cancer development and cellular functions.

In the case of breast cancer, the isolation of tumor suppressor genes *BRCA1* and *BRCA2* has had an impact on several aspects of cancer treatments as well as cancer research. *BRCA1* and *BRCA2* genes can be utilized for identification of individuals at high risk of developing breast cancers, which has been crucial in the development of risk assessment. There are also differences in clinical outcomes between breast cancers with *BRCA1* mutations and those with *BRCA2* mutations. These findings suggest that specific types of breast cancers require specific screening methods and therapeutic approaches. However, tumor suppressor genes responsible for sporadic breast cancer remain to be discovered.

We have isolated a previously uncharacterized gene, *DBC2* (*d*eleted in *b*reast *c*ancer 2) that is likely to be a tumor suppressor gene for sporadic breast cancer. It is homozygously deleted in 3.5% (7/200) of breast tumor samples and silenced in 55% (12/22) of breast cancer cell lines. Activation of *DBC2* in a breast cancer cell line resulted in growth arrest of the cells, whereas naturally occurring *DBC2* mutants did not suppress tumor growth.

Functional Analysis of *DBC2*

M. Hamaguchi, J. Meth, D. Roberts, C. von Klitzing
[in collaboration with M. Wigler, Cold Spring Harbor Laboratory, and S. Powers, Tularik Inc.]

Knowledge about this new tumor suppressor *DBC2* will definitely facilitate clarifying the mechanisms of breast cancer development. To study *DBC2* functions, we utilized an inducible gene expression system that enables us to switch on/off a gene of interest as we like. This system utilizes an insect hormone, ecdysone, or its homolog, Muristerone A. Since these chemicals do not normally exist in mammalian cells, they will not affect most mammalian cells by themselves. To examine the function of interesting genes, they are cloned into a specially designed vector containing ecdysone-responsive elements so that the genes will be activated only when the hormone is administered. Wild-type (without mutations) and mutated *DBC2* genes were independently induced in breast cancer cells and the consequences were monitored. When wild-type *DBC2* is induced, the breast cancer cells stopped growing, indicating that *DBC2* can suppress the growth of tumor cells. In contrast, induction of mutated *DBC2* did not hinder the growth of tumor cells (Fig. 1). Since the mutants discovered in clinical specimens (naturally occurring) were used in this study, it is likely that breast cancer cells exploit

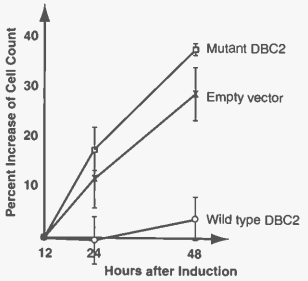

FIGURE 1 Growth curve of tumor cells. Growth curves of a breast cancer cell line, T-47D, were drawn after induction of various genes. The ordinate is the percent increase of cell count. The abscissa is hours after induction. After induction of empty vector (x) and a *DBC2* mutant (*squares*), the tumor cells kept growing. In contrast, the tumor cells stopped growing after induction of wild-type *DBC2* (*circles*).

inactivation of *DBC2*. These findings warrant important research projects to answer intriguing questions. Which types of breast cancers are sensitive to *DBC2* tumor suppressor function? Besides breast cancer, which cancer takes advantage of *DBC2* inactivation? How does *DBC2* suppress the growth of tumor cells? We are continuing our effort to answer these questions.

One of our approaches is a microarray analysis. The microarray we utilize is a microscope slide carrying 46,000 spots in 1-inch by 2-inch area. Each spot contains a DNA probe representing a gene. When a color-labcled sample is incubated with the microarray, DNA fragments in the sample seek matching sequences with the probe. As a result, the color intensity of a probc corresponds to the amount of the matching sequcnccs in the samplc. When cDNA from a specimen is used, the color intensity indicates the expression level of the gene represented by the probe. This is particularly useful when we want to isolate genes that are expressed differently in the two spccimens. If samples from two specimens are labeled with different colors and analyzed by the microarray, the intensity ratio of most genes will be close to one. However, there will be genes with high- or low-intensity ratios sincc gene expression patterns are different among cells. Using this system, we analyzed breast cancer cells with and without *DBC2* induction. After induction of wild-typc *DBC2*, approximately 100 genes demonstrated increased expression by more than threefold (Fig. 2). We are currently studying how *DBC2* regulates the expression of these genes (directly or indirectly) and what roles *DBC2* plays in the biological pathways involving these genes.

Expression of *DBC2*

M. Hamaguchi, J. Meth [in collaboration with
D. Broek, University of Southern California]

Tumor suppressor genes are inactivated by transcriptional suppression in some cascs. In fact, *DBC2* expression is extinguished in approximately half of breast and lung tumors, whereas its expression is detected in other types of cancers. Modification of the promoter region has been shown to cause transcriptional inactivation. The most common mechanism for such modification is methylation. Therefore, a number of cancer cell lines derived from breast, lung, and

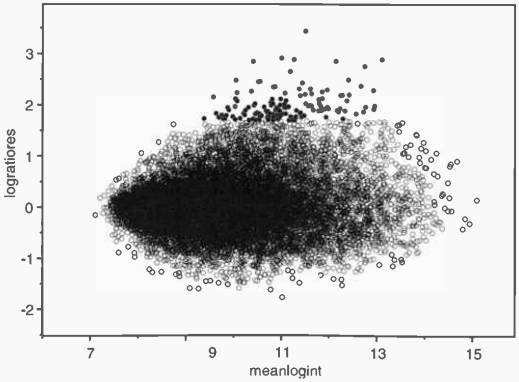

FIGURE 2 Comparison of expression patterns of the cells with and without *DBC2* induction. The ordinate is "log ratio of signal intensity," which represents changes of gene expression level. The base of log is two (e.g., value two indicates a fourfold increase and three indicates an eightfold increase). The abscissa is "log of mean intensity," which represents the abundance of the gene in the cell. For example, highly expressed genes are displayed at the right side of the chart. Solid circles represent genes whose expression is increased greatly (more than threefold) by *DBC2* induction.

other cancers were screened for methylation status of the *DBC2* promoter. In several breast and lung cancer cell lines that do not express *DBC2*, the promoter region was found to be hypermethylated. Transcriptional inactivation by hypermethylation can be reversed in many cases when methylation is chemically blocked. A commonly used chemical for this purpose is 5-aza-2′-deoxycytidine (5-Aza-C). We cultured these cells with 5-Aza-C and demonstrated that *DBC2* expression was restored. Our findings suggest that methylation of the *DBC2* promoter is the main mechanisms for silencing.

Structure of *DBC2*

M. Hamaguchi, N. Kobayashi, D. Roberts, R. Shudo,
[in collaboration with L. Van Aelst, Cold Spring Harbor Laboratory]

Computational analysis revealed that *DBC2* encodes a protein with significant RAS homology and contains known protein-protein interaction domains (BTB). Presence of RAS domain implies *DBC2*'s role in signal transduction. However, its novel combination of structural domains, its lack of a membrane anchor sequence at the carboxyl terminus, and its divergence from the RAS proteins with known functions suggest that its function may be distinct from other members of the RAS superfamily.

Since *DBC2* is likely to interact with other proteins through the BTB domains, it is critical to isolate these proteins. We utilized a yeast two-hybrid system that allows identification of interacting proteins in yeast cells. In this system, a collection of yeast cells containing exogenous proteins are prepared. After transfection of *DBC2* into the yeast cells, they are cultured under special environments where interaction of an exogenous protein with *DBC2* is essential for yeast survival. Millions of yeast cells containing different proteins were examined. Several proteins were found to interact with *DBC2* in yeast cells. These candidates are currently being verified for interaction in mammalian cells.

Generation of *DBC2* Knockout Mice

M. Hamaguchi, M. Kanis, J. Meth, R. von Roeschlaub
[in collaboration with the Genomic Targeting and Transgenomic Mouse Shared Resouce, and M. Zhang, Cold Spring Harbor Laboratory]

Analysis of a mouse model has advantages. First, more information has been accumulated from mouse genetics than any other vertebrate. Second, gene functions can be studied in vivo. Analysis of tumor suppressor genes has often been facilitated by studies of tumor-suppressor-deficient mice.

We genetically engineered mouse embryonic stem (ES) cells so that they had a disrupted *DBC2* allele. These ES cells were used to produce chimeric mice that are partially originated from the ES cells. The chimeric mice were examined, and successful introduction of the engineered *DBC2* allele was confirmed. We will continue our efforts to establish a *DBC2*-deficient mouse model from offspring of these mice hoping that it will facilitate understanding the roles of *DBC2* in cancer development.

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RNA INTERFERENCE: MECHANISMS AND APPLICATIONS

G. Hannon S. Aruleba, E. Bernstein, M. Carmell, A. Caudy, D. Conklin, A. Denli, J. Du, A. Hotta, G. Katari, J.D. Liu, H. Mizuno, P.J. Paddison, K. Scobie, Y.R. Seger, J.M. Silva, I. Sujka

The interests of my laboratory remain twofold. First, we are striving to understand the biochemical mechanism and biological function of a sequence-specific silencing phenomenon, known as RNA interference (RNAi). Second, we are exploiting RNAi as an experimental tool in a large-scale effort to understand gene function.

During the last two years, we have made significant progress toward understanding how double-stranded RNA (dsRNA) can trigger the silencing of homologous genes at the posttranscriptional level. In the past year, we have extended our understanding by the identification of new subunits of the complex, including a *Drosophila* homolog of a human disease gene. As we gain a deeper understanding of the players in this silencing process, we gain opportunities to use genetics to address the biological functions of RNAi. Toward this end, we have begun the process of creating mutant mice lacking known RNAi genes. The first of these to be completed is the Dicer mutant mouse, which is beginning to reveal key roles for the RNAi machinery in regulating mammalian development. Finally, we have continued our efforts to determine how dsRNA can induce gene silencing in so many different ways, ranging from mRNA degradation to translational suppression to chromatin remodeling. Efforts to address the particular aspects of this process are moving forward on several fronts.

The past year has also seen a revolution in the application of RNAi to mammalian systems. With our development of approaches that permit stable silencing of genes by RNAi in mammalian cells, we have begun to move toward the creation of a genome-wide library of RNAi-inducing vectors. Initially, we will use this in a search for novel anticancer targets through genetic screens. However, this project will also create a vast public resource that can be applied to an enormous variety of biological questions. As genetic

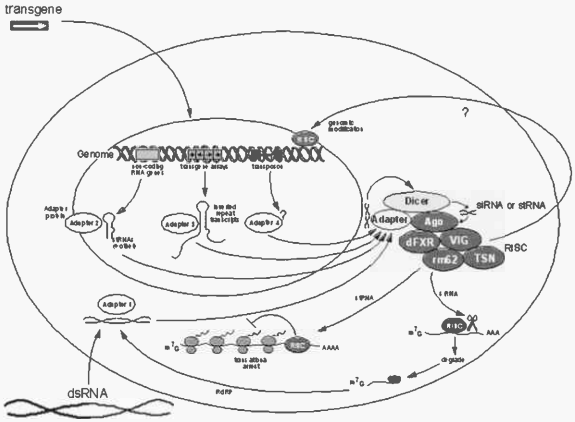

FIGURE 1 The mechanistic model for RNAi that we have developed over the past few years.

approaches in cell culture begin to yield substantive information, it is critical to be able to extend our analyses into animal systems. During the past year, we have collaborated with a number of different investigators, including Mark Kay at Stanford, Tom Rosenquist at SUNY Stony Brook, and Scott Lowe, here at CSHL, to probe the ability of RNAi to manipulate gene function in vivo. These studies have demonstrated not only that RNAi can be used to silence gene expression in mice, but also that this silencing can be used to create biological phenotypes that permit a more detailed study of tumorigenesis. When coupled with our continuing studies of the transformation of human cells in vitro and of key elements of cell cycle control, we hope to exploit the interdisciplinary nature of the programs in our lab to identify new approaches to cancer therapy.

This year, we were joined by Ikuko Hotta as a graduate student from the MCB program at SUNY Stony Brook and by Kim Scobie and Hana Mizuno, who began her CSHL career as a high school student in Yuri Lazebnik's lab. Ji Dong Liu arrived as a postdoctoral fellow from Yue Xiong's lab (University of North Carolina, Chapel Hill). We were also visited by Sarah Whitcombe as part of our undergraduate research program and a student from Columbia University. Scott Hammond became the first person to depart from the lab for a faculty position at the University of North Carolina, Chapel Hill. Patrick Paddison and Michelle Carmell both received predoctoral fellowships from the U.S. Army Breast Cancer Research Program, joining Yvette Seger, our previous recipient of this fellowship, and Jian Du received a postdoctoral fellowship from this same program. Amy Caudy was honored by the receipt of the Harold Weintraub Graduate Student Award in recognition of her outstanding thesis work.

The Biochemical Mechanism of RNA, Connections to Human Disease

A. Caudy

RNAi silences genes in response to a specific trigger. Our work has shown that the triggering dsRNA initiates formation of a nuclease complex that specifically degrades homologous mRNAs in the cell. This year, Scott Hammond and I discovered three additional components of this nuclease complex: Tudor-SN, *Drosophila* Fragile X mental retardation protein (dFMR), and Vasa intronic gene (VIG). Tudor-SN is the first component identified in the RNA-induced silencing complex (RISC) that has a known nuclease activity. Although Tudor-SN was discovered in flies, our early data suggest it may have a similar role in mammals.

Our discovery of the dFMR protein as a part of RNAi complexes may open new paths to approach the understanding of Fragile X, the most common inherited form of mental retardation. Our work shows that the fly homolog of the human Fragile X mental retardation protein is important for RNAi, which could imply that some of the problems in Fragile X patients could stem from defects in RNAi pathways.

The Biology of RNAi: RNAi Is Essential for Mammalian Development

E. Bernstein

A key player in the RNAi phenomenon, Dicer, is responsible for the initial steps of this gene silencing pathway by "dicing" dsRNA into short interfering RNAs (siRNAs) that join the RISC complex to subsequently degrade their cognate mRNAs. This enzyme exists in all organisms known to perform RNAi and is quite unique in its structure. It contains a helicase domain, a PAZ domain (also found in the Argonaute homologs, which have been demonstrated to have various roles in RNAi), two RNase III catalytic domains, a dsRNA-binding domain, as well as a domain of unknown function (DUF283).

The *Caenorhabditis elegans* Dicer null worm was shown to be RNAi-deficient, as well as having various developmental phenotypes that correlated strongly with those of a *let7*$^{-/-}$ worm. As it turns out, Dicer is responsible for processing microRNAs (miRNAs), such as *let7*, which are endogenously encoded RNA hairpins that when processed bind the 3′-untranslated region (3′UTR) of target genes to inhibit translation. Thus, Dicer was found to have roles both in the processing of dsRNA, which results in mRNA degradation, and in the processing of miRNAs to inhibit translation.

We therefore set out to create a Dicer null mouse in order to investigate the function of this ribonuclease, and of the RNAi machinery in general, in a mammalian system. The null mouse is an embryonic-lethal, as expected, dying at approximately day 7 during development. The mutant embryo does not appear to have proper polarity formation and does not undergo the gastru-

lation process. Embryonic stem cell lines have been produced and will be characterized for loss of RNAi capabilities, the accumulation of miRNA sequences, and the derepression of repetitive sequences such as transposons and centromeres (the latter of which has been demonstrated in *Schizosaccharomyces pombe* Dicer knockout strains). This null mouse will allow us to decipher not only the role Dicer has in RNAi, but also its critical role in mammalian development.

RNAi and the Genome

A. Denli

The term RNAi refers to the sequence-specific gene silencing induced by dsRNA. Although it was initially discovered as a response to exogenous dsRNA, further experiments suggested a role for RNAi in antiviral, antitransposon defense and also endogenous gene regulation. One interesting aspect of endogenous gene regulation is the formation of heterochromatin, a term used for the compact chromatin structures in the cells. Heterochromatin in the cells is found mainly in centromeres and telomeres, and methylation of histones at lysine 9 is a hallmark for this chromatin structure. Recent genetic experiments suggest a link between centromere function and RNAi. On this end, I am trying to reveal this link biochemically in the fission yeast *S. pombe* and to identify protein complexes associated with RNAi proteins in *S. pombe*. Since a good system is established for heterochromatic silencing in fission yeast, any new components that are identified through biochemistry can be tested genetically and functionally in this powerful model system. In the future, the results from these studies can be helpful in shedding light onto similar pathways in humans.

RNAi: The New Somatic Cell Genetics

P.J. Paddison, D. Conklin

Since the 1970s, the war on cancer has been based on the notion that studying the disease will lead to the discovery of vulnerabilities, which can be exploited in the clinic. Although many underlying genetic determinants of cancer have been identified, this knowledge has failed to translate into new therapeutic strategies, with only a handful of exceptions. This is largely due to the genetically intractable nature of cultured mammalian cells.

In other model systems, the use of genetic screens to explore functional dependencies has been an enabling feature of countless discoveries. For example, analysis of temperature-sensitive mutants in bacteriophage T4 led to the discovery of viral morphogenesis modules (Edgar and Wood, *Proc. Natl. Acad. Sci. 55:* 498 [1966]). Similar approaches in yeast revealed functional hierarchies among genes regulating cell cycle progression (Hartwell et al., *Science 183: 46* [1974]; Hartwell and Weinert, *Science 246:* 629 [1989]). The key to such discoveries has been the ability to create recessive, genetically defined lesions in molecular pathways.

Since cancer arises from genetic lesions in somatic cells, the concept of synthetic lethality has been heralded as way to functionally define vulnerabilities in cancer cells (Hartwell et al., *Science 278:* 1064 [1997]). Synthetic lethal interactions occur when mutations in two or more nonallelic genes synergize to kill cells. For example, a mutation in gene *A* or gene *B* may be tolerated when singly present in cells but, when combined, result in a loss of viability. Thus, synthetic lethal interactions reveal situations in which cellular homeostasis is altered by a molecular lesion so that the action of another gene or pathway is required to compensate. The fact that cancer cells arise from genetic alterations makes synthetic lethality ideally suited for identifying cellular targets required by cancer cells for viability.

The emergence of RNAi as a technology to silence gene expression in virtually every experimental eukaryotic system, including mammals, holds promise for identifying cancer lethal targets. My own work has revolved around (1) demonstrating that dsRNA induces sequence-specific gene silencing in mammalian cells (Paddison et al. 2002a); (2) refining the method of triggering RNAi so that it is useful in most cellular contexts (Paddison et al. 2002b; Paddison and Hannon 2002); and (3) constructing genome-wide RNAi libraries to make genetic screens feasible in mammals.

The Search for New Approaches to Cancer Therapy Using Genetics

J.M. Silva

RNAi is probably the most powerful molecular tool for gene function discovery in mammals. My research is focused in the use of this phenomenon for large-scale analysis of gene function. The approach is based on the in vivo production of silencer hairpins encoded by a transfected plasmid. Using this system, I will identify methods to modulate the growth of tumor cells.

The first step is the transient transfection of the tumor cells lines with single hairpins. Tumor cells that are affected by the action of a hairpin may go to apoptosis or be arrested in a cell cycle checkpoint. To detect the first possibility, cells transfected with individual hairpins from the library are cultured during several doubling cycles, and the activation of caspase-3 (apoptosis-specific pathway) is measured. The induction of apoptosis is quantified by a time-resolved fluorescence caspase-3 detection assay.

I have set up the conditions for this assay and the first hairpins are currently being tested. This approach has been validated with 200 genes that are involved in pro-apoptotic or anti-apoptotic affects, and, as expected, several caspases have revealed apoptotic protective effects.

In addition, I am also developing an assay to detect growth arrest in tumor cells. The assay is based on the facts that in our assay, whole-cell populations cannot be analyzed by traditional cell-cycle-phase detection methods, because the variability of transfection efficiency may produce a high background. To solve this problem, I am cotransfecting with the short hairpin RNA (shRNA)-interfering vector, a gene that produces a tagged protein that will be specifically phosphorylated in G_2/M phase (histone H3 and Nucleolin). The capture of this protein with tag-specific antibodies and the detection with phosphospecific antibodies will show the cell cycle status of the hpRNA-transfected cells.

Comparison of these data with control hairpin assays will identify genes that arrest tumor cells in G_2/M. To detect other types of arrest, cells are treated with nocodazole for 24 hours, and phosphorylated forms are detected as described previously. In this assay, cells arrested in G_1 or S cell cycle phases will be detected as absence of G_2/M phosphorylated forms after nocodazole treatment. Several genes involved in dNTP synthesis and in histone acetylation/deacetylation are currently being tested for their ability to mimic the S and G_1 phase arrest produced by HU and TCA.

Heritable RNAi in Mice: The Road toward the Creation of Mouse Models

M. Carmell

After the demonstration that RNAi in mammalian cells can be mediated by vectors encoding shRNAs, we sought to develop a system by which to create transgenic mice using this technology. We demonstrate that a stable, heritable RNAi trigger in the form of a short hairpin was successfully passed through the mouse germ line. These observations open the way to the use of RNAi as a complement to standard knockout methodologies and provide a means to rapidly assess the consequences of suppressing a gene of interest in a living animal.

In addition to exploring RNAi as a tool for creation of mouse models, conventional mouse knockouts of the Argonaute gene family are under way. Argonaute family members have been shown to be essential for RNAi/PTGS (posttranslational gene silencing) in several organisms, including *Neurospora* (QDE-2), *Arabidopsis* (AGO1), and *C. elegans* (rde-1). Evidence is also mounting demonstrating a role for *Argonaute* genes in silencing at the level of chromatin. Interestingly, Argonaute proteins have been implicated in control of development in several organisms, and at least a subset have particular roles in stem cell maintenance.

Human Cell Transformation: What Does It Have to Do with RNAi?

Y.R. Seger

One of the long-standing interests of our lab is the study of proliferation control in mammalian cells and the development of molecular tools to determine how these intricate cell cycle control mechanisms go awry in cancer progression. Many cancer studies use actual tumor samples as the starting material, but we have taken the reverse approach, starting with genetically normal human cells and using retroviral gene transduction to manipulate these cells into cancer cells. This method allows us to determine the minimum pathways that must be disrupted in order to convert a normal cell into a cancer cell. Utilizing this approach, we have shown that primary human fibroblasts are readily transformed by the coexpression of adenoviral E1A, MDM2, and constitutively activated Ras (Ha-RasV12) (ERM).

Although E1A is typically not expressed in human tumors, it is an extremely well-characterized oncoprotein and can thereby be used as a genetic tool to determine a combination of cellular oncogenes capable of transforming human cells. Coexpression of E1A functional mutants with MDM2 and Ha-RasV12 has indi-

cated that the interactions between E1A and Rb, p300, and p400/TRRAP are essential for transformation. We are currently testing the ability of several cellular oncogenes to rescue the transformation defects of these E1A-binding mutants. For example, E1AΔ26-35, which is defective for p400/TRRAP binding, should be able to be rescued by c-Myc, a protein that also interacts with p400/TRRAP.

This well-defined transformation model can also serve as a useful tool for testing the range of RNAi in cancer biology and therapcutics. Since we know precisely what genetic alterations resulted in transformation, we can use shRNAs to target and silence pathways that could make these cells initiate apoptotic or senescence programs, or increase their sensitivity to chemotherapeutic drugs.

Alterations in the Regulation of Centrosome Duplication Produce Aneuploidy

J. Du

The ccntrosome is the critical cell apparatus that determines thc correct partition of the genome during mitosis. The eukaryotic centrosome, the cell's microtubule organizing center and the counterpart of yeast spindle pole body, is composed of two perpendicularly positioned centrioles and surrounding amorphous pericentriolar materials. It normally replicates once and only once per cell cycle. The centrosome participates in most mitotic events such as dctermining spindle bipolarity, assembling spindle microtubules, regulating actin cytoskelcton dynamics, and determining the plane for cytokinesis.

Among the protcins associated with ccntrosome, protein kinases and phosphatases have important roles in the centrosome replication cycle, including centriole duplication, maturation, and separation. There are four groups of protein kinases found to associate and regulate these processes: cyclin-dependent kinases, Polo-like kinascs, NIMA kinases, and Aurora kinases.

Among these centrosomal kinases, only Aurora-A has been identified as an oncogene candidate. Aurora-A is overexpressed at both thc mRNA and protein levels in a number of cancer cell lines, as well as in breast bladder cancer tissues. Increasing data show that Aurora-A protein lcvel correlates with the progress and malignance of cancers and is suggested to be used as a diagnostic parameter. Overexpression of STK15 in 3T3 and Rat1 cells causes cellular transformation and growth in soft agar. The injection of the cells from the soft agar colonies induces cancer in nude mice. The overexprcssion of the protein in human near-diploid MCF79 cells causes centrosome amplification and aneuploidy/genomic instability, a plausible contributor to cancer progression.

Another centrosome-associated protein, p160ROCK, is a member of the ROCK family of Rho-associated scrine-threonine protein kinases. ROCK kinases are involved in various cellular functions downstream from Rho, such as smooth muscle contraction, stress fiber formation, and cytokinesis. p160ROCK can bc phosphorylated by Rho and then further phosphorylates LIM kinase and mDia. ROCK inactivates myosin phosphatase and cofilin to inhibit actin depolymerization to stabilize actin cytoskeleton.

In 2002, we continued our studies on the mechanisms by which STK15 may contribute to tumorigenesis. We showed that p160ROCK associates with STK15 in a protein complex (*S*TK15-*a*ssociated-*f*actors, SAF) both by immunoprecipitation and by FPLC purification. Kinase analysis revealed that p160ROCK is a substrate of STK15 kinasc in vivo. Half of the total endogenous p160ROCK associates with STK15, and STK15 is a major kinase responsible for p160ROCK phosphorylation in vivo. Suppression of p160ROCK by RNAi induces genomic instability, as evidenced by the accumulation of polyploid cells and super mitotic spindle reassembly capacity in human HeLa cells. Knock out of STK15 by siRNA induces G_2/M delay and tetraploidy, as well as apoptosis in HeLa cells. However, further deletion of p160ROCK by siROCK together with siSTK15 can release the G_2/M cell cycle delay and alleviate the apoptosis defect, thus leading to more stable accumulation of aneuploidy. Thus, biochemical and genetic evidence indicates that p160ROCK is a substrate of STK15 kinase, and the two proteins interact genetically to induce the aneuploidy phenotype.

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SPORADIC HUMAN GENETIC DISEASE

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T. Lee C. Lindsay

Recent progress in the field of human genetics has largely been limited to Mendelian (familial) disorders because a powerful algorithm exists for tracking genes in families (linkage analysis). No such general approach exists for disorders that are primarily sporadic. Mounting evidence, however, suggests that one fruitful approach in this group of disorders is analysis of genomic copy number, as many sporadic syndromes which have been solved (almost always as a result of serendipitous discoveries) are associated with copy number abnormalities.

Microarrays have become ubiquitous in biological research. However, most applications have been in the field of gene expression analysis, and relatively few have focused on direct analysis of the genome. Analysis of gene expression in sporadic disorders is unlikely to be useful for two main reasons: (1) It is often difficult to define the primary tissue that is affected, as most of these conditions are multi-system, and (2) in cases where the relevant tissue is known (e.g., the central nervous system), access is usually impossible.

A number of different approaches to genome copy number analysis currently exist:

- Large clone arrays, based on bacterial artificial chromosomes (BACs) from the library used to sequence the human genome. Total human genomic DNA is analyzed directly. BAC arrays are our currently favored method (see below).
- The use of total genomic DNA on cDNA arrays. This approach is difficult to implement in practice, largely because the complexity of the human genome results in poor signal:noise ratios at individual elements of the array, although copy number changes can be detected when local averages are calculated for genes that map to the same chromosomal region.
- The use of reduced-complexity representations of the human genome (usually generated by amplification using polymerase chain reaction [PCR]) onto microarrays whose elements are designed to "match" sequences in the representation (oligonucleotides or PCR products may be arrayed). This approach suffers from two related drawbacks: (1) The unprovable assumption that all sequences within the representation will have been amplified equally and be present at the same relative copy number as in the starting genome. (2) The analysis is of a representation of the genome, not the genome itself.

We are studying individuals with a range of disorders:

- Congenital heart disease (CHD). We are collaborating with pediatric cardiologists in both the United States and the United Kingdom to search for copy number variations in children with CHD, who are 22q11 normal (22q11 deletions are the most common cause currently known of CHD).
- Complex phenotypes, mostly involving mental retardation in association with widespread physical anomalies, in individuals with normal karyotypes.
- Individuals with sporadic phenotypes in association with de novo balanced translocations.
- Autistic spectrum disorder.

We are also interested in studying a cohort of normal individuals, in order to assess the frequency of some of the deletions/duplications that we have already detected in those individuals we have been using as "normal" controls when analyzing patients. We estimate that, on average, each individual will have approximately half a dozen cryptic genomic rearrangements, and it is vital to characterize these changes in the normal population, in order that the significance of rearrangements detected in patients can be interpreted intelligently.

Finally, we are cognizant of the fact that genomic microarray copy number analysis is relatively novel and that, in the short term at least, it will be necessary to use complementary methods to confirm suspected deletions/duplications, both in normals and in patients. Traditional approaches to confirming copy number changes include:

- Fluorescence in situ hybridization (FISH) analysis. This is the approach of choice, as chromosomal homologs are visualized separately and loss/gain can be demonstrated directly. However, live cells

are required, and these are not always available, particularly when studying archival DNA.

- Quantitative PCR. Detection of 2:1 changes is difficult using Q-PCR, which is mostly used for detection of higher-fold changes.
- Microsatellite analysis for deletions. This approach may demonstrate stretches of homozygosity (compatible with but not proof of hemizygosity) or failure of inheritance, where parental samples are available and the rearrangement is de novo. For duplications, this method may be diagnostic where three distinct alleles are demonstrable, but this convenient situation will not always be present.
- Dosage analysis by Southern blotting, which is labor-intensive and requires relatively large amounts of genomic DNA.
- Specific amplification of junction fragments. This is an ideal method but requires the rearrangement endpoints to be cloned and is not a short-term solution.

We are developing a PCR-based method that will allow robust and rapid initial analysis of putative deletions/duplications in tiny DNA samples.

JOUBERT SYNDROME IN A PATIENT WITH A DE-NOVO-BALANCED TRANSLOCATION

We have studied, using conventional and array-based methods, an individual with Joubert syndrome associated with a de-novo (apparently)-balanced translocation [46,XY,t(2;22)(q13;q11.1)]. Joubert syndrome is a rare disorder that results in partial agenesis of the cerebellar vermis, mental retardation, and episodic hyperpnea. It is thought to be autosomal-recessive, but thus far, no genes have been cloned for this disorder. It is known to be genetically heterogeneous, with one locus mapped to 9q34 in a consanguineous Arabian-Iranian family; this locus has been excluded in other families.

We have mapped the 2q13 breakpoint in our patient to a 324-bp LINE (long interspersed nuclear element) fragment, using a combination of microarray analysis and STS-PCR of flow-sorted material (see Fig. 1). This breakpoint interrupts a predicted gene, and we have recently demonstrated that this gene is real and expressed both in fetal brain and (at lower levels) in adult brain, consistent with a likely developmental role.

We are currently searching for point mutations in Joubert patients who show no evidence of linkage to 9q34 and who do not harbor obvious 2q13 rearrangements.

A COMMON MICRODELETION POLYMORPHISM AT 8q24.3

We initially discovered a heterozygous microdeletion at 8q24.3 when investigating a patient with Kabuki syndrome, a rare sporadic disorder manifest by short stature, mental retardation, and characteristic facies. This was detected in a genome-wide analysis using BAC arrays and was subsequently confirmed by FISH analysis of our index patient. Further analysis of a cohort of Kabuki patients revealed the presence of the same deletion in a further one individual (a total of 2/40 analyzed), but in each case, the deletion was also present in a normal parent (a mother in the first case and a father in the second case). After characterization of the microdeletion in our index case, we cloned the breakpoint and designed a PCR-based assay that rapidly reports the genotype of any individual with respect to this microdeletion. The microdeletion has been found to be present in approximately 5% of the normal population, although higher rates have been seen in some groups, including Iraqi Jews (~10%). The significance, if any, of this microdeletion polymorphism is unknown. However, the importance of this discovery is twofold:

1. To the best of our knowledge, it is the first microdeletion polymorphism described, and serves to highlight the potential variation that is present in normal genomes.
2. As with other rearrangement polymorphisms that will be discovered, it is critical to evaluate the frequency of this rearrangement in that population, so that potentially disease-specific lesions can be assessed intelligently.

GENOME-WIDE BAC ARRAYS

We have successfully generated, at relatively low cost, two BAC arrays, each based on a distinct subset of the RPCI-11 BAC library. One subset was defined by the Cheung laboratory (http://genomics.med.upenn.edu/genmapdb/) by genome-wide STS screening, and it consists of 4200 BACs. The other was defined by the Cancer Chromosome Aberration Project (CCAP; http://cgap.nci.nih.gov/Chromosomes/CCAP_BAC_Clones), and it consists of 1300 FISH-mapped BACs that were selected on the basis that they had been completely sequenced or else anchored to the consensus genome by virtue of end sequences. We generate our BAC arrays as follows:

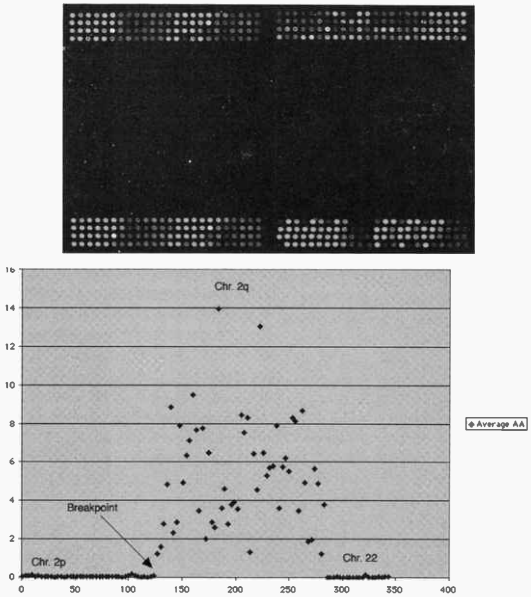

FIGURE 1 (*Top*) The result of array hybridization using differentially labeled material from two flow-sorted derivative chromosomes. The experiment was conducted in both *dark/light gray* directions. Shown here is the experiment in which the chromosome 2p derivative was labeled with Cy3 (depicted as *light gray*) and the chromosome 2q derivative was labeled with Cy5 (depicted as *dark gray*). The BAC array contains clones spanning both chromosomes 2 and 22, arrayed in triplicate. In this experiment, clones from chromosomes 2p and 22 are seen as *light gray* and those from chromosome 2q (below the breakpoint) are seen as *dark gray*. Using *dark/light gray* as the default ratio (i.e., Cy5:Cy3 ratios, after appropriate background correction, etc.), chromosome 2p clones have a ratio close to 0, whereas chromosome 2q clones have high ratios (see *Bottom*). (*Bottom*) Graphical output of analysis of Cy5:Cy3 ratios from the array experiment depicted at the top. Cy5, Cy3 intensities were corrected for background, and average ratios were obtained for each triplicate set. The position of the breakpoint is clearly visible on chromosome 2. All the chromosome 22 BACs yielded very low ratios, implying that the breakpoint on chromosome 22 lies centromeric to the most proximal BAC arrayed.

- BAC DNA preparation from small cultures (200 µl to 1 ml) in 96-well plates, most recently using an automated robot.
- Universal PCR of about 1 ng of BAC DNA, using a method that we have developed.
- Secondary PCR to attach an amino link to our products, followed by arraying onto aldehyde slides, ensuring a covalent attachment between DNA and the surface of the slide.

We have generated BAC arrays at resolutions of less than 1 Mb (for one set) and about 2 Mb for another. We have commenced analysis on some of the patient groups we are interested in and are currently evaluating our results, particularly with respect to

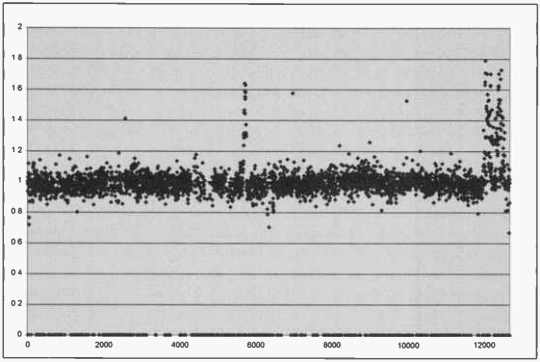

FIGURE 2 BAC array output of male vs. female controls. Cy3:Cy5 ratios are plotted after appropriate background corrections, removal of low-quality data, and averaging over triplicates. The vast majority of elements report a ratio between 0.9 and 1.1. All of the outliers seen are believed to represent true copy number changes, either deletions or duplications (they have been observed in other individuals). The elements are plotted starting at 1pter on the extreme left and ending with Yqter at the extreme right.

apparent deletions/duplications in normals. It is clear that some of the copy number changes that we see are the result of deletions, whereas others are the result of duplications. Furthermore, in some cases, we have observed subtler copy number changes but are uncertain of their significance currently, although we believe that there are two main possibilities: (1) The lesion is present mosaically and (2) the lesion overlaps one of our large clones, such that only a portion of the clone is involved, thus "blunting" the reported ratio. Our goal in 2003 is to generate a tiling path of BAC arrays, using high-throughput methods, in collaboration with workers at the National Cancer Institute, National Institutes of Health.

EPIGENETICS AND MONOZYGOTIC TWINS: ANALYSIS USING *Hpa*II REPRESENTATIONS

To a rough approximation, monozygotic twins share identical germ-line genomes (although somatically they are likely to differ). However, little is known of the epigenetic similarity between monozygotic twins. This is an area of importance when considering possible reasons for phenotypic discordance in monozygotic twins, such as occurs in schizophrenia (50% concordance). As an initial foray into techniques to approach this problem, we have decided to look for methylation differences between pairs of monozygotic twins, using two approaches:

- Representational difference analysis (RDA) using *Hpa*II (a methylation-sensitive enzyme that cleaves at CCGG unless the internal cytosine is methylated).
- cDNA microarray analysis using *Hpa*II representations.

We have performed RDA on a set of twins and are currently evaluating the output.

REGULATION OF APOPTOSIS IN CANCER CELLS

Y. Lazebnik D. Duelli, P. Lassus, S.-C. Lin, A. Matapurkar, X. Opitz-Araya, J. Raychaudhuri, J. Rodriguez, M. Yuneva

Apoptosis is a type of cell death that is critical for maintaining tissue homeostasis. An intense effort to understand apoptosis has been fueled in large part by the hope that the apoptotic machinery will be used for curing cancer. The accumulated knowledge is sufficient to design and implement tools that kill cells quickly and efficiently by inducing apoptosis. However, because the apoptotic machinery is present in most if not all mammalian cells, the major problem is how to induce apoptosis in cancer cells selectively. One approach to solving this problem is to learn how apoptosis is induced by oncogenic transformation. This approach is based on a paradoxical observation that some oncogenes, including *myc* and adenovirus *E1A*, either induce apoptosis or sensitize cells to cytotoxic agents, including those used for chemotherapy. One implication of this observation is that some oncoproteins are pro-apoptotic activities that are specific for transformed cells. We think that understanding how the apoptotic machinery is activated in response to the expression of oncogenes, and how apoptosis is prevented in transformed cells, will help to develop ways to kill cancer cells selectively.

Central to the apoptotic machinery are caspases, a family of cysteine proteases. Caspases are activated at the onset of apoptosis and cause death by cleaving a number of proteins in coordinated manner. If caspase activation is prevented, a cell can become drug-resistant. Caspase activation occurs in two steps. At the first step, pro-apoptotic signals lead to autocatalytic activation of caspases that are called initiators. Activated initiator caspases process effector caspases, which in turn cause cell collapse by cleaving a specific set of substrates. Each initiator caspase is activated in response to a subset of signals, indicating that a prerequisite for understanding how a specific signal activates apoptosis is finding the initiator caspase that mediates it. We investigate which caspases are involved in apoptosis, and study how they are activated, how this activation leads to cell death, and what prevents this activation in drug-resistant cells. The ultimate goal is to understand how caspases can be activated selectively in cancer cells.

To investigate how caspases are activated by oncogene expression, we previously developed a cell-free system that mimics apoptosis dependent on expression of *E1A*, an adenoviral oncogene. Using this system, we found that *E1A*-dependent apoptosis is mediated by caspase-9 and that the expression of *E1A* sensitizes cells to apoptosis by facilitating activation of this caspase. This is achieved by at least two ways: facilitating the release from mitochondria of cytochrome *c*, a cofactor required for caspase-9 activation, and regulation of an unidentified subsequent step in caspase-9 processing.

This model was consistent with the prevailing view that cytotoxic stress, such as DNA damage, induces apoptosis by regulating the permeability of mitochondria. Mitochondria sequester several proteins that, if released, kill by activating caspases, the proteases that disassemble the cell. Another way to activate caspases, which is used by cytokines, is to assemble receptor complexes that activate caspases directly, although the subsequent mitochondrial permeabilization accelerates cell disassembly by amplifying caspase activity. We found that cytotoxic stress causes activation of caspase-2 and that this caspase is required for permeabilization of mitochondria. Therefore, we concluded that cytokine- and stress-induced apoptosis acts through conceptually similar pathways in which mitochondria are amplifiers of caspase activity, rather than initiators of caspase activation.

Our findings have several implications. One is related to a link between apoptosis and cancer. Although strong evidence indicates that a failure of apoptosis contributes to cancer progression in experimental systems, the evidence is much weaker for such a relationship in human cancers. Evidence for the latter has been primarily gathered by correlating tumor properties with deficiencies in the apoptotic machinery. Our findings imply that a critical part of a major apoptotic pathway is yet to be considered by such studies. Indeed, survival of cancer cells might be enhanced by any changes that prevent caspase-2 activation. How this activation is regulated is unknown, which indicates that even basic pathways of apoptosis

are yet to be sufficiently explored to allow the efficient modulation of apoptosis to a therapeutic end. Our study also highlighted truly novel opportunities provided by RNA interference (RNAi) to study cancer biology without relying only on observations made with genetically modified mice.

Another implication is related to the basic design of apoptotic pathways. Genetic studies in the nematode *Caenorhabditis elegans* provided an outline of the apoptotic machinery. In this organism, *CED-4*, an activator of the caspase *CED-3*, is sequestered by a Bcl-2 family member *CED-9*. *EGL-1*, another Bcl-2 family member, releases *CED-4* from *CED-9,* thus triggering *CED-3* activation and cell death. Our finding that caspase-2 is required early in apoptosis provides an experimental model to test the hypothesis that in mammals, as in the nematode, the Bcl-2 proteins control apoptosis by directly sequestering molecules required for activation of a caspase.

Another implication is related to the current model of how mitochondria permeability is regulated during apoptosis. It is thought that cytotoxic stress, such as DNA damage, activates Bax and other pro-apoptotic proteins of the Bcl-2 family, which permeabilize mitochondria, thus releasing factors required for caspase activation. Our observations argue that at least in some cell types, the roles of Bax and caspase activation are reversed, in that a caspase activity is required for Bax translocation. This conceptual change may help to understand how Bax is activated.

This year, we also continued our collaborations with several laboratories. A collaboration with Scott Lowe's laboratory here at CSHL, which was initiated several years ago by former graduate student Lavina Faleiro, came to fruition by uncovering a link between oncogenic transformation and expression of caspases. A collaboration with Emad Alnemri resulted in identifying and characterizing a mitochondrial protease that promotes apoptosis, and caspase-14, whose function remains to be determined.

Overall, our studies continued to investigate how apoptosis can be used to kill cancer cells.

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REGULATION OF APOPTOSIS AND SENESCENCE BY CANCER GENES

S. Lowe A. Bric, E. de Stanchina, J. Fridman, M. Hemann, M. McCurrach, Z. Nahle, M. Narita, M. Narita, S. Nunez, S. Ray, C. Rosenthal, A.M. Spector, H. Wendel, J. Zilfou

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 typically exploit simple cellular systems to study cancer gene function. More recently, we have studied human tumors and animal models to confirm the relevance of our simple systems for tumor development and cancer therapy in vivo.

Oncogene-induced Apoptosis

A. Bric, E. de Stanchina, M. McCurrach, Z. Nahle, J. Zilfou [also involving former Laboratory member A. Samuelson and in collaboration with Y. Lazebnik, Cold Spring Harbor Laboratory; D. Bar-Sagi, SUNY Stony Brook; T. Mak, Ontario Cancer Institute; and S. Powers, Tularik, Inc.]

Normal cells possess natural fail-safe mechanisms that limit the consequences of aberrant proliferation. One of these mechanisms involves their ability to couple excessive proliferation to cell death. 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 appears to limit tumor development, and, as a consequence, mutations that disable oncogene-induced apoptosis can contribute to tumorigenesis. Consistent with this view, 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 promote oncogenic transformation in vitro and tumor development in vivo (reviewed in S. Lowe and C.J. Sherr, in press). We are currently interested in identifying additional components of the process, and understanding how they function in a "tumor suppressor network."

Oncogenes can also contribute to cell death via p53-independent mechanisms. Indeed, we recently showed that deregulation of E2F by adenovirus E1A, Rb loss, or enforced E2F-1 expression results in the accumulation of caspase pro-enzymes through a direct transcriptional mechanism (Nahle et al. 2002). Increased caspase levels appear to potentiate cell death in the presence of p53-generated signals that trigger caspase activation, for example, the presence of cytosolic cytochrome *c*. Hence, p53-independent signals leading to caspase up-regulation cooperate with p53-dependent signals, leading to caspase activation during oncogene-induced cell death. Our results reveal one way in which cell cycle progression can be directly coupled to the apoptotic machinery.

We also have been involved in several collaborations relating to our apoptosis research. With Tak Mak, we showed that Smac/DIABLO—a mitochondrial protein with potent pro-apoptotic activity—is dispensable for apoptosis in oncogene-expressing cells (Okada et al. 2002). This result is consistent with the observation that Smac-deficient mice develop normally and suggests that Smac is not important for apoptosis or that there is redundancy at this level of the apoptotic program. In another collaboration with Tak Mak, we showed that Chk2 is dispensable for DNA-damage-induced apoptosis in oncogene-expressing cells (Hirao et al. 2002). This contrasts with the requirement for Chk2 for p53-dependent apoptosis in other cell types, and highlights the complexity of stress signaling to p53. Finally, we contributed to Scott Powers' study that identified a novel oncogene known as *PPM1D* (Li et al. 2002). This gene is amplified in a substantial fraction of breast cancers and acts as a phosphatase that can inactivate p53 and suppress apoptosis.

Control of Cellular Senescence

E. de Stanchina, J. Fridman, M. Narita, M. Narita, S. Nunez
[also involving former laboratory member C. Schmitt]

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. This has led us to propose that senescence acts in parallel to apoptosis as a cellular response to stress. Thus, our studies on cellular senescence are guided, in part, by lessons learned from our work on apoptosis.

Cellular senescence has been proposed to have opposing effects on organismal aging and cancer, although it has been difficult to demonstrate that the process actually occurs in vivo. One of our most significant advances in the last year was the demonstration that cellular senescence is relevant to tumor behavior (Schmitt et al. 2002). This conclusion stemmed from our efforts to uncover biologic and molecular determinants of sensitivity to cancer chemotherapy in the *Eμ-myc* transgenic mouse. Specifically, we showed that *Eμ-myc* lymphomas can respond to cancer chemotherapy by inducing both apoptosis and cellular senescence and that lymphomas with *p53* or *INK4a/ARF* mutations respond poorly to cyclophosphamide therapy in vivo. Moreover, tumors harboring a Bcl2-mediated apoptotic block undergo a drug-induced cytostasis involving the accumulation of p53, $p16^{INK4a}$, and senescence markers and typically acquire *p53* or *INK4a* mutations upon progression to a terminal stage. Finally, mice bearing tumors capable of drug-induced senescence have a much better prognosis following chemotherapy than those harboring tumors with senescence defects. Therefore, cellular senescence contributes to treatment outcome in vivo.

We are currently trying to identify other factors that modulate drug-induced senescence and are making a strong effort to understand the underlying genetic and molecular mechanisms that control senescence. For example, we are continuing to work on the regulation and activity of the promyelocytic leukemia protein (PML), and have uncovered a new link between PML and the p53 tumor suppressor that we are characterizing in more detail. We have also noticed dramatic changes in chromatin structure accompany senescence and are determining whether these changes contribute to the unique gene expression pattern of the senescent state. Finally, we are pursuing strategies to reverse cellular senescence and hope that these approaches will provide insights into how the process is maintained. Together, we hope that these studies will provide new insights into the regulation of senescence and the activity of various tumor suppressor genes.

New Mouse Models of Cancer

J. Fridman, M. Hemann, C. Rosenthal, H. Wendel [also involving former laboratory member C. Schmitt and in collaboration with R. Hoffman, AntiCancer, Inc.; G. Hannon, Cold Spring Harbor Laboratory; and C. Cordon-Cardo, Memorial Sloan-Kettering Cancer Center]

During the last several years, we have been adapting retrovirus-mediated gene transfer and infection of tumor cells or premalignant stem cells as an alternative to germ-line transgenic or knockout mice to rapidly produce tumors with compound genetic lesions. In one approach, we use retrovirus-mediated gene transfer to introduce genes into stem cells and then reconstitute the hematopoietic compartment of lethally irradiated mice (for review, see Schmitt and Lowe 2002). As a consequence, normal mice are produced that contain a "transgenic" hematopoietic system. Both the transgene and the genotype of the stem cells can be varied, allowing the rapid production of chimeric animals with multiple genetic changes. Moreover, since our retroviral vectors often co-express a green fluorescent protein (GFP) reporter, it is possible to track the infected cells in vivo by whole-body fluorescence imaging. We have used this system to study the action of various oncogenes and tumor suppressor genes on the development and treatment of leukemia and lymphoma.

In one study, we used this technology to examine the p53 effector functions that are important for tumor development in vivo (Schmitt et al. 2002). Although the p53 tumor suppressor acts in a plethora of processes that influence cellular proliferation and survival, it remains unclear which p53 functions are essential for tumor suppression and, as a consequence, are selected against during tumor development. By reconstituting the hematopoietic compartment of recipient mice with *Eμ-myc*-derived stem cells infected with anti-apoptot-

ic genes, we showed that disruption of apoptosis downstream from p53 by Bcl2 or a dominant-negative caspase 9 confers—like p53 loss—a selective advantage and completely alleviates pressure to inactivate p53 during lymphomagenesis. Despite their p53-null-like aggressive phenotype, apoptosis-defective lymphomas that retain intact *p53* genes do not display the checkpoint defects and gross aneuploidy that are characteristic of *p53* mutant tumors. Therefore, apoptosis is the only p53 function selected against during lymphoma development, whereas defective cell cycle checkpoints and aneuploidy are mere by-products of p53 loss.

In collaboration with G. Hannon here at CSHL, we investigated whether RNA interference (RNAi) technology could be used to suppress gene expression in stem cells and produce phenotypes in reconstituted mice (Hemann et al. 2003). As a proof-of-concept experiment, we asked whether short hairpin RNAs (shRNAs) against p53 could recapitulate the phenotype of complete p53 deficiency during *myc*-induced lymphomagenesis. We introduced several *p53* shRNAs into hematopoietic stem cells derived from *Eμ-myc* mice and monitored tumor onset and overall pathology in lethally irradiated recipients. Different *p53* shRNAs produced distinct phenotypes in vivo, ranging from benign lymphoid hyperplasias to highly disseminated lymphomas that paralleled the nullizygous setting. In all cases, the severity and type of disease correlated with the extent to which specific shRNAs inhibited p53 activity. Therefore, RNAi can stably suppress gene expression in stem cells and reconstituted organs derived from those cells. Moreover, intrinsic differences between individual shRNA expression vectors targeting the same gene can be used to create an "epiallelic series" for dissecting gene function in vivo.

Molecular Genetics of Drug Sensitivity and Resistance

J. Fridman, C. Rosenthal, S. Ray, M. Spector, H. Wendel, J. Zilfou [also involving former laboratory member C. Schmitt and in collaboration with R. Hoffman, AntiCancer, Inc., San Diego, California]

A major goal of our research is to understand the biological and molecular basis of drug sensitivity and resistance in tumors (Johnstone et al. 2002). Conventional approaches to identify factors that dictate treatment sensitivity rely on human tumor cell lines treated in vitro or as ectopic xenografts. As an alternative approach, we are using transgenic mouse models to study drug action in spontaneous tumors. Our system exploits the *Eμ-myc* transgenic mouse, which develops B-cell lymphomas at short latency with high penetrance. Using this system, we have identified biologic and genetic determinants of treatment sensitivity in vivo.

This year, we published a comprehensive study on the effects of the chemotherapeutic drug cyclophosphamide (CTX) in *Eμ-myc* lymphomas. CTX is an alkylating agent that has been successfully used to treat many human malignancies. Although CTX is not subject to resistance mechanisms involving enhanced drug efflux, many human tumors respond poorly or become resistant to this agent. We showed that the antitumor activity of CTX depends on its ability to induce both apoptosis and senescence, which was controlled by important cancer genes such as *p53*, $p16^{INK4a}$, and *bcl-2* (see Fig. 1). Tumors lacking both programs rapidly progress to a lethal stage, suggesting that post-damage responses are almost completely responsible for CTX action. Our results established a new paradigm for understanding drug action, as well as the interconnections between tumorigenesis and drug resistance. This paradigm will undoubtedly apply to other conventional anticancer agents, and perhaps many "targeted therapeutics."

It is worth noting that the *Eμ-myc* system provides a model of human cancer that is remarkably simple. Each tumor analyzed is initiated by the same oncogene (*myc*), arises in the same strain (C57BL/6), and is treated with the same drug (CTX). Yet, these tumors display an extraordinary heterogeneity in treatment responses, ranging from cure to progression under therapy depending on their genotype. Given the complexities of human oncology, it is no wonder that treatment responses are remarkably variable and difficult to predict. Undoubtedly, this inherent variability will remain a problem, even with targeted therapeutics. Still, our studies provide a new paradigm to understand drug action and inherent variations in treatment sensitivity and provide support for the view that tumor genotype is the most important determinant of treatment outcome. These principles, when applied to human tumors, provide a strong rationale for individualized cancer therapy based on knowledge of drug action and tumor genotype.

In addition to using the *Eμ-myc* system to identify new processes and genes that modulate treatment sensitivity, we are also using this as a model to test new agents that might overcome or circumvent traditional resistance mechanisms. We are also beginning to apply genomics technology to the problem of drug

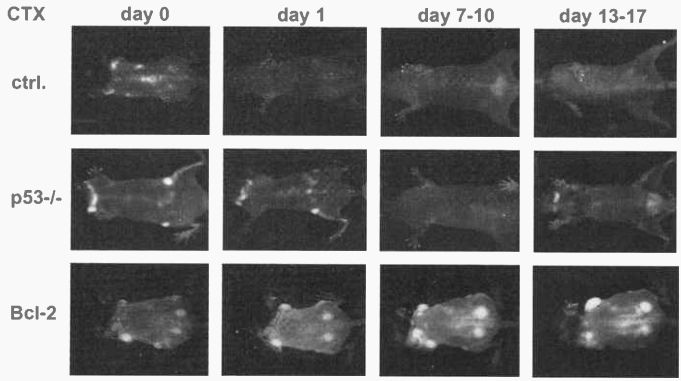

FIGURE 1 Tumor genotype and treatment responses visualized by fluorescence imaging. Mice harboring GFP-tagged lymphomas are treated with cyclophosphamide (CTX) and monitored by whole-body fluorescence imaging. Note that control tumors harboring an intact p53 pathway (ctrl.) show a durable response to CTX, whereas those with *p53* mutations (p53–/–) or overexpressing Bcl-2 relapse or fail to respond altogether. The images were produced in collaboration with R. Hoffman (AntiCancer, Inc., San Diego, California).

resistance, as well as developing new models to study drug action in malignancies besides lymphoma. Ann and Herbert Siegel, who have provided funding for some of these initial studies, have generously supported these efforts. We intend to expand this area of research over the coming year.

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GENOMIC MICROARRAY ANALYSIS

R. Lucito A. Brady
S. Gass
F. Oszolak

This work was carried out in collaboration with M. Wigler, J. Healy, A. Reiner, B. Mishra, and R. McCombie here at CSHL. Cancer is a disease of mutation of the genome. Mutations occur in the genome of the precancerous cells and accumulate until the growth of these cells proceeds unchecked. The genes responsible for cancer must be identified if we are to understand why a cell becomes cancerous. We propose to survey the genome of the cancer 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. Once these regions are identified and tumor suppressors and oncogenes are found, their function can be investigated to understand the role that these genes have in the progression to tumorigenesis. An innovative strategy for the treatment for cancer can be seen by the development of Herceptin for the treatment of breast cancers with *erbB2* gene amplification or for Gleevec for the treatment of CML (chronic myeloid leukemia) that has the Bcr-Abl translocation. Both of these molecules target specific genes that are mutated in the respective cancer. If the cancer has these lesions, the patient becomes a candidate for treatment from these modalities. We intend to take the first step by identifying gene candidates that will later be the targets of such innovative therapies. In addition, the regions themselves can be used for studies in prognosis and diagnosis.

We have developed a genomic microarray technique to identify copy number fluctuations, which borrows the methodology of complexity-reducing representations developed for representational difference analysis (RDA) 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 *Bgl*II, ligation of adaptors, and polymerase chain reaction (PCR) amplification. Amplification in the presence of many fragments results in preferential amplification of the smaller fragments, so that the size range of the reprcsentation is 200–1200 bp. Many of the restricted fragments are lost during this preferential size selection caused by the PCR amplification, resulting in a reduction in complexity of approximately 3% of the genomc.

The array is composed of oligonucleotides based on the sequence of the human genome. An in-silico-based *Bgl*I representation was derived from the sequence of the human genome. The 70-mer oligonucleotides were derived from the predicted representational fragments. Oligonucleotides were chosen based on uniqueness within the genome. The set of oligonucleotides were culled by distribution across the genome, maximizing oligonucleotide quality so that the total numbered 30,000. The number 30,000 was set due to the constraints of the number of fragments that can be arrayed on a single slide with our currcnt format. The average resolution on the array will be 1 probe every 100 kb. More oligonucleotides can be added, increasing resolution further; however, a second array would be produced.

*Bgl*II representations of tumor and normal that were differentially labeled are compared on such an array to identify copy number fluctuations as microarray features having an intensity ratio deviating from 1:1 for tumor to normal. Although we will be using this microarray method to categorize the mutations that occur in many tumor types, we will be primarily analyzing two tumor types initially. The first is pancreatic caner. There will be an estimated 30,000 cases of pancreatic cancer this year. Of these, 29,700 patients will succumb to the disease. Although this number 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. We are collaborating with Dr. Daniel Von Hoff of the Arizona Cancer Center, who will provide pancreatic specimens and invaluable clinical

information and expertise. We will analyze 100–150 primary pancreatic tumor specimens using the 30,000 probe representational oligonucleotide microarray analysis (ROMA) array to identify regions of the genome that have undergone copy number fluctuations. We have already analyzed close to 10 samples and have confirmed that the INK4a-ARF locus is deleted at a very high frequency in this type of tumor. In addition, several other regions are deleted frequently in this cancer, and we are continuing to study these regions to identify possible gene candidate tumor suppressors. Eventually, the results from this study will be combined with clinical information associated with the patient to determine whether there are any parameters that associate with specific tumor mutation patterns.

The second cancer we will focus on is ovarian cancer, which has a relatively high incidence and approximately 50% survival rate. In many patients, the cancer is diagnosed late due to location; often, the tumor has already metastasized, the first symptoms being an accumulation of fluid in the abdominal cavity. There are few genes discovered that have a role in the progression of ovarian cancer.

We will collaborate with Dr. Michael Pearl of SUNY Stony Brook for access to tissue and clinical information. We will be performing ROMA on ovarian cancer samples to identify gene copy number fluctuations to determine candidate tumor suppressors and oncogenes. We will compare samples of the primary cancer , metastatic tissue from the cell wall, metastatic tissue found in the fluid in the abdominal cavity, and in some cases, the metastatic tissue after chemotherapy. We intend to analyze the tumors to trace the accumulation of mutations from primary to metastasis with the hope of identifying not only genes involved in the development of the primary tumor, but also those genes that may increase metastatic capability and genes involved in chemotherapy resistance.

The regions identified from these studies will serve as valuable diagnostic and prognostic indicators. Of equal importance, these regions will serve to identify new oncogenes and tumor suppressor candidate genes. The advantage to using genome-wide analysis on a large set of tumors is that the time-consuming step of positional cloning will have already been done, although it will be necessary to compile the data. Once compiled, the epicenter, or common region of mutation, will be located in collaboration with B. Mishra, who is developing algorithms and informatics tools. Once the epicenter is identified, candidate genes will be identified informatically in collaboration with R. McCombie here at CSHL.

Colon Cancer

A. Brady, S. Gass [in collaboration with IM. Wigler and R. McCombie, Cold Spring Harbor Laboratory]

We are currently investigating a possible tumor suppressor gene candidate located on chromosome 20p12 that is deleted homozygously in approximately 4% of gastrointestinal tumors. This region was used for the identification of gene candidates as possible tumor suppressors. The region defined as the epicenter or common region of deletion was used for gene candidate identification. On the initial freeze of the golden path that was used for gene candidate searching, no RefSeq genes were found and one spliced expressed sequence tag (EST) was identified. Unfortunately, homology searches performed have not revealed any striking similarities that would yield clues to the function of this gene. The sequence of the EST was used to generate primers for PCR analysis of exon deletion status in the cell lines identified to have homozygous deletion of the 20p12 region, finding exon 1 and 2 frequently deleted. This EST is a 5′ read and presumably represents the 5′ end of the gene. Therefore, it is likely that the gene is not expressed in these tumor cell lines.

Northern blots were performed to determine the tissue distribution of the EST and to identify the full-length product. This EST appears to be expressed predominantly in the skeletal muscle. Reverse transcriptase-PCR (RT-PCR) was utilized to determine that the EST is expressed in several colon tumor cell lines. Northern blots of these cell lines are currently being performed. Upon inspection of a more recent genome build, two additional ESTs have been mapped to the deleted region. Both of the ESTs are at least partially deleted in the cell lines originally identified to harbor deletion of the chromosome 20p12 region. The sequence of these ESTs is currently being used for RT-PCR of several colon tumor cell lines to determine whether they are expressed in this tissue, and later for northern blotting.

Maize Mutagenesis Library Screening

R. Lucito (in collaboration with R. Martienssen, P. Rabinowicz, and E. Vollbrecht, Cold Spring Harbor Laboratory)

We are developing a high-throughput microarray screen of a maize insertion mutagenesis library. The probes on the array are picked from a gene-enriched methyl-filtering library. The basis of this library relies on the fact that gene-rich regions of the maize genome are not methylated, but repetitive regions are methylated. The methyl-filtered clone library was further screened to remove clones with remaining repetitive content, and redundant clones. A mutant plant library was produced by random insertion of a transposable element. DNA from these plants was isolated, and pooling was performed. Representations are prepared from these samples by PCR amplification using the sequence of the transposable element. The representations are then used for hybridization to the arrays to identify which individual plants have a gene insertion. This information will be cross-referenced to phenotypic data to identify the gene regions with transposon insertions. We are optimizing the representational procedure and labeling procedures to improve the accuracy of the technique to identify pools with mutants.

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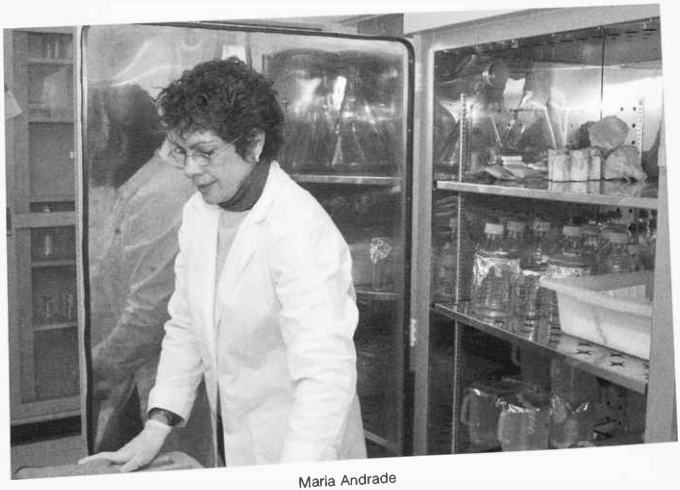

Maria Andrade

MAMMALIAN FUNCTIONAL GENOMICS

A. Mills A. Bagchi W. Keyes
S. Cheloufi T. Riemels
E. Garcia Y. Wu

Our goal is to understand the genetic basis of human disease. Although genome sequencing efforts have generated an impressive amount of information about the content of the mammalian genome, we know relatively little about how specific genes function within the organism. Thus, functional genomics—dissecting the genome by determining the role of specific genes—is essential for understanding normal development and the disease process.

We are using the mouse as a model system to probe the function of human genes. The mouse provides an excellent opportunity to explore gene function because of its reproductive, anatomical, physiological, and genomic similarity to humans. Our ability to genetically modify the mouse genome makes it possible to create mouse models that allow us to address a multitude of specific biological questions.

In our laboratory, we use gene targeting in embryonic stem cells to generate mouse strains that have precise modifications of the genome; these strains serve as experimental models that allow us to define gene function. We are using two different approaches to achieve this goal: (1) analyzing the role of *groups of genes* within specific regions of the genome, and (2) analyzing the role of *individual genes* known to be associated with the disease process. The first approach uses chromosome engineering to generate mouse strains that have precise chromosome rearrangements that mimic those found in human diseases. These mouse strains are useful for providing models of human disease and for determining the function of genes within specific regions of the genome. The second research emphasis in the laboratory is to perform an in-depth exploration of a single gene known to be causal in several human disease syndromes. This gene, *p63*, is related to the *p53* tumor suppressor gene, a gene that is mutated in approximately one half of all human cancers. Understanding the molecular and genetic mechanisms of *p63* action will allow us to gain an insight into the processes of development, differentiation, stem cell maintenance, and cancer.

Generation of Megabase Chromosome Rearrangements: Human Chromosome 1p

A. Bagchi, T. Riemels, Y. Wu

What do our genes normally do? How do chromosome abnormalities lead to disease?

A substantial number of human diseases are associated with chromosomal rearrangements such as deletions, duplications, and inversions. These abnormalities are complex because they affect large numbers of genes within specific chromosomal regions. To understand how these rearrangements cause disease, we can make mouse strains that have the same rearrangement as those found in human patients. Defined chromosome rearrangements are made using chromosome engineering, an approach that combines the power of gene targeting with Cre/*loxP* technology. Similarities between the human and mouse genome make it possible to generate mouse models of human disease. We are currently using this approach to generate models for hereditary cancer syndromes. We are focusing our efforts on human chromosome 1p, a region containing several as yet unidentified tumor suppressor genes. A diverse array of human tumors have deletions at 1p, suggesting that genes underlying the tumorigenic process are located in this region of the genome. We are creating models of hereditary cancer by generating mouse strains that have the same deletions as those found in human tumors. We first make specific deletions in embryonic stem cells, and then use these cells to generate mouse models that transmit the modified allele in the germ line. To make this approach feasible on a genome-wide scale, we have created a system that greatly reduces the substantial amount of effort required for generating gene targeting constructs. This system is composed of two genomic libraries of essentially pre-made gene targeting vectors that contain all of the features required for generating Cre-induced

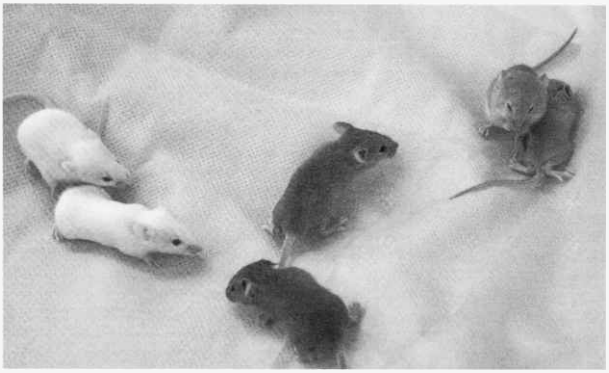

FIGURE 1 Coat color markers are used to "tag" mice containing chromosome rearrangements. Mice haploid (*right*) or diploid (*center*) for a 23-cM inversion on the distal portion of mouse chromosome 4 are visually distinguishable from wild-type albino mice (*left*). The *Tyrosinase* transgene that was inserted into the rearranged chromosome produces a slight graying of the coat when a single copy is present (*left*), and a more intense coat color when two copies are present (*middle*).

chromosome rearrangements. In addition, constructs isolated from these libraries contain genes that alter the color of mice that harbor them; this feature has the advantage that mice containing a specific rearrangement in their genomes are visibly distinguishable from normal mice (Fig. 1). This greatly reduces the cost and labor normally required for maintaining mouse colonies and also allows these models to be implemented in genetic screens.

Analyzing the Role of Specific Disease Genes: The *p63* Locus

S. Cheloufi, E. Garcia, W. Keyes

How does p63 keep us young? What is the role of p63 in human cancer?

The *p53* tumor suppressor gene has an important role in cancer progression. Approximately one half of all human cancers either have lost *p53* altogether or have inactivated it by mutation. Although mice lacking *p53* are viable, they develop tumors at a very early age. Thus, *p53*-deficient models are extremely valuable for investigating the molecular and genetic events associated with tumor formation.

We discovered *p63*—a gene that has striking similarity to the *p53* tumor suppressor gene. We used gene targeting to create mice that lack *p63*; these mice have severe defects that affect craniofacial, limb, and skin development. Mice lacking *p63* are devoid of all structures that are normally derived from the ectoderm during development: They lack hair, teeth, mammary and sebaceous glands, nails, and prostate. These observations provided an important clue which led to the discovery that mutations in *p63* cause five different human developmental disease syndromes: EEC (ectrodactyly, ectodermal dysplasia, clefting), AEC (ankyloblepharon, ectodermal dysplasia, clefting), and ADULT (acro-dermato-ungual-lacrimal-tooth) syndromes, as well as LMS (limb-mammary syndrome) and SHFM (split hand/foot malformation). These syndromes are characterized by a spectrum of clinical features that affect development of the limbs, skin, and the craniofacial region. How mutations in *p63* bring about the striking defects in these patients is not yet known.

We are currently investigating the role of *p63* in morphogenesis of the ectoderm and its related structures. This year, we generated a conditional *p63* model that allows us to first generate viable mice and then to ablate *p63* function within specific tissues at particular stages of development (Mills et al. 2002). Using this approach, we are able to inactivate *p63* within specific tissues of the adult. This is the first system that provides a tool for direct genetic analysis of the role of *p63* in aging and tumorigenesis.

We are also using several mutagenesis approaches to determine how specific alterations of *p63* affect its function. The first approach is to use site-specific mutagenesis to generate mouse models that have the same *p63* mutations that are present in EEC patients. We used gene targeting to replace the normal copy of *p63* with this mutated version. This model will allow us to determine how mutations in *p63* lead to the abnormalities found in individuals afflicted with EEC syndrome.

We are also investigating the role of *p63* using a phenotype-driven mutagenesis strategy—one that makes random mutations throughout the entire genome. The mutagen that we use is ENU; it is a potent mouse mutagen that typically generates single-nucleotide mutations. If an ENU-induced mutation happens to occur within the *p63* gene, this is detectable in the screen because a visible phenotype can be observed. This approach is extremely valuable because it is random; it is unbiased because it is based entirely on the resulting phenotype and not on our understanding of gene structure. This approach will provide a series of mouse models that harbor different *p63* mutations. This resource will be invaluable for assessing the role of *p63*.

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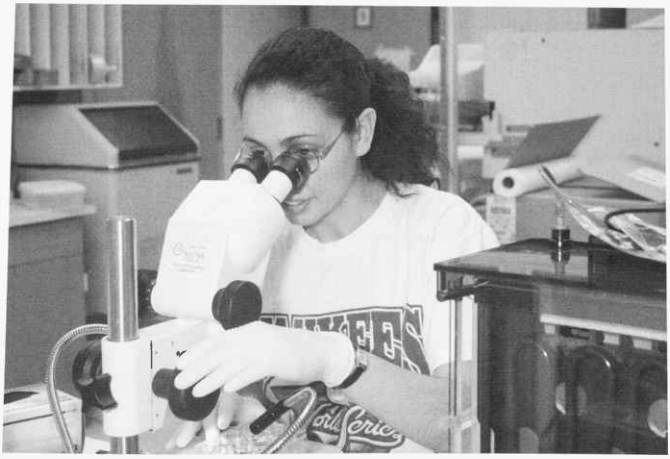


Sihem Cheloufi

FUNCTIONAL GENOMIC APPROACHES IN BIOLOGY

V. Mittal J. Egan A. Kennedy K. LaVine
R. Kumar R. Schoer C. Catapano
S. Gupta R. Lindsay O. Morris

During the past year, our laboratory has been carrying out a systematic approach that combines the strengths of methods such as RNA interference (RNAi)-mediated suppression of gene expression, DNA microarrays for gene expression profiling, and functional analysis to dissect the basic helix-loop-helix (bHLH) *Id* gene regulatory pathways. Our approach is multidisciplinary; it utilizes both mouse genetic models and cultured primary cells. An understanding of these pathways will have implications in defining novel strategies for blocking the process of angiogeneis as an effective therapeutic modality.

We have recently begun to use similar functional genomic approaches to dissect gene regulatory pathways by which components of an innate and adaptive immune system (T cells and dendritic cells) respond to immunity potentiating drugs. Below is a brief description of individual projects.

Using RNAi and DNA Microarrays to Delineate *Id*-mediated Angiogenic Pathways

S. Gupta, K. LaVine, A. Kennedy, R. Kumar, R. Schoer
[in collaboration with M. Ruzinova and R. Benezra,
Memorial Sloan-Kettering Cancer Center, New York]

The bHLH *Id* gene family of transcription factors (*Id1, Id2, Id3, Id4*) are important regulators of key cellular processes such as cell cycle regulation, cell differentiation, and angiogenesis and are deregulated in a variety of cancers. In adults, *Id1* and *Id3* are expressed exclusively in the endothelium of the blood vessels of tumors, and mice lacking *Id1* or *Id3* do not support growth and metastasis of tumors. Thus, *Id* and its downstream targets serve as attractive candidates for anti-angiogenic drug therapy.

IDENTIFICATION OF Id TARGETS IN ENDOTHELIAL CELLS

To understand how *Id* regulates formation of intact vasculature in tumors, we used DNA microarrays to compare gene expression in hyperplastic lymphoid tissue from *Id* wild-type ($PTEN^{+/-}$ $Id1^{+/+}$) and *Id* mutant ($PTEN^{+/-}$ $Id1^{-/-}$) mice. Various control hybridizations were performed, for example: (1) three separate pairs of wild-type and mutant mice provided control for variability; (2) each comparison was performed in duplicate and color reversal (reciprocal labeling); (3) at least four correlated data sets (coefficient of correlation of >0.9 or more in normalized and filtered data) were used for further analysis; and (4) tumors isolated from two separate wild-type mice and two mutant mice were used to measure variability from mouse to mouse. The data obtained from these control experiments were used to make an error model to identify genes that showed random fluctuations in their ratios. These were considered noise and were eliminated from the final analysis. On average, per array hybridization of a total of 10,000 features, 7,000 passed thresh filters set during analysis. Of these, about 1% showed significant up-regulation and down-regulation. A hierarchical clustering analysis filtered data set was used to visualize for genes showing consistent expression profiles across individual experiments.

DOWNSTREAM ANALYSIS OF Id TARGETS

In the initial phase, our priority was to study target genes that were pro-angiogenic and whose expression was down-regulated in *Id1* mutant samples. We reasoned that this approach would validate our experimental strategy and identify candidate genes with a potential for anti-angiogenic therapy targets. The following genes qualified our set criteria: β4 integrin (AA407046), α6 integrin (AW556992), fibroblast growth factor receptor-1 (FGFR1, AW557998), metalloprotease-2 (MMP-2, M84324), pleiotrophin (AW550271), and chemokine (C-X-C) receptor 4 (AA409357). The down-regulation of these genes was not due to the decrease in numbers of endothelial cells in an $Id1^{-/-}$ background since endothelial markers such as CD146 were not down-regulated. Semiquanti-

tative polymerase chain reaction (PCR) showed that the relative amounts of transcripts for each gene correlated well with the microarray-based experiments, suggesting that our microarray provides reliable measures of gene expression. Almost all except pleiotrophin and C-X-C receptor 4 localized exclusively to the endothelium of the blood vessels in *Id* wild-type cells and not the contaminating tumor cells. Down-regulation of MMP-2 expression was previously shown in the defective tumor vasculature of *Id1* mutant animals, consistent with the array data. Genes that did not show endothelium-specific localization were not investigated further.

FUNCTIONAL VALIDATION OF THE DOWNSTREAM TARGETS

The biological relevance of target gene analysis was demonstrated by performing an in vivo Matrigel assay. This assay was used to establish whether loss of function of the gene was sufficient to account for the angiogenic defects in *Id* mutant mice. Control experiments showed that VEGF-impregnated Matrigel plugs implanted into *Id1* wild-type animals form multiple channels by endothelial cells. As expected, Matrigel plugs from *Id1* mutant animals failed to form endothelial tubes. Strikingly, blocking antibodies to integrin α6 and FGFR1 prevented endothelial cells from forming luminized channels. Inhibition of MMP-2 activity resulted in a more dramatic phenotype; there was absence of endothelial cell invasion of Matrigel plugs. Thus, it seems that the inhibition of FGFR1 prevents formation of endothelial tubes while MMP-2 blocks invasion of endothelial cells into Matrigel plugs. Thus, combined down-regulation of these two factors may account for the reduced endothelial cell invasion and tube formation seen in *Id*-deficient mice. We showed that α6 integrin also has a role in endothelial tube formation, but the effect may be due to its dimerization with either β4 or β1 integrins. We could not study inhibition of β4 integrin because of a lack of reliable antibodies. Currently, we are using RNAi to suppress expression of β4 integrin gene suppression, and this will be followed by functional analysis.

Previous findings suggest that pro-angiogenic molecules identified in our studies may lie in the same pathway. bFGF up-regulates the expression of α6β4 integrins in endothelial cells and MMP-2 induces the migration of epithelial cells by cleaving and regulating the function of laminin-5, a recognized ligand for α6β4 integrin. Thus, if the same pathway operates in endothelial cells, loss of *Id* may short-circuit it by down-regulation of three of its crucial components.

In summary, our studies show how functional genomic approaches combined with the strength of a physiologically relevant mouse tumor model can be used to define novel strategies for the development and use of anti-angiogenic therapeutic drugs. Such an approach should be directly applicable to the functional analysis of other disease models. A major interest in our laboratory now is to identify components of the *Id* gene network, analyze their mechanism of action, and determine their relative impact on tumor progression. We have started generating loss of function of *Id* genes (*Id1* and *Id3*) by RNAi in murine and human endothelial cell lines.

A Method of Screening Effective siRNA Probes

R. Kumar

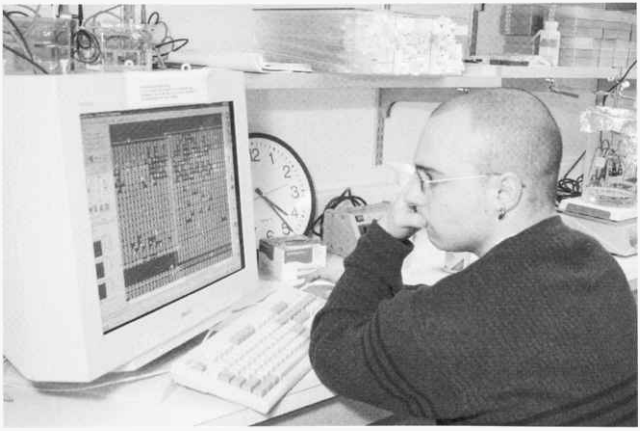
RNAi is a process of sequence-specific posttranscriptional gene silencing mediated by double-stranded RNA. More recently, RNAi has emerged as a powerful genetic tool to analyze gene function in mammalian cells. However, the power of this method is limited by the uncertainty in determining which region of the mRNA can serve as the most effective target for short interfering RNA (siRNA)-mediated gene silencing. This feature has imposed serious limitations not only for small-scale, but also for high-throughput RNAi screening initiatives in mammalian systems.

We have developed a method for rapid and efficient screening for a most effective siRNA against any gene. The strategy is based on a rational assumption that the expression of endogenous protein will be as efficiently silenced as that of ectopically expressed reporter-tagged mRNA by an effective siRNA candidate. We have shown correlation in the ability of siRNA probes to suppress expression of ectopically expressed and cognate endogenous counterparts using a variety of genes with diverse biological functions, in both murine and human cell types. Using microarray-based cell transfections, we have shown that it is possible to identify very rapidly effective RNAi candidates in large parallel screens.

Dissection of Gene Pathways of the Immune System

J. Egan

The immune system is a highly adaptive defense system that has evolved in vertebrates to protect them from both invading pathogens and cancer. This is accomplished by the elegant interaction of two unique branches referred to as the innate and adaptive immune systems. A functional breakdown in either of these systems due to genetic lesions, disease, or age has catastrophic consequences for the organism. Additionally, it is becoming apparent that a potentially powerful and specific therapy for diverse diseases such as malaria, influenza, AIDS, and even cancer is to harness the power of the immune system to combat these diseases. Of the several compounds that stimulate both the innate and adaptive arms of the immune systems, inosine 5′-methyl monophosphate (MIMP) is classified as a "thymomimetic drug" based on the fact that it mimics the activity of hormones derived from the thymus. Specifically, it augments the proliferative and secretory functions of mature T cells, a critical cellular component of the adaptive immune system, and it provides protection against a variety of pathogens. MIMP has also been shown to reverse the effects of aging on the immune system and overcome various immunosuppressive influences. We are interested in further understanding the biological properties of MIMP as well as elucidating the signal transduction pathways that MIMP stimulates. We are using human $CD4^+$ and $CD8^+$ T lymphocytes (derived from peripheral blood) as a model for adaptive immune system activation, coupled with DNA microarray technology, to study the global gene expression profile changes induced by MIMP. Several candidate genes have been identified and are currently undergoing validation by independent methods. Parallel studies are also being conducted on cellular components of the adaptive immune system, such as dendritic and natural killer cells. A more comprehensive insight into the mechanism of action of MIMP in immune function will allow for the design of more specific and efficacious therapies for diseases as disparate as AIDS and cancer, as well as provide prophylactic therapies in the form of more potent vaccines.



Christopher Catapano

MAMMALIAN CELL GENETICS

M. Wigler J. Alexander, A. Brady, K. Chang, M. Chi, J. Douglas, D. Esposito, S. Gass, H. Grasmo-Wendler, J. Healy, R. Lucito, A. Reiner, M. Riggs, L. Rodgers, S. Rostan, E. Thomas, J. Troge, J. West

It is a poor and unnecessary gamble to act as though either our theory or our knowledge of cancer is nearly complete. Future progress in detection, prognosis, and treatment of cancer will depend on the accuracy and completeness of our understanding of its specific molecular causes. This knowledge is likely to become increasingly important as cancers, or suspected cancers, are detected at earlier and earlier stages.

There are simple tests for the completeness of our understanding of how cancers survive in and kill their hosts. If our knowledge were complete, we would see a plateau in the number of genes commonly found mutated in cancers. If the principles were few, even advanced cancers with a large number of accumulated genetic lesions would show only a small number of commonly affected pathways. It follows from this that if mutation in a single gene were sufficient to affect a given pathway, then even advanced cancers would show only a small number of commonly affected genes, the remainder of lesions being more-or-less random.

We have developed a microarray-based method that can partially address these issues. We can readily identify loci in the genome that undergo amplification, deletion, and imbalanced breaks. Although there are many other possible mechanisms that alter critical genes, such as point mutations, balanced translocations, and possibly stable epigenetic changes, many if not most oncogenes and tumor suppressor genes will eventually be found in the types of lesions that we can readily detect. Moreover, if a region is commonly found altered in cancers, that region harbors a good candidate cancer gene. Therefore, the application of our method to a large series of cancers, and the comprehensive comparative analysis of such data, should reveal the position and number of candidate cancer genes in cancers.

Using our methodology, we have also discovered, as we predicted last year, that there are many examples of copy number polymorphisms in the human gene pool, i.e., large regions of the human genome that are present in individuals in unequal amounts. It remains to be shown that these regions of copy number variation are inherited as such, but it seems plausible to us that some of these regions will be shown to be associated with disease resistance and sensitivity. We have made great strides in developing our technology. We are now at the beginning stages of accumulating data. We also report on our progress at cancer gene identification, genome mapping techniques, and other genomic studies.

THE TECHNOLOGY

The major obstacle to the implementation of our technology has been financial. The principles and pilot demonstrations were established in previous years. Fortunately, due mainly to the contributions of many extraordinary philanthropic groups and individuals, and through additional support from corporations and the National Cancer Institute, we have been able realize our plans on a large scale only this year. But financial considerations aside, this work became possible because of the publication of the assembled human sequence.

The basis of our technology, representational oligonucleotide microarrays, or ROMA, has been explained over the past years. It involves making complexity-reducing representations of genomic DNA and hybridizing such to microarrays of oligonucleotide probes designed informatically from the published human genome assembly. The probes are chosen from the genome so that they are complementary to the parts of the genome that are in the representations and are further selected so that they have a minimal overlap with unrelated regions of the genomes.

The algorithms that minimize overlap were described briefly in last year's Annual Report, and were developed by John Healy in our group. They have now been submitted for publication. The algorithms allow counts of exact matches of sequences of any length throughout a sequenced genome and are based on a Burrows-Wheeler transform of the genome sequence and the construction of auxiliary data structures. The unintended uses for these algorithms are described below.

We use two forms of oligonucleotide microarrays, the printed form that we make ourselves, and a form in which oligonucleotides are synthesized in situ on the array surface using laser-directed photochemistry. A

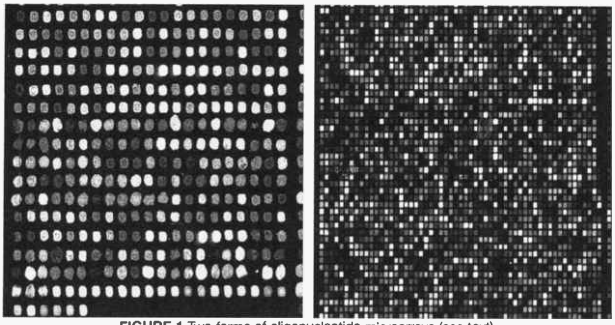


FIGURE 1 Two forms of oligonucleotide microarrays (see text).

company called Nimblegen makes the latter, and their technology has given us substantially greater flexibility in the design of arrays and the selection of representations. Pictures of these microarrays are shown in Figure 1, with the printed array on the left and the Nimblegen array on the right. With Nimblegen, we can array 85,000 probes (85K format), average one probe per 30 kb, and even greater densities could be achieved readily. We have shown that each format yields very similar measures, probe for probe. This work was conducted in collaboration with Robert Lucito here at CSHL.

CANCER LESIONS

We have applied our method to both tumor biopsies and cancer cell lines and have observed gross chromosomal copy number alterations, and highly localized amplification, imbalanced chromosome breaks, and deletions. In the latter case, we expect that we have observed both hemizygous and homozygous deletions. Representative examples of these lesions are illustrated in Figure 2. Clockwise from upper left, we have an imbalanced chromosome break, a complex series of amplifications, a homozygous deletion, and a presumed hemizygous deletion. Ratios of probe intensity are roughly proportional to copy number alteration and are plotted for each probe in the order that they occur on the genome. Averages of probe ratios for segments with similar behavior are indicated. In our data analysis, we have used algorithms for statistical segmentation designed by Adam B. Olshen and E.S. Venkatraman of the Memorial Sloan-Kettering Cancer Center. We observe a large number of lesions, of varying sizes, per cancer. We do not yet have enough examples of genomes analyzed at 85K resolution to allow us to make generalizations, but in the two genomes that we have analyzed in great detail, we observe on the order of 80 lesions each, a number greatly exceeding our expectation.

In collaboration with Rob Lucito here at CSHL, and L. Norton and W. Gerald of Memorial Sloan-Kettering Cancer Center (MSKCC), we have shown that detection of amplification at the ErbB-2 locus in breast cancer by ROMA is roughly comparable to its detection by fluorescence in situ hybridization (FISH). We therefore have initiated a far-ranging collaboration with Anders Zetterberg at the Karolinska Institute of Stockholm on a program to convert the findings of ROMA into FISH assays. Dr. Zetterberg is one of the world's foremost experts on FISH analysis of cancer.

Our future plans include a survey of a large number of breast, ovarian, and pancreatic tumors and cell lines, and leukemias and lymphomas, after which we will determine by epicenter mapping the minimal regions of frequently recurring amplifications and deletions and breakpoints. Candidate genes from these regions will then be further explored by functional analysis and sequencing. To complete this program, we need to automate data processing and gene annotation, so that our efforts for the next few years will focus on computational methods. In these efforts, we are currently collaborating with clinicians and researchers at the MSKCC, the Karolinska Institute, University of Toronto, Stony Brook University, Stony Brook, New York University, LI–North Shore University Hospital, and Johns Hopkins University School of Medicine.

NORMAL VARIATION

We have applied our method to the comparison of normal genomes and have discovered that there are a large

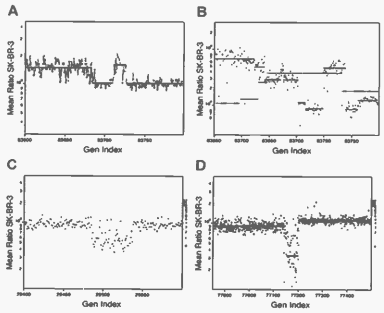


FIGURE 2 Representative examples of cancer lesions (see text).

number of extensive regions of copy number variation between any two genomes. These regions could result from polymorphic gene duplications or deletions, and in further collaboration with Dr. Zetterberg, we are in the process of determining precisely this question. These regions must be categorized, otherwise they will be mistaken for recurring cancer lesions in our cancer surveys. Furthermore, these normal variations may be associated with inherited disease susceptibility or resistance. In a collaboration with Conrad Gilliam of Columbia University College of Physicians & Surgeons, we are planning to carry out a large survey of normal genomes and genomes from families with children with mental or psychiatric disorders.

CANCER GENES

We have continued our work on the relationship of PTEN expression and TGF-β, a growth factor. We have demonstrated that the expression of PTEN squelches the effects of TGF-β on gene expression and are trying to determine the mechanism of action. Work with collaborator Masaaki Hamaguchi here at CSHL has led to the discovery of the *DBC2* tumor suppressor (Hamaguchi et al. 2002). *DBC2* expression is lost in the majority of breast cancer cell lines, and in lung cancers as well. Its function is unknown, but its forced expression arrests the growth of cancer cell lines that have lost expression of the endogenous gene. For more details, see Hamaguchi's annual report. Work with the collaborator Scott Powers at Tularik has led to the discovery of a new class of oncogene, *KCNK9* (Mu et al. 2003). *KCNB* (a.k.a. *KCNK9*) encodes a potassium channel, and work from the Tularik group suggests that it is involved in resistance to death by oxygen limitation. *PTEN, DBC2*, and *KCNB* were all discovered through the use of representational difference analysis (RDA), a method that was developed by us in past years (Lisitsyn et al., *Nat. Genet. 6:* 57 [1994]; *Proc. Natl. Acad. Sci. 92:* 151 [1995]) and is now supplanted by ROMA.

CELL TALK

We continue to examine the question of whether we can use microarray expression analysis and other means to detect "conversations" between cancer cells and cancer cells and hosts. Our rationale is that cancer-host interaction remains a large unexplored chapter of cancer biology. We initiated with pairs of sarcoma-carcinomas and found evidence that the transcriptional state in one is affected by the presence of the other. We are now exploring the effect that these cells have on each other in co-tumorigenesis experiments. In collaboration with Jan Kitajewski at Columbia University College of Physicians & Surgeons, we are testing whether microarrays can be used to detect the interaction between the notch receptor and its ligands. These interactions depend on direct cell-cell contact.

GENOME MAPPING, ASSEMBLY AND EVOLUTION

In collaboration with Bud Mishra here at CSHL and the Courant Institute for Applied Mathematics at New York University, we have developed uses of microarray hybridizations for the mapping of probes in genomes. A graduate student, Joseph West, has recently collected a full set of data in a model organism, *Schizosaccharomyces pombe*, which will enable us to test these ideas. John Healy in our group and a Watson School graduate student, Elizabeth Thomas, have found that our exact matching algorithms have had some unexpected uses beyond probe design, namely, monitoring inconsistencies between human genome assemblies and the discovery of new genomic repeats.

PUBLICATIONS

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Mu D., Chen L., Zhang X., See L.-H., Koch C.M., Yen C., Tong J.J., Spiegel L., Nguyen K.C.Q., Servoss A., Peng Y., Pei L., Marks J.R., Lowe S., Hoey T., Jan L.Y., McCombie W.R., Wigler M.H., and Powers S. 2003. Genomic amplification and oncogenic properties of the *KCNK9* potassium channel gene. *Cancer Cell* **3:** (in press).

David Helfman's lab is working to understand how oncogenes disrupt a cell's cytoskeleton, the internal framework that gives a cell its shape and strength and controls its interactions with neighboring cells. Cancer is due in part to the loss of such controlled interactions among neighboring cells, a loss of control which enables cells to break free from their biological moorings and become metastatic.

Most cancers arise from epithelial cells, which line organs and body cavities. Senthil Muthuswamy's lab is investigating how cells in healthy breast epithelium form hollow spherical structures, and how cancer-causing genes, called oncogenes, cause the cells in these structures to proliferate out of control and metastasize.

Tatsuya Hirano's lab studies the function of proteins that act to modulate large-scale chromosome structure and behavior. One of these proteins, called condensin, is required for the condensation or compaction of chromosomes that occurs prior to cell division. Another, called cohesin, holds chromosome pairs together until the time is right for the pairs to separate and for each newly freed chromosome to migrate into one or the other cell as one cell divides and becomes two.

To better grasp how genes direct cell function, David Spector's lab has engineered a cell line that allows them to watch DNA, RNA, and proteins in concerted action. Carefully designed molecules allow them to see RNA transcribed from DNA and then to visualize proteins translated from the RNA. Spector and his colleagues have provided the first glimpses of the entire process of gene expression in living cells.

Arne Stenlund focuses on the events that trigger DNA replication and is collaborating with X-ray crystallographer Leemor Joshua-Tor to unravel the crucial first steps during the replication of papillomavirus DNA. Papillomavirus is associated with virtually all cases of cervical cancer. By studying the biochemical and structural changes that initiate viral DNA replication, Stenlund and Joshua-Tor have revealed key details not only about this cancer-causing virus, but also about the process of DNA replication in general. Moreover, these studies have uncovered specific molecular targets for the development of drugs to prevent or treat cervical cancer.

Bruce Stillman's lab explores events that initiate cellular rather than viral DNA replication. One focus of their research is a protein complex called ORC that attaches to DNA and both triggers DNA replication and silences gene expression. This year, Stillman's lab has discovered new roles for ORC in chromosome segregation (the sorting of chromosomes during cell division such that each cell receives a complete set of chromosomes) and in cytokinesis (the process in which cells divide as one cell becomes two). As the researchers piece together the multiple roles of ORC and its various protein partners, they are uncovering vital clues concerning how the control of DNA replication and cell proliferation is lost in cancer cells.

Nicholas Tonks's lab studies particular enzymes—protein tyrosine phosphatases—which typically function to counteract the effects of growth factors and hormones, thereby helping to control cell growth. Some protein tyrosine phosphatases, or PTPs, have been linked to diseases including cancer and diabetes. This year, Tonks's lab has shown that decreasing the level of PTP1B in cells may be an effective approach to treating cancer.

Proteins called Ras and Rap have well-defined, antagonistic growth-promoting and growth-inhibiting functions in non-neuronal cells which, when altered, can lead to cancer. Linda Van Aelst and Roberto Malinow have discovered intriguing roles for Ras and Rap in cells of the nervous system. Van Aelst, Malinow, and their colleagues have shown that Ras mediates the addition of AMPA receptors to synapses during LTP (long-term potentiation of synaptic activity), whereas under conditions that induce LTD (long-term depression of synaptic activity), Rap mediates the removal of AMPA receptors from synapses. Moreover, Van Aelst and Malinow established a differential effect of Ras and Rap on different types of AMPA receptors. The resulting dynamics of AMPA receptor replacement at synapses is likely to be a principal mechanism of learning and memory.

THE CYTOSKELETON AND ONCOGENIC TRANSFORMATION

D.M. Helfman E. Araya S.-W. Lee
L. Connell G. Pawlak
E. Kim

We are interested in the role of the cytoskeleton in oncogenesis and tumor suppression. The importance of the cytoskeleton in oncogenesis was first inferred from studies almost 30 years ago showing that disruption of actin filament bundles (stress fibers) is a common feature following transformation by various oncogenes. These changes in the cytoskeleton are a result of changes in the expression of both specific cytoskeletal proteins and signaling molecules, e.g., small GTPases and kinases. The changes in the cytoskeleton are more than simply a generic consequence of cell reorganization characteristic for oncogenic transformation. For example, work by our lab and others has shown that ectopic expression of specific cytoskeletal proteins can suppress many features of transformation, including disruption of microfilament bundles and focal adhesions, loss of contact-inhibited cell growth, ability to grow in soft agar, and tumorigenicity in nude mice, indicating that the loss of specific cytoskeletal proteins has a direct role in oncogenesis. However, the mechanisms by which specific components of actin structures are targeted and deregulated by oncogenes are unknown, and, more importantly, how the accompanying changes in actin filament organization contribute mechanistically to oncogenesis remains to be established. We are studying the molecular mechanisms involved in oncogene-mediated disruption of the cytoskeleton and how the actin cytoskeleton participates in pathways linked to transformation. Below is a description of our studies during the past year to better understand the role of the cytoskeleton in normal and transformed cells.

Anchorage-independent Activation of ERK1/2 Depends on MLCK-mediated Regulation of Myosin II

G. Pawlak

It has been suggested that oncogenes induce anchorage-independent growth by substituting for cell adhesion, therefore causing aberrant activation of signaling pathways that are normally regulated by adhesion. Activation of the ERK1/2 pathway notably becomes adhesion-independent in Ras-transformed fibroblasts (NRK/*ras* cells). Since adhesion-dependent signaling itself is regulated in part by the actin cytoskeleton, we investigated the potential role played by the actin cytoskeleton in the anchorage-independent activation of ERK1/2 observed in NRK/*ras* cells. For that purpose, we used specific pharmacological agents to inhibit myosin II function or disrupt the actin cytoskeleton. We then studied activation of ERK1/2 in NRK/*ras* cells. We found that activation of ERK1/2 was strictly dependent on myosin light-chain kinase (MLCK) activity, since inhibition of this kinase by ML7 or ML9 leads to a complete inhibition of ERK1/2 phosphorylation, even when NRK/*ras* cells are kept in suspension. On the other hand, inhibition of Rho-kinase did not have any effect on the level of ERK1/2 phosphorylation. We also found that this effect was specific for ERK1/2 since phosphorylation of Akt by phosphatidylinositol 3-kinase (PI3K) was not affected by inhibition of MLCK. MLCK is a known substrate of ERK1/2, raising the interesting possibility that an autocrine loop takes place in NRK/*ras* cells, in which the MLCK-dependent actomyosin contractility allows constitutive activation of the ERK pathway, which, in turn, keeps the MLCK active, irrespective of the adhesion state of the cells. We are currently trying to define the step along the Ras-Raf-MEK-ERK pathway that is dependent on MLCK activity to obtain some insight into the underlying mechanism.

The Cytoskeleton and Programmed Cell Death

L. Connell

The association of cells with the extracellular matrix (ECM) has an important role in maintaining cell survival. This interaction between a cell and the ECM generates signals from focal adhesions that promote growth and survival. Activation of myosin II is necessary for the formation of focal adhesions. When normal cells detach from the ECM, they undergo a par-

ticular kind of apoptosis due to loss of survival signals, called anoikis. Transformed cells do not undergo anoikis, and loss of anchorage dependence in cells correlates with tumor growth. Presumably, transformed cells can activate signals that would normally emanate from cell attachment to the ECM. We are interested in elucidating the pathway between cell attachment to the ECM and activation of actomyosin contractility (myosin II) and generation of survival signals. By utilizing pharmacological agents and mutant proteins that can disrupt actin filament organization and inhibit myosin function, we are studying the role of the actin cytoskeleton and myosin in cell survival in normal and transformed epithelial cells. Integrins also have an important role in the generation of adhesion-dependent signaling. The expression and activation states of different integrins in normal and transformed epithelial cells are also being investigated to understand what role integrins may have in anchorage-independent cell survival.

Mechanisms Underlying Ras-induced Morphological Transformation and Inactivation of Rho-dependent Stress Fiber Formation

S.-W. Lee

Studies in our lab during the past year have investigated the signaling pathways by which oncogenic *ras* and v-*src* disrupt the actin cytoskeleton (Pawlak and Helfman 2002a,b). These studies demonstrated that the sustained MAPK signaling in response to both types on oncogenic stimuli results in down-regulating the activity of Rho-kinase, a Rho effector required for stress fiber formation (see Fig. 1). Thus, a critical function of sustained MAPK signaling is to uncouple Rho-GTP from stress fiber formation. Furthermore, these studies demonstrate that these functionally different oncogenes, namely, the small GTPase *ras* and the tyrosine kinase v-*src*, use the same signaling pathway to achieve morphological transformation of fibroblasts, i.e., inactivation of Rho-mediated stress fiber assembly through sustained activation of the ERK pathway. However, the mechanism responsible for suppression of Rho-kinase activity was not fully established. We have continued to study the mechanism by which activation of MAPK leads to diminished Rho-kinase activity. During the past year, we have found a potential role for $p21^{Cip1/Waf1}$ in the regulation of cytoplasmic Rho-kinase (ROCK).

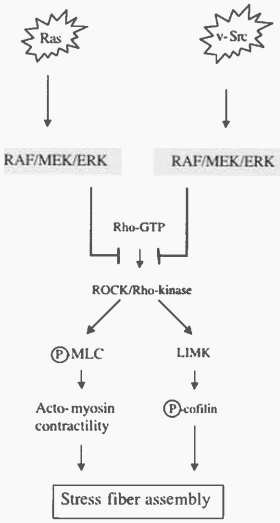

FIGURE 1 Constitutive MAP-kinase signaling uncouples Rho-GTP from stress fiber formation.

$p21^{Cip1/Waf1}$ is best known as a broad-specificity inhibitor of cyclin/CDK complexes, but, paradoxically, the level of $p21^{Cip1/Waf1}$ expression is highly increased in various human cancers. In recent years, accumulating evidence has suggested that $p21^{Cip1/Waf1}$ located in the cytoplasm might have a role in promoting transformation and tumor progression. Using NIH-3T3 fibroblasts, we have found that activation of Ha-RasV12 causes a loss of actin stress fibers and focal contacts. Ras-mediated transformation was also accompanied by induced expression of $p21^{Cip1/Waf1}$. Immunofluorescence studies showed that more than 90% of the cells expressed $p21^{Cip1/Waf1}$ in the cytoplasm as well as in the nucleus. Recently, Tanaka and colleagues reported that cytoplasmic $p21^{Cip1/Waf1}$ has a role in regulation of neurite remodeling by inhibiting ROCK activity in the cytoplasm. Accordingly, we examined the hypothesis that $p21^{Cip1/Waf1}$ contributes to the disruption of actin stress fibers in Ras-transformed cells by targeting the ROCK/LIMK/cofilin pathway. We consistently observed a decrease in the phosphorylation level of cofilin in Ha-RasV12-transformed cells, which is indicative of a compromised ROCK/LIMK/cofilin

pathway. Interestingly, inhibition of MEK using U0126, in Ha-RasV12-transformed NIH-3T3 cells, caused a decrease in $p21^{Cip1/Waf1}$ protein expression, followed by an increased level of phosphorylated cofilin, which is indicative of a restored ROCK/LIMK/cofilin pathway, resulting in the restoration of actin stress fibers. Furthermore, restoration of stress fiber by inhibition of MEK in Ha-RasV12 cells was blocked following ectopic expression of a mutant form of $p21^{Cip1/Waf1}$ that did not have the nuclear localization signal and therefore was localized in the cytoplasm. Coimmunoprecipitation experiments also showed that $p21^{Cip1/Waf1}$ interacts with ROCK in vivo. Taken together, these results suggest that $p21^{Cip1/Waf1}$ is a novel regulator of actin remodeling by inhibiting ROCK activity in Ras-transformed cells.

Characterization of the Metastasis-associated Protein, S100A4

E. Kim

Elevation in expression of the small calcium-binding protein S100A4 has been correlated with increased invasiveness and with a worse prognosis in the case of breast cancer. In vitro biochemical assays have suggested calcium-dependent protein-protein interactions between S100A4 and three cytoskeletal proteins: actin, tropomyosin, and myosin. We have confirmed a calcium-dependent interaction between S100A4 and the heavy chain of nonmuscle myosin in vivo. Specifically, we have demonstrated that S100A4 interacts with the myosin heavy-chain II-A isoform preferentially over the II-B isoform in vivo. A mutant S100A4 that is incapable of binding to calcium is unable to interact with myosin heavy-chain II-A. Through the use of mutants singly defective in calcium binding at either of the two EF-hand motifs, we have found that the lower-affinity site dictates full function. Furthermore, we have determined through the study of these mutants deficient in calcium binding that S100A4 homodimerization is dependent on the monomeric calcium-binding status.

To further study the S100A4 protein, we have performed immunofluorescence studies of the endogenous protein in the aggressively turmorigenic breast cancer cell line, MDA-MB-231. When plated on collagen, these cells demonstrate a polarized morphology indicative of motile cells. S100A4 localizes to the leading edge of these polarized cells. It also consistently colocalizes with known markers or effectors of lamellipodial formation. Interestingly, sublines stably expressing tagged forms of either wild type or a calcium-binding-deficient mutant also localize to the same leading-edge region. Further evidence that this localization is not through a calcium-dependent interaction with myosin heavy-chain II-A comes from preliminary immunofluorescence studies in COS cells which lack myosin heavy-chain II-A, yet demonstrate similar localization to the leading edge.

Molecular Organization and Regulation of Actin Filaments

E. Araya

In nonmuscle cells, the formation of actin filaments and their assembly into various structures, e.g., stress fibers, contractile ring, filopodia, and lamellipodia, are dynamic processes. How these different structures are formed and regulated within a single cell is not known. In addition to actin and its associated motor molecules (myosin I and II), other actin-filament-associated proteins, such as tropomyosin, have essential roles in the assembly, function, and regulation of these structures. We have begun to use RNA interference (RNAi) to inhibit the expression of specific isoforms of tropomyosin as well as other cytoskeletal proteins. By using RNAi technology, we plan to carry out a systematic screen targeted to structural and regulatory components of the cytoskeleton. These studies will determine the effects of disrupting the expression of specific proteins. For example, we will determine if loss in the expression of specific proteins leads to changes in the structure of the actin cytoskeleton, focal contacts, and cell-cell contacts, as well as the motile and growth properties of cells. It is anticipated that these studies will identify key components of the cytoskeleton involved in cell motility and growth control.

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HIGHER-ORDER CHROMOSOME DYNAMICS

T. Hirano O. Cuvier A. Losada
P. Gillespie T. Ono
M. Hirano

Our laboratory is interested in understanding the molecular mechanisms by which mitotic chromosomes are assembled and segregated during the cell cycle. Our efforts are focused on the structural and functional analyses of two multisubunit protein complexes—cohesin and condensin—that have central roles in sister chromatid cohesion and condensation, respectively. Each of the complexes contains a different pair of SMC (structural maintenance of chromosomes) ATPase subunits, implicating a mechanistic similarity between cohesion and condensation. SMC proteins are also conserved in most bacterial and archaeal species. It is therefore anticipated that elucidating the action of this class of proteins is the key to understanding the evolutionarily conserved, fundamental aspects of higher-order chromosome dynamics.

Regulation of Cohesion and the Establishment of Metaphase Chromosomes

A. Losada, T. Hirano

The establishment of metaphase chromosomes is an essential prelude to the synchronous and accurate segregation of sister chromatids in anaphase. In metazoans, this event involves at least two distinct processes. The first process is the loosening of sister chromatid cohesion, which accompanies the dissociation of most cohesin from chromatin in prophase. The second is the initiation of compaction, which is triggered by the association of condensin with chromatin. Although it is widely believed that the former process may be the prerequisite of the latter, this idea has never critically been tested before. We have used *Xenopus* egg cell-free extracts to address the mechanistic relationship between the two processes. We show that the prophase release of cohesin is completely blocked when two mitotic kinases, polo-like kinase (Plx1) and aurora B, are simultaneously depleted from the extracts. To our surprise, we find that condensin is loaded onto chromatin despite the presence of abnormally high levels of cohesin and that rod-shaped chromosomes are produced with an apparently normal degree of compaction (Fig. 1). Importantly, however, the resolution of sister chromatids within these chromosomes is severely compromised. Depletion of Plx1 and aurora B has little effect on the replication of chromosomal DNA or on the activity of topoisomerase II that decatenates intertwined sister DNAs. It is therefore most likely that the observed defect in sister chromatid resolution is a direct consequence of the failure of cohesin release from chromatin. Plx1 and aurora B phosphorylate cohesin and histone H3, respectively, in vitro, suggesting that the two kinases cooperate to destabilize sister chromatid linkage through distinct mechanisms. Thus, our results strongly suggest that the bulk dissociation of cohesin is essential for sister chromatid resolution, but not for condensin-mediated compaction.

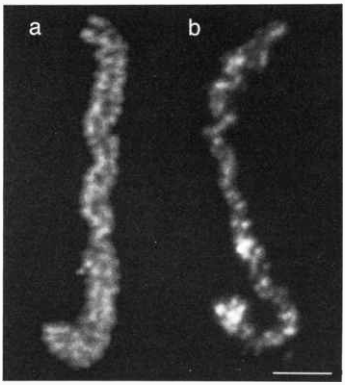

FIGURE 1 The resolution of sister chromatids is defective in the absence of Plx1 and aurora B. Sister chromatids are well-resolved in a metaphase chromosome assembled in the control extract (*a*), whereas they cannot be distinguished from each other in a chromosome assembled in the Plx1/aurora B-depleted extract (*b*). Bar, 2 μm.

Establishment of Sister Chromatid Cohesion

P. Gillespie, T. Hirano

In eukaryotic cells, the physical linkage between duplicated chromatids (sister chromatid cohesion) is constructed during S phase, and the cohesin complex is part of the molecular "glue" that holds the sister chromatids together. Current models suggest that this process involves at least two steps: the binding of cohesin to chromatin, and the establishment of cohesion that presumably accompanies a conformational change or activation of cohesin. Although regulatory components required for each step have been identified genetically in yeast and fungi, it remains to be determined how the process is accomplished at a mechanistic level. To address this question, we take advantage of *Xenopus* cell-free extracts in which the whole process of sister chromatid cohesion can be reconstituted in vitro. Our effort is now focused on the functional characterization of two conserved cohesion factors, Scc2 and Eco1. Scc2 is implicated in directing cohesin's association with chromatin, whereas Eco1 facilitates the establishment step by an unknown mechanism. As an initial attempt, we have cloned the corresponding *Xenopus* cDNAs by reverse transcriptase–polymerase chain reaction (RT-PCR). Interestingly, we find that *Xenopus* has two isotypes of each protein. A database search reveals that this is also the case in humans. We have prepared a panel of antibodies against individual polypeptides to further investigate the cellular localization and functions of Scc2 and Eco1. Moreover, we set up a series of experiments to determine how the establishment of cohesion might functionally be coupled to DNA replication. Preliminary evidence suggests that cohesin is loaded onto chromatin at a stage between replication licensing and elongation.

Role of Topoisomerase II in Linking DNA Replication to Chromosome Condensation

O. Cuvier, T. Hirano

Topoisomerase II (topo II) is an ATP-dependent, DNA-strand-passing enzyme that passes one duplex through a transient break in another and rejoins the break in vitro. The condensin complex has a different activity that introduces a superhelical tension into DNA in an ATP-dependent manner. Previous studies suggested that topo II and condensin are both required for mitotic chromosome condensation in *Xenopus* egg cell-free extracts. We are interested in understanding how the two different molecular activities might contribute to chromosome condensation and what the exact roles of condensin and topo II might be in organizing higher-order chromosome structure. To this end, we have used the egg extracts in which mitotic chromosome assembly can be induced in the absence or presence of preceding DNA replication. When unreplicated chromatin is directly incubated with a mitotic extract, condensin and topo II must function together to assemble mitotic chromosomes. In contrast, when chromosome assembly is induced after DNA replication, the requirement for topo II can temporarily be separable from that of condensin. This experimental setting allows us to find that in the absence of condensin, topo II becomes enriched in an axial structure within uncondensed chromatin. Subsequent addition of condensin converts this structure into mitotic chromosomes in an ATP-hydrolysis-dependent manner. Strikingly, prevention of DNA replication by the addition of geminin or aphidicolin disturbs the formation of topo-II-containing axes and alters the binding property of topo II with chromatin. Our results suggest that topo II has an important role in an early stage of chromosome condensation and that this function of topo II is tightly coupled with prior DNA replication.

Condensin Functions In Vivo

T. Ono, T. Hirano

The condensin complex is composed of two SMC subunits (CAP-E/SMC2 and CAP-C/SMC4), and three non-SMC subunits (CAP-D2, CAP-G, and CAP-H). Although our previous work demonstrated that condensin has a central role in establishing and maintaining mitotic chromosome structure in *Xenopus* egg cell-free extracts, much less is known about how the complex contributes to these processes in vivo. We have taken three different approaches to address this question in vertebrate cells. First, immunofluorescent microscopy is used to obtain more insight into the spatial and temporal regulation of condensin. Our previous work using a phosphospecific antibody showed

that threonine-1314 of XCAP-D2 (*Xenopus* CAP-D2) is phosphorylated in a mitosis-specific, *cdc2*-dependent manner in vitro. Immunofluorescent staining of *Xenopus* tissue culture cells shows that this phosphorylated epitope appears on chromosomes in prophase and is maintained until metaphase. Interestingly, however, the epitope becomes undetectable in anaphase, suggesting that the phosphorylation status of condensin is changed at the metaphase-anaphase transition. Second, we have established stable HeLa cells expressing epitope-tagged subunits of the condensin complex. We are currently expressing mutant forms of the subunits to test whether different mutations may cause specific, dominant-negative phenotypes. Third, we use an RNA interference (RNAi) technique to repress the expression level of individual condensin subunits. We find that cells depleted of different subunits show distinct abnormalities, suggesting that each subunit may have a different role in the formation of higher-order chromosome structure in mitosis.

Structural and Functional Dissection of SMC Proteins

M. Hirano, T. Hirano

SMC proteins are highly conserved among the three phyla of life. To understand the basic action of this unique class of chromosomal ATPases, we use the SMC homodimer from the gram-positive bacterium *Bacillus subtilis* (BsSMC) as a simple model system. As judged by electron microscopy, BsSMC is composed of two antiparallel, coiled-coil arms with a flexible hinge. It has not been established, however, how the two subunits dimerize to make such a two-armed molecule. By using a site-directed cross-linking approach, we show that dimerization of BsSMC is mediated by a hinge-hinge interaction between self-folded monomers. A coiled-coil interaction assay shows that this architecture is also conserved in the eukaryotic SMC2-SMC4 heterodimer. Analysis of different deletion mutants of BsSMC unexpectedly reveals that the major DNA-binding activity does not reside in the catalytic ATPase domains located at the ends of a dimer. Instead, point mutations in the hinge domain that disturb dimerization of BsSMC drastically reduce its ability to interact with DNA. Furthermore, we find that proper hinge function is essential for BsSMC to recognize distinct DNA topology and that mutant proteins with altered hinge structure cross-link double-stranded DNA in a nucleotide-dependent manner. We propose that the hinge domain of SMC proteins is not a simple dimerization site; rather, it acts as an essential determinant of dynamic SMC-DNA interactions.

Single-molecule Manipulation

T. Hirano, M. Hirano [in collaboration with T. Strick, Cold Spring Harbor Laboratory]

In collaboration with Terence Strick's group here at CSHL, we use a single-molecule manipulation technique to study the interaction of condensin and BsSMC with DNA. This powerful technique should provide us with the mechanistic information as to how the SMC protein and protein complexes change the structure of DNA at an unprecedented resolution.

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EPITHELIAL CELL BIOLOGY AND CANCER

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EARLY EVENTS IN BREAST CANCER

Almost all malignant breast cancers originate from epithelial cells. Several oncogenes and tumor suppressor genes have been implicated in breast cancer; however, we do not understand the mechanisms by which oncogenes initiate transformation of mammary epithelial cells (MECs) that line the ducts and alveoli in the breast. Early lesions, characterized by uncontrolled proliferation and disruption of epithelial architecture, are currently defined by histological features, and their prognosis is imprecisely determined. Although lesions within a category look alike, they must possess molecular differences that allow some to become malignant and others to remain benign. Understanding the molecular mechanisms involved in initiation of carcinoma will not only identify early diagnostic markers, but also identify drug targets for treating premalignant disease.

Conventional transformation assays (foci formation, soft agar growth, and tumor formation in nude mice) do not allow us to investigate the cell and molecular changes that take place in polarized epithelia that line the ducts and alveoli in vivo. We have adapted a cell culture technique where nontransformed MECs form three-dimensional acinus-like structures containing a single layer of polarized, growth-arrested epithelial cells when grown within a matrix rich in laminin and collagen (Matrigel). The epithelial cells in these structures, unlike cells grown on plastic dishes, share several properties with those that line the ducts and lobules in an adult breast. Interestingly, tumor-derived epithelial cell lines or epithelial cell lines transformed in culture fail to form acinus-like structures in three dimensions. They either form filled spheroids or do not growth-arrest or both, suggesting that acinus-like structures formed by nontransformed MEC possess unique properties that are lost during transformation.

Oncogenes in the ErbB family of receptor tyrosine kinases have important roles in breast cancer. Among its four members, ErbB2 overexpression is observed in 25–35% of breast cancers and correlates with poor clinical prognosis. ErbB2 is targeted for therapy using anti-ErbB2 antibodies, i.e., Herceptin. ErbB3 is coverexpressed with ErbB2. Despite the compelling evidence for a role of the ErbB family in breast cancer, it has been challenging to decipher the signaling and biological specificities of ErbB receptor activation, because MECs express multiple ErbB receptors and homo- and heterodimerization of ErbB receptors complicate our ability to determine how specific ErbB dimers transform mammary epithelia.

We have developed a novel method to control dimerization and activation of ErbB receptors without contribution from endogenous receptors. This method, for the first time, will allow us to dissect out the mechanisms by which specific ErbB homo- and heterodimers transform normal mammary epithelial cells. We have successfully integrated the controlled dimerization strategy and the three-dimensional cell culture method. Using this powerful and unique system, we can now activate specific ErbB dimers of choice in three-dimensional epithelial structures that share several properties with cells that line the ducts and alveoli in vivo. We believe that this approach will take us a step closer to conditions in vivo and will allow us to uncover molecular mechanisms involved in initiation of transformation that cannot be readily determined under standard culture conditions or with the use of animal models.

ONCOGENESIS USING THREE-DIMENSIONAL ACINI

Activating ErbB Receptors in Three-dimensional Acini: We have previously analyzed the differential effects of activating ErbB1 and ErbB2 receptors in preformed, growth-arrested, polarized acinar structures to mimic the conditions in which ErbB receptors are amplified and activated in vivo. Activation of ErbB2 in preformed mammary epithelial acini results in reinitiation of proliferation, loss of polarized organization, and formation of structures containing multiple acinar units. Each acinus within these structures had a filled lumen, surrounded by an intact basement membrane, and did not display any invasive properties. The phenotype displays aspects of a premalignant stage of breast cancer in vivo referred to as carcinoma

in situ, suggesting that activating oncogenes in three-dimensional acini in culture will allow us to identify the mechanisms that are involved in early stages of cancer.

Our recent studies suggest that coactivation of ErbB2 and ErbB3 not only induces reinitiation of proliferation and formation of multi-acinar structures, but also induces invasive behavior. Coactivation of ErbB1 and ErbB3 did not induce invasive properties, suggesting that ErbB2/ErbB3 dimers possess the unique ability to induce tumor progression. Our results are consistent with clinical reports suggesting that coexpression of ErbB2 and ErbB3 in primary breast tumors correlates with poor clinical prognosis. Thus, three-dimensional epithelial acini can be used to identify early events in cancer and also to investigate mechanisms involved in cancer progression.

Effect of Activating Receptor Tyrosine Kinases in Three-dimensional Acini: We have begun to systematically analyze the effect of activating different receptor tyrosine kinases in three-dimensional epithelial cell acini. It is likely that we will be able categorize receptor tyrosine kinases on the basis of the ability to affect the three-dimensional organization of acini, reinitiate proliferation, induce filling of the lumen, and induce invasive properties and disruption of epithelial cell polarity. Such categorization will allow us to identify the mechanisms shared by receptor tyrosine kinases to transform three-dimensionally organized epithelial cells.

Cell Cycle Regulation on Three-dimensional Acini: The mechanism by which oncogenes reinitiate proliferation in growth-arrested epithelial acini is not understood. We have begun to investigate how activation of ErbB2 induces reinitiation of proliferation. Our initial observations suggest that activation of ErbB2 induces down-regulation of a cell cycle inhibitor p27/Kip1 and a concomitant up-regualtion of Skp2, a component of the SCF/Cullin/F-Box (SCF) complex. It has been shown that growth-promoting factors such as serum induce down-regulation of p27 by regulating the function of SCF complex. Whether ErbB2 regulates the SCF complex to effect proliferation of epithelial cells is not known. The relationship between ErbB2 and the SCF complex in primary breast tumors is not well understood.

ErbB RECEPTORS AND EPITHELIAL CELL POLARITY

Activation of ErbB2 but not ErbB2 induces disruption of cell polarity as monitored by its ability to induce mislocalization of tight-junction-associated proteins ZO-1 and AF-6 and apical membrane-associated protein GP135.

Recent studies have identified three protein complexes conserved in *Drosophila, Caenorhabditis elegans*, and mammals that regulate establishment of epithelial cell polarity: the Par3/Par6/aPKC/CDC42 complex, crumbs/Pals1/PATJ complex, and Scribble/Lgl/Dlg complex. It is thought that these protein complexes regulate formation of polarity in an interdependent manner. It is not known whether oncogenes that disrupt the architecture of epithelial cells regulate protein complexes involved in the formation of cell polarity. Our results suggest that activation of ErbB2 induces mislocalization of the Par complex in polarized epithelial cells. In addition, activation of ErbB2 also regulates the biochemical composition of the Par3/Par6/aPKC complex. We are in the process of investigating how activation of ErbB2 affects the composition of the Par complex and will determine the role the Par complex has in ErbB2-induced transformation of epithelial cells.

We propose that understanding how oncogenes deregulate proliferation and disrupt polarized architecture of growth-arrested, polarized epithelial cells will allow us to identify novel mechanisms that regulate early events in epithelial cancers.

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CELL BIOLOGY OF THE NUCLEUS

D.L. Spector Y.-C.M. Chen, L. Denis, Y. Fang, S. Hearn, S.M. Janicki, P. Kannanganattu, G. Lark, P. Sacco-Bubulya

Most cellular processes can trace their beginnings to the nucleus where a gene is activated resulting in the production of an RNA molecule which must get processed and transported to the cytoplasm. Although many genes are constitutively expressed, a large number are up-regulated and down-regulated at various times during the cell cycle and development. *Trans*-acting factors have the daunting task of locating and gaining access to specific genes within the three-dimensional nuclear space. Altering the transcriptional status of a particular gene involves a series of events including chromatin remodeling and the recruitment of a large number of factors involved in either transcription/RNA processing/export or silencing events. However, a failure of the correct set of factors to associate with their target substrates at the correct time will result in changes in the gene expression program which can lead to a disease phenotype. Although much biochemical information is available regarding some of the factors involved in these processes, the spatial and temporal aspects of the regulation of gene expression/silencing and the dynamics of the nuclear domains that they occupy are less well understood. During the past year, we have focused a significant amount of our efforts on two main areas: (1) the development of a system to directly visualize gene expression in living cells, and (2) characterization of nuclear speckles and PML nuclear bodies.

VISUALIZING GENE EXPRESSION IN LIVING CELLS

We have developed a mammalian cell line that allows us to directly visualize a stably integrated inducible genetic locus as well as its RNA and protein products in living cells. The locus is based on a 20-kb plasmid that contains at its 5′ end *lac* operator repeats that when bound by a cyan fluorescent protein (CFP)-Lac repressor fusion protein allows the integration site to be visualized. A tetracycline-responsive element enables the minimal cytomegalovirus (CMV) promoter to be regulated. Transcription by RNA polymerase II (pol II) results in an RNA containing stem loop structures that are specifically recognized by the bacteriophage MS2-binding protein. Expression of MS2-binding protein fused to yellow fluorescent protein (YFP) containing a nuclear localization signal allows us to see the RNA as it is being transcribed. The RNA encodes CFP with a peroxisome-targeting signal to allow the protein product of this transcription unit to be visualized as it concentrates in cytoplasmic peroxisomes. Using this system, we have observed the dynamics of proteins associated with inactive (i.e., HP1, Suv39h1) as well as active (i.e., VP-16, RNA pol II) chromatin. Activator arrival results in extensive chromatin decondensation as well as initiation of transcription (Fig. 1). Within 5–10 minutes of transcription initiation, we can detect nascent RNA at the transcription site in living cells. During this time period, the nucleoplasmic pool of MS2-binding protein changes from a diffuse distribution to a granular distribution suggestive of the formation of mRNP particles. In addition, we have observed components of the RNA processing machinery, which localize in different nucleoplasmic regions, recruited to the transcription site. In addition to visualizing the dynamics of transcription/RNA processing, we have also been able to characterize the reorganization of the genetic locus during its transition from a transcriptionally inactive state to an active state. This system provides a powerful approach to examine the dynamics and coordination of gene expression in the context of the living cell. Ongoing studies in the laboratory are utilizing this system to examine the spatial and temporal events of gene expression, silencing, and DNA replication.

NUCLEAR BODIES

Although the cell nucleus was once thought of as an amorphous milieu containing little organization or

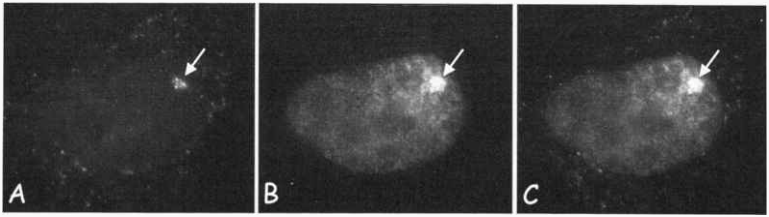

FIGURE 1 Visualization of a stably integrated genetic locus in the transcriptionally active state. The 4-Mb locus appears decondensed (arrow, *left panel*) and the protein product of the locus is concentrated in cytoplasmic peroxisomes. Messenger ribonucleoprotein (mRNP) particles are observed concentrated at the locus (arrow, *middle panel*) as well as distributed in a granular pattern in the nucleoplasm. Overlay of the two images is visualized in the right panel. Photo provided by Susan M. Janicki (CSHL).

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: PML (promyelocytic leukemia) nuclear bodies and nuclear speckles.

PML nuclear bodies 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 bodies 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 differentiate between these possibilities, we have taken a biochemical approach and have developed a strategy to purify and biochemically characterize these nuclear bodies. Thus far, we have obtained a nuclear fraction enriched in these bodies, and ongoing studies are attempting to further purify PML bodies and determine their complete protein composition using mass spectrometry.

RE-ESTABLISHING THE INTERPHASE NUCLEUS AFTER MITOSIS

Pre-mRNA splicing factors are localized in a speckled pattern, set within a diffuse nuclear distribution, when mammalian cell nuclei are examined by immunofluorescence microscopy using anti-splicing-factor antibodies. At the electron microscopic level, this localization pattern corresponds to interchromatin granule clusters and perichromatin fibrils. Splicing factors are recruited from interchromatin granule clusters to regions of active transcription (perichromatin fibrils), where they function in cotranscriptional pre-mRNA processing. We have previously observed this recruitment in living cells by time-lapse microscopy of green fluorescent protein (GFP)-tagged splicing factors. Although there is a continuous flow of GFP-tagged splicing factors in and out of nuclear speckles, these structures, as well as their positions within the nucleus, are maintained for many hours. We would like to understand how interchromatin granule clusters (IGCs) are established and organized within the nucleus and elucidate their relationship to specific aspects of gene expression.

Interestingly, mammalian cell nuclei undergo many changes upon entry into mitosis, including chromatin condensation, termination of transcription, and disassembly of nuclear structures such as the nuclear lamina, nucleoli, nuclear speckles, and other nuclear

bodies. When mitosis is completed, the chromatin decondenses, transcription begins, and nuclear structures are reassembled in daughter nuclei. We have been interested in how the pre-mRNA processing machinery is reorganized into nuclear speckles in daughter nuclei, and how this reassembly is coordinated with reactivation of gene expression. We have discovered a novel pattern of splicing factor localization in daughter nuclei during telophase, prior to reformation of nuclear speckles. Splicing factor SF2/ASF transiently localizes in a ring of patches surrounding transcriptionally active nucleolar organizing regions (NORs) that contain UBF, fibrillarin, and rRNA transcripts. The patches differ from mature nuclear speckles in that they do not contain stable poly(A)$^+$ RNA or hyperphosphorylated SR proteins. A HeLa cell line stably expressing YFP-SF2/ASF was used to examine the dynamics of the patches in living cells. Fluorescence recovery after photobleaching (FRAP) has revealed a turnover of YFP-SF2/ASF within this structure. As YFP-SF2/ASF nuclear entry proceeds, we observe a "wave" of YFP-SF2/ASF entry that originates at the patches and progresses through the nucleus. We have also observed that YFP-SF2/ASF telophase patches fragment, traverse the nucleoplasm, and develop into nuclear speckles in G_1 nuclei. Intriguingly, we colocalized protein phosphatase 1 with SF2/ASF in the patches. On the basis of these findings, we propose that localized phosphatase activity may dephosphorylate SR proteins upon their nuclear entry, initiating RS domain–RS domain interactions and facilitating formation of the patches as a "seed" for nuclear speckle assembly in daughter nuclei.

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MOLECULAR BIOLOGY OF PAPILLOMAVIRUSES

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

An impediment to the study of papillomaviruses has been the inability to define simple in vitro cell culture systems for analysis of the viral life cycle. These viruses normally require specialized differentiating cells that only with difficulty can be generated in cell culture. However, for a bovine papillomavirus (BPV-1), a convenient cell culture system exists where viral gene expression, oncogenic transformation, and viral DNA replication can be studied. Thus, BPV has become a useful model for these aspects of the viral life cycle. The DNA replication properties of the papillomaviruses show some unique and interesting characteristics. As part of their normal life cycle, these viruses can exist in a state of latency, which is characterized by maintenance of the viral DNA as a multicopy plasmid in infected cells. The copy number of the viral DNA is tightly controlled, and the viral DNA is stably inherited under these conditions. Papillomaviruses therefore provide a unique opportunity to study plasmid replication in mammalian cells. In addition, the viral DNA replication machinery represents one of the most promising targets for antiviral therapy.

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

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

STRUCTURAL CHANGES IN THE ORI INDUCED BY BINDING OF E1

The E1-binding site in the ori consists of an 18-bp inverted repeat. This E1-binding site actually is composed of multiple E1 recognition sequences that are arranged in an overlapping array, and we have demonstrated that a monomer of E1 recognizes the hexanucleotide sequence AACAAT, a sequence which, with slight variations, occurs six times in the 18 bp. Four of the sites are arranged pair-wise and are bound pairwise by dimers of E1 DBD (DNA-binding domain). To determine whether the overlapping sites could bind E1 simultaneously. we examined DNA binding by the E1 DBD utilizing high-resolution hydroxyl radical footprinting. The results from these studies demonstrated that binding of the E1 DBD to the E1-binding sites follows a particular sequence. At low concentration, a dimer of E1 DBD binds to sites 2 and 4. At

higher concentrations of E1 DBD, the overlapping sites 1 and 3 are also filled, generating a structure where two dimers of the E1 DBD bind to the ori but on different faces of the DNA helix. Two types of observations indicate that the binding of four molecules of E1 results in significant changes in the DNA structure. First, formation of a stable complex containing four molecules of E1 occurs only on short probes, indicating that the ability to deform the DNA is of importance for complex formation. Second, the binding of four E1 DBD molecules results in significant OH radical hypersensitivity outside the ori. These results indicate that DNA binding by the E1 DBD provides initial structural changes in the template likely related to subsequent melting of the ori. Although full-scale melting depends on ATP hydrolysis by E1, limited base opening can be observed upon DNA binding by the full-length E1 protein even in the absence of ATP.

STRUCTURE OF E1 DBD-DNA COMPLEXES

In collaboration with E. Enemark and L. Joshua-Tor here at CSHL, we have determined the cocrystal structures of E1 DBD bound to the ori DNA as a dimer and as a tetramer. These structures provide information about several aspects of E1 DNA binding. Most importantly, specific information about how E1 DBD recognizes its binding can be obtained. The majority of the contacts between E1 DBD and the DNA are phosphate backbone contacts, which provide little specificity. In excellent agreement with the mutational analyses which have indicated that only a single position in the hexanucleotide sequence is invariant, the base-specific contacts are largely confined to one single position, which is contacted via van der Waals interactions by multiple side chains. Furthermore, the binding of the E1 DBD causes significant alterations in the structure of the ori DNA. The DNA structure

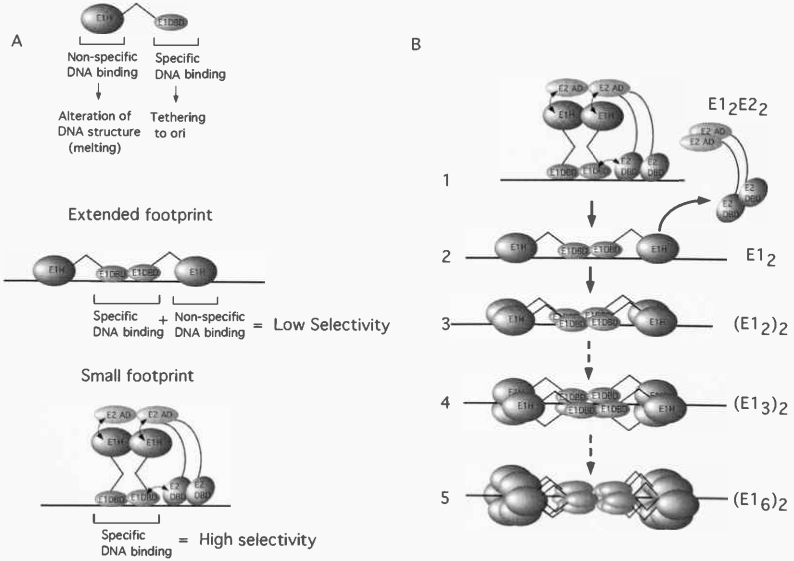

FIGURE 1 (*A*) A summary of the dual DNA-binding activities in E1 and the consequences for DNA-binding specificity. (*B*) A model for the assembly of E1 monomers to form a double hexamer. See text for details.

becomes progressively more deformed as additional E1 DBD molecules are bound to the DNA, indicating that the binding of the E1 DBD may provide the first steps in melting of the ori.

GENERATION OF SEQUENCE SPECIFICITY FOR INITIATOR DNA BINDING

Viral initiators of DNA replication are dependent on the ability to find and recognize the viral origin of DNA replication in a vast excess of host-cell DNA in the infected cell. Interestingly, many viral initiators appear to bind DNA with modest to low selectivity, raising the question of how the ori is recognized. For the papillomaviruses, the E2 protein contributes to highly selective DNA binding by binding cooperatively with E1 to the ori. We, as well as others, have believed that the role of E2 is to recruit E1 to the ori. Interestingly, we have recently determined that the E2 protein functions by an entirely different molecular mechanism. By analyzing the DNA-binding properties of the E1 protein, we found that although the full-length E1 protein binds DNA with low specificity, the DBD of E1 binds with high specificity. The explanation for this apparent paradox is that two different DNA-binding activities are present in the full-length E1 protein (Fig. 1A). The E1 DBD is capable of highly sequence-specific DNA binding, but a different DNA-binding activity, present in the helicase domain of the E1 protein, binds DNA nonspecifically. The net result of the presence of both a nonspecific and a specific DNA-binding activity is that full-length E1 binds DNA with low specificity. We now also understand how E2 acts to provide high-specificity DNA binding. E2 interacts with the E1 helicase domain and prevents this domain from contacting DNA. As a result, in the presence of E2, E1 binds DNA only via its DBD, resulting in highly specific DNA binding. As E2 is displaced, the E1 helicase domain is free to engage the sequences flanking the E1-binding sites, generating a complex with intrinsically low sequence specificity, but bound to a specific site. The reason for this complex arrangement is clearly that the nonspecific DNA-binding activity that resides in the E1 helicase domain has an important role in later events where the E1 protein melts and unwinds the ori DNA. This raises the interesting possibility that initiator proteins in general may employ similar procedures for the generation of highly sequence-specific DNA binding. Furthermore, this mode of E1 binding provides us with a simple model for how larger initiator complexes are assembled on the ori (Fig. 1B). By addition of dimers in ordered fashion, the final form, a double hexamer of E1, can be assembled without rearrangement of the individual subunits.

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DNA REPLICATION AND CHROMATIN INHERITANCE

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Our research focuses on the inheritance of chromosomes and chromatin structures in eukaryotic cells. The primary interest is how DNA replication occurs, how it is regulated, and how it is coordinated with other events such as chromosome segregation during mitosis. Coupled to duplication of the DNA is the copying of chromatin structures during S phase, the period in cells when DNA is replicated. In the past year, we have continued to study the mechanisms of initiation of DNA replication at origins and the proteins that function in this process. One of these, the origin recognition complex (ORC) associates with origins of DNA replication and is the initial landing pad on DNA for all subsequent protein complexes that participate in replicating DNA. ORC is also involved in a number of other processes, such as silencing of gene expression. This year, we have discovered new functions for ORC in human cells, including unexpected roles for the smallest ORC subunit, Orc6 in chromosome segregation and cytokinesis, the process in which cells actually divide after chromosomes are separated at mitosis.

ORC is also required for silencing of gene expression in yeast and associates in *Drosophila* with heterochromatin proteins. In collaboration with Rui-Ming Xu's laboratory here at CSHL, we have determined the three-dimensional structure of a domain in the Orc1 subunit of ORC, called the BAH domain. This domain interacts with one of the silent information regulatory proteins, Sir1, only at the *cis*-acting sites of ORC binding within silencer DNA elements that lie adjacent to the silent genes. In contrast, the other Sir proteins, Sir2, Sir3, and Sir4, were found to localize over the entire silent domain, not just at the silencers. The resulting structures resemble heterochromatin found in mammalian cells, supporting the suggestion that ORC functions in organizing heterochromatin in higher eukaryotes.

THE PREREPLICATION COMPLEX IN YEAST AND HUMAN CELLS

We continue to study the formation and regulation during the cell division cycle of prereplication complexes (pre-RCs) in both yeast and human cells. In yeast, the proteins required for formation of the pre-RC include the ORC, Cdt1, Cdc6, and the minichromosome maintenance (MCM) proteins. One of the final steps in forming the pre-RC is loading of the MCMs onto DNA at replication origins in a manner dependent on ORC, Cdc, and Cdt1. To better understand this process, each of these proteins has been expressed in either bacteria or baculovirus expression vectors and the proteins purified. Using these proteins, we have begun to study the detailed biochemistry of formation of pre-RCs. It is still not known, however, whether these proteins are sufficient for formation of the pre-RC.

Once the pre-RC is formed, subsequent steps require a number of proteins to be loaded onto the DNA at origins that allow initiation of DNA replication to proceed. Proteins such as Cdc45, which is loaded only after S-phase-specific cyclin-dependent protein kinases are activated, are necessary before actual DNA synthesis can occur. We have purified the Cdc45 protein from yeast cells and have shown that it is contained in a complex of proteins that includes, among others, the MCM proteins. The precise nature of this protein complex is under investigation.

The principal effort in the last year, however, has been to translate our knowledge of initiation of DNA replication in yeast to understanding the function of these proteins in human cells. In pursuing this project, we have encountered surprising results that expand the known functions of the individual ORC subunits. Last year, we reported that in rapidly proliferating cells, the human Orc1 subunit was loaded onto chromatin as cells exited mitosis and remained bound throughout the G_1 phase of the cell division cycle. As cells entered into S phase, Orc1 was degraded and remained absent from cells until late in mitosis when the pre-RC was formed. Newly synthesized Orc1 bound to chromatin just prior to the time when the MCM proteins were loaded onto chromatin during the very last stages of telophase in mitosis, just before the cells divided. Thus, like in yeast, in rapidly proliferating human cells, the pre-RC formed as cells exited mitosis and was inherited into the new daughter cells.

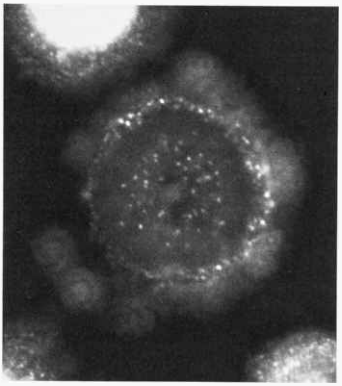

FIGURE 1 View of a human cell metaphase plate viewed from one centrosome that organizes the microtubules that attach to the chromosomes. Anti-Orc6 antibodies stain two ring-like structures that are shown by the white spots. The center ring of spots represents Orc6 at the kineticores at each centromere on the chromosomes, whereas the outer ring of staining represents association of Orc6 with a structure at the plane of cell division.

Using specific antibodies against the Orc6 subunit of the human ORC, we have shown that this protein associated with the nuclear chromatin during interphase of the cell division cycle, but surprisingly, at prophase, there was a dramatic reorganization of this protein into two separate structures. Orc6 associated with kinetochores at centromeres during prophase and remained associated with these structures throughout the subsequent metaphase and anaphase stages of mitosis, but was then removed from kinetochores during telophase. Immunofluorescence colocalization studies suggested that Orc6 associated with outer kinetochore proteins, such as CENP-E, Mad3, and BubR1, the latter two being a subset of proteins that ensure that all chromosomes are aligned at the metaphase plate before initiation of anaphase. The localization of Orc6 suggests that it may have a role in chromosome segregation during mitosis.

Concomitant with the association of some of the Orc6 proteins with kinetochores at prophase of miosis was the reorganization of the remaining Orc6 to a reticular-like pattern that formed a ring around the condensed chromosomes that were aligned at the metaphase plate. As cells underwent anaphase, this reticular fraction of Orc6 protein aligned in the mid plane of the cell division plane where cytokinesis would ultimately occur. As cells divided, this fraction of Orc6 protein remained at the mid plane until the very end stages of cell division. During the very last stages of cytokinesis, the majority of Orc6 associated with the newly formed nucleus where it probably had a role in formation of new prereplication complexes, although we have not yet demonstrated this point. A small fraction of Orc6 remained associated with the mid body that joined the two separated cells through a thin tube-like structure called the Fleming body. Orc6 localized in the middle of this structure that also contained tubulin, actin, surviving and number of other proteins. This localization suggested that Orc6 might have a role in cytokinesis.

To further extend the cell biology observations that Orc6 localized to two interesting structures and test the possibility that it might be involved in the later stages of cell division, we turned to a genetic analysis of Orc6 function using short interfering RNA (siRNA) that eliminated Orc6 from human cells. Two short double-stranded synthetic RNA oligonucleotides directed against different segments of the Orc6 messenger RNA were introduced into human cells and were compared with a control siRNA that did not eliminate Orc6 from the cell. The first and obvious phenotype upon elimination of Orc6 from human cells was the formation of binuclear cells probably due to the failure of cytokinesis after chromosome segregation and nuclear formation. This phenotype is consistent with the localization of Orc6 to the mid plane of cell division and to the Fleming body and suggested that Orc6 was required for correct cytokinesis. A second phenotype observed was a reduction in the number of cells incorporating the nucleotide analog bromodeoxyuridine (BrdU) into DNA, indicating a problem with DNA synthesis in cells with reduced Orc6 protein. Later, two prominent phenotypes were observed. The first was the formation of multinuclear cells containing two or more nuclei per cell, and some of the nuclei in these cells contained less than a normal complement of DNA. A second late phenotype was the formation of abnormal mitoses containing multipolar spindles and misaligned chromosomes at the metaphase plate. This later phenotype may be due to a reduction in DNA synthesis in the cell since there was a reduction in the BrdU incorporation prior to accumulation of the aberrant microtubules. However, because Orc6 associated with kinetochores at

prophase, some of the misalignment of chromosomes may be due to the participation of Orc6 in correct regulation of kinetochore binding to microtubules during the early stages of metaphase.

Prompted by the unexpected localization of Orc6 during mitosis, we have begun to look at the other ORC subunits and have made the surprising observation that Orc2 associated with centrosomes during mitosis. We are currently completing a genetic analysis of Orc2 function using siRNA genetics in human cells; however, initial results suggest that the Orc2 siRNA phenotype was significantly different from the phenotype described for Orc6 siRNA, except for the similar reduction in DNA synthesis by elimination of these proteins from cells.

ANALYSIS OF THE BIOCHEMISTRY OF CHECKPOINT SIGNALING IN HUMAN CELLS

When investigating the biochemistry of the replication fork using human cell extracts that support SV40 DNA replication, we discovered replication factor C (RFC), a five-subunit protein that loads the proliferating cell nuclear antigen (PCNA) clamp onto DNA. When loaded onto DNA, PCNA tethers DNA polymerase δ onto the template DNA as it synthesizes both leading and lagging strands at the replication fork. The trimeric PCNA clamp protein encircles the double-stranded DNA and is loaded on by an ATP-driven clamp loader protein RFC. Each RFC subunit is a member of the AAA^+ family of ATPases, and the four small subunits (Rfc2–5) form a complex that associates with a fifth and larger subunit (Rfc1) to form the active clamp loader. During the past year, we have demonstrated that the four small RFC subunits are also associated with other RFC-like proteins, including the human Rad17 protein and the Ctf18 protein. The former protein has been implicated by genetic studies in yeast as being involved in a DNA-damage response during S phase that is coupled to checkpoint signaling. Damage to the replicating DNA during S phase causes replication fork arrest and establishes a signal that prevents further progression of the cell cycle until the damage is repaired or replication forks are restored.

In the last year, we have purified both the Rad17-Rfc2–5 and Ctf18-Rfc2–5 complexes from human RKO cells. We have demonstrated that the Rad17-Rfc2–5 subunits form a complex that loads a DNA-damage-induced PCNA-like clamp onto DNA. The PCNA-like clamp consists of a heterotrimeric structure containing the Rad1, Hus1, and Rad9 proteins that form a protein complex we called RHR trimer. Loading of the RHR trimer onto DNA requires ATP, the five-subunit RHR loading complex containing Rad17 and the Rfc2–5 subunits. Loading of the RHR complex optimally requires the presence of the single-stranded DNA-binding protein replication protein A (RPA). RPA was previously shown to interact with RFC and for loading of PCNA onto primer-template DNA junctions. Although there are significant similarities between the Rad17-Rfc2–5 loading of the RHR complex and the loading of PCNA by RFC, there are, however, differences, including the types of DNA on which the RHR complex is loaded and the ATPase activities.

The Ctf18-Rfc2–5 complex from yeast was previously shown to be required for sister chromatid cohesion, although the mechanism of action of this protein is unknown. We have detected a similar complex in human cells, although we have not analyzed the biochemistry of this complex in great detail.

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PROTEIN TYROSINE PHOSPHATASES AND THE CONTROL OF SIGNAL TRANSDUCTION

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The phosphorylation of tyrosyl residues in proteins is a key component of the regulation of signaling pathways that control many fundamental physiological processes including cell proliferation 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 expanding 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 utilizing a variety of 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.

PTPs FROM A GENOMIC PERSPECTIVE: DEFINING THE "PTP-OME"

With the availability of a near complete (>99%) draft of the sequence of the human genome, it is now possible to define the complement of PTPs in humans. The PTP family of enzymes are recognized by the presence of a signature sequence [I/V]HCXXGXXR[S/T] and can be divided into two broad categories: the Tyr-specific PTPs and the dual specificity phosphatases (DSPs), the latter having the ability to dephosphorylate Ser/Thr and Tyr residues in proteins. Previously, in collaboration with Niels-Peter Moller and his colleagues at Novo Nordisk, we had searched the NCBI Genbank database for the Tyr-specific (a.k.a. classical) PTPs and identified cDNA sequences corresponding to 37 distinct PTP genes in humans, which could be divided into 17 subtypes. Now, having searched the assembly and draft sequence of the human genome for its complement of PTP-like sequences, we have identified 1 additional, novel human PTP gene, bringing the total to 38, and 12 PTP pseudogenes. We believe that this is the first complete catalog of the classical PTP-like sequences present in the human genome.

In conjunction with this analysis, we are in the process of collating chromosomal mapping data for all known and novel PTP-like sequences for correlation with cytogenetic data published in the "pregenomic" era and an investigation of linkages between PTP loci and genetic disease. We have also examined the exon structure of PTP domains to explore the evolutionary history of this enzyme family and to provide further insight into the diversity and complexity within the family that arises from mRNA splicing. We have set up a Web Site describing our previous analysis (Andersen et al. *Mol. Cell. Biol. 21:* 7117 [2001]) at the following links, http://ptp.cshl.edu and http://science.novonordisk.com/ptp, and this will be updated as our studies are completed.

We are currently conducting a similar analysis of the DSPs in collaboration with Ravi Sachidanandam and Jeremiah Faith here at CSHL. Although the DSPs contain the PTP signature motif, these enzymes display greater overall sequence diversity than the classical PTPs. An obvious major difference is in the design of the active site. In the classical PTPs, the active site is present as a deep cleft on the surface of the protein, the depth of which is defined by a conserved Tyr residue that restricts specificity only to phosphotyrosine residues. In the DSPs, this Tyr residue is absent and the active site cleft is shallower, allowing it to accommodate the shorter side chains of phopho-Ser and phospho-Thr. The first DSP to be described was the enzyme VH1, which is essential for infectivity of the virions of vaccinia virus. In mammals, DSPs have been implicated in the control of MAP kinase signaling pathways and in regulating transition through the cell cycle. The PTEN tumor suppressor, which is fre-

quently mutated in glioblastoma and prostate cancer, is also a DSP, and the analysis of this enzyme expanded the repertoire of potential physiological substrates of members of the PTP family. PTEN recognizes phosphatidylinositol phospholipids as substrates, thereby regulating PI3 kinase-dependent signaling pathways associated with cell survival and apoptosis. By aligning published and expressed sequence tag (EST)-predicted sequences, we have defined a minimal DSP catalytic domain of 130 residues. Our search of the human genome yielded 43 DSPs that contain this domain, and we have mapped the chromosomal location of each gene. Currently, we are performing similar searches in other sequenced genomes to identify orthologs of human DSPs and investigate the evolution of these enzymes.

In addition to the VH1-like DSPs, 11 myotubularin family DSPs have been identified. These enzymes also recognize phosphatidylinositol phospholipids as substrates, and disruption of their function has been linked to human disease. The Cdc25s, A, B, and C, regulate cell cycle transition through the dephosphorylation of cyclin-Cdk protein kinases. Finally, the enzyme termed Low Mr PTP has been implicated in regulation of receptor PTK (protein tyrosine kinase) signaling. Thus, the total number of PTPs currently stands at 96—large enough to introduce diversity in structure and function, but small enough to study from a family-wide perspective.

PTP1B: A THERAPEUTIC TARGET FOR TREATMENT OF DIABETES AND OBESITY

It is now apparent that PTP1B, the prototypic member of the PTP family, is a major regulator of signaling events induced by insulin and leptin. In fact, PTP1B is now recognized in the pharmaceutical industry as an important target for development of novel strategies for therapeutic intervention in diabetes and obesity. Previous studies from this lab defined in molecular detail the interactions between PTP1B and the activation loop of the insulin receptor as substrate, leading to the identification of the sequence E/D-pY-pY-R/K as a consensus optimal substrate recognition motif for the phosphatase.

These analyses were instrumental in identifying the JAK family of PTKs, which regulate the signaling response to leptin, as substrates for PTP1B. The active site of members of the PTP family is highly charged, consistent with its recognition of a charged, phosphorylated residue as substrate. This, and the fact that the essential, nucleophilic Cys residue at the active site is prone to oxidation, has presented challenges to the design of therapeutic inhibitors, suggesting that alternative methods for manipulating PTP1B levels may be of benefit. Therefore, we have been characterizing the *PTP1B* promoter with the goal of identifying alternative targets through which to regulate PTP1B function.

Changes in the levels of PTP1B have been noted in several human diseases, particularly those associated with dysfunctional tyrosine phosphorylation. Previously, we had shown that the expression of PTP1B is induced by the p210bcr-abl oncoprotein, a PTK that is directly responsible for the initial manifestations of chronic myelogenous leukemia. Interestingly, PTP1B can antagonize p210bcr-abl-induced transformation. The effects of p210bcr-abl on PTP1B expression are manifested at the transcriptional level, and previously, we had reported the identification of a *p*210bcr-abl-*r*esponsive *s*equence (PRS) in the *PTP1B* promoter. In extending these analyses, we have now identified an enhancer sequence within the *PTP1B* promoter upstream of the PRS. Through a protein purification strategy, culminating in a DNA affinity chromatography step that utilized the *PTP1B* enhancer sequence, and various binding analyses, we demonstrated interaction between the enhancer and the transcription factor Y-box-binding protein-1 (YB-1). Y-box-binding proteins comprise a hydrophilic carboxy-terminal domain, which has been implicated in protein-protein interactions, and an amino-terminal nucleic-acid-binding domain, which is implicated in transcriptional regulation.

This nucleic-acid-binding domain is highly conserved throughout evolution from bacteria to humans, displaying 43% identity with the cold shock response protein (cs7.4) from *Escherichia coli*, suggestive of a fundamentally important function. Y-box-binding proteins have the ability to bind single- and double-stranded DNA and RNA. They have been implicated in transcriptional activation and repression, functioning directly as transcription factors and indirectly by altering promoter conformation, through recognition of single-stranded and H-form triplex DNA. Although originally identified in terms of their ability to recognize a DNA sequence termed a Y box, the Y-box proteins are now known to recognize motifs with little similarity to the classical Y box. In fact, there is no recognizable Y-box motif in the *PTP1B* enhancer, and so this interaction could not have been predicted from analysis of the promoter sequence.

We observed that overexpression of YB-1 led to an increase in the levels of PTP1B. Furthermore, depletion of YB-1 protein, by expression of a specific antisense construct, led to an approximately 70% decrease in expression of PTP1B, but no change in the level of TC-PTP, which is the closest relative of PTP1B. Expression of antisense YB-1 resulted in increased sensitivity to insulin and enhanced signaling through the cytokine receptor gp130, which was suppressed by re-expression of PTP1B. These data illustrate that by targeting YB-1, the levels of PTP1B and signaling through insulin and cytokine receptors can be manipulated. Interestingly, we have observed a correlation between the expression of YB-1 and that of PTP1B across a panel of cancer cell lines. Furthermore, we demonstrated that the reported increases in the expression of PTP1B in skeletal muscle of Goto-Kakazaki non-obese, insulin-resistant type-II diabetic rats, compared to control nondiabetic rats, coincided with increased expression of YB-1. These data are consistent with a functional relationship between PTP1B and YB-1 under both physiological and pathophysiological conditions and suggest that it may be possible to manipulate PTP1B activity therapeutically by controlling its level of expression.

REVERSIBLE OXIDATION AND THE REGULATION OF PTP FUNCTION

One major area of interest in the lab is a characterization of the regulation of PTP function by reversible oxidation. The signature motif [I/V]HCXXGXXR[S/T], which defines the PTP family of enzymes, contains an invariant Cys residue, which functions as a nucleophile in catalysis. Due to the unique environment of the PTP active site, this Cys residue displays an unusually low pKα, which enhances its nucleophilic properties but renders it susceptible to oxidation. It is becoming apparent that a wide variety of physiological stimuli that result in stimulation of tyrosine phosphorylation are also accompanied by the production of reactive oxygen species (ROS). Furthermore, ROS production has been shown to be required for an optimal tyrosine phosphorylation response (Fig. 1). Work from several labs has demonstrated that PTPs are an important target of ROS in this context. The operating principle is

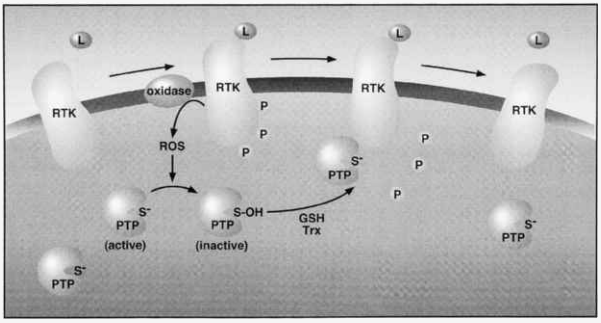

FIGURE 1 Regulation of PTP activity by reversible oxidation. Ligand-dependent activation of a receptor protein tyrosine kinase (RTK) triggers the activity of a Rac-dependent NADPH oxidase, leading to production of reactive oxygen species (ROS). ROS oxidizes the active-site Cys residue of members of the PTP family, converting it from a thiolate ion (the active form) to sulfinic acid. Oxidation results in inhibition of PTP activity, thereby promoting tyrosine phosphorylation. However, oxidation of the PTPs is transient. Restoration of PTP activity following reduction back to the thiolate form of the active-site Cys residue terminates the tyrosine-phosphorylation-dependent signal. A variety of growth factors, hormones, and cytokines induce ROS production and stimulate tyrosine phosphorylation. We are developing methods to identify the PTPs that become oxidized in response to a physiological stimulus as a way of establishing links between particular PTPs and the regulation of defined signaling pathways.

that the stimulus (hormone, growth factor, etc.) enhances tyrosine phosphorylation directly, by activation of a PTK and/or indirectly by inactivation of a PTP. Thus, one function of ROS produced following agonist stimulation is to inactivate transiently the critical PTP that provides the inhibitory constraint upon the system, thus facilitating the initiation of the signaling response to that stimulus. We reasoned that by identifying which PTPs are oxidized in response to a particular stimulus, we would reveal which PTPs are critical for down-regulating the signaling response to that stimulus. The application of state-of-the-art technologies, such as RNA interference (RNAi) and the use of substrate-trapping mutant PTPs, would allow us then to define the function of the PTPs that were identified in this way.

Continuing with the study of insulin signaling, we have demonstrated that stimulation of Rat1 fibroblasts with insulin leads to the production of ROS, in particular hydrogen peroxide. Expression of catalase inhibited both tyrosine phosphorylation of the insulin receptor and downstream signaling to PKB/Akt, thus highlighting the importance of ROS production for an optimal signaling response. Using a modified in-gel phosphatase assay that we have developed, we showed that two PTPs were rapidly and reversibly oxidized in response to insulin in this system, and we have identified these enzymes as PTP1B and TC45. A role for PTP1B in the regulation of insulin receptor (IR) signaling is now well-established and provides validation of this strategy. A role of TC45 in insulin signaling, however, had not been appreciated. To examine this role, we used RNA interference and generated short interfering RNA (siRNA) oligonucleotides targeted against TC45, demonstrating ablation of the PTP in both Rat1 fibroblasts and HepG2 cells, which express higher levels of the insulin receptor. Specificity of the siRNA was confirmed by the lack of an effect on expression of PTP1B, the closest homolog of TC45. In both Rat1 and HepG2 cells, we observed that TC45 siRNA enhanced the activation of PKB/Akt induced by insulin.

Focusing on HepG2 cells, we observed that the substrate-trapping mutant form of TC45, but not the wild-type enzyme, formed a complex with the β-subunit of the insulin receptor (IR-β). Furthermore, RNA interference with siRNA to TC45 led to hyperphosphorylation of IR-β, and blotting of immunoprecipitates of IR-β with phospho-specific antibodies suggested that TC45 displays preferential recognition of a particular site(s) within the receptor.

In a parallel series of studies, in collaboration with Tony Tiganis (Monash University, Australia), we examined insulin signaling immortalized mouse embryo fibroblasts (MEFs) generated from TCPTP–/– and control TCPTP+/+ mice. Consistent with the data generated by RNA interference, we observed that ablation of TCPTP led to enhanced and sustained activation of PKB in response to insulin. Furthermore, insulin-induced tyrosine phosphorylation of the IR-β subunit was enhanced in TCPTP–/– MEFs. Importantly, these effects were suppressed by reexpression of TCPTP to physiological levels. Therefore, these data illustrate that in addition to PTP1B, the PTP TC45 is a negative regulator of signaling in response to insulin and that one of its substrates in this respect is the β-subunit of the insulin receptor.

IDENTIFICATION OF THE PTK MET, THE RECEPTOR FOR HGF/SF, AS A SUBSTRATE OF THE RECEPTOR PTP DEP-1

When we first identified the receptor protein tyrosine phosphatase DEP-1 (*d*ensity-*e*nhanced *P*TP-1), we noted that its expression is increased as cells in culture approach confluence, suggesting a role as an inhibitor of cell proliferation. This PTP has been implicated in the regulation of cell growth, differentiation, and transformation and, most recently, has been identified as the product of a tumor suppressor gene mutated in colon, lung, and breast cancers. We generated constructs comprising the cytoplasmic domain of DEP-1 fused to the maltose-binding protein to identify potential substrates, using our substrate-trapping mutant technology, and thereby suggest a physiological function for DEP-1.

We have shown that the substrate-trapping mutant form of DEP-1 interacted with a small subset of tyrosine-phosphorylated proteins from lysates of human breast tumor cell lines. We have identified the hepatocyte growth factor/scatter factor (HGF/SF) receptor Met as well as the adapter protein Gab1 and the junctional component p120 catenin as potential substrates of DEP-1 in MDA-MB-231, T-47D, and T-47D/Met cell lysates. Following ligand stimulation, phosphorylation of specific tyrosyl residues in Met induces mitogenic, motogenic, and morphogenic responses. When coexpressed in 293 cells, the full-length substrate-trapping mutant form of DEP-1 formed a stable complex with the chimeric receptor CSF.MET and wild-type DEP-1 dephosphorylated CSF.MET. Furthermore, we

observed that DEP-1 preferentially dephosphorylated the Gab1-binding site (Tyr^{1349}) and a carboxy-terminal tyrosine implicated in morphogenesis (Tyr^{1365}), whereas tyrosine residues in the activation loop of Met (Tyr^{1230}, Tyr^{1234}, Tyr^{1235}) were not preferred targets of the PTP. The tight regulation and appropriate execution of Met signals are vital for normal growth and differentiation, whereas aberrant signaling through Met can lead to transformation. The ability of DEP-1 preferentially to dephosphorylate particular tyrosine residues that are required for Met-induced signaling suggests that DEP-1 may function in controlling the specificity of signals induced by this PTK, rather than as a simple "off-switch" to counteract PTK activity. This functional interaction between Met and DEP-1 raises the possibility that up-regulation of Met may be coupled with down-regulation of DEP-1 in the progression of certain human tumors.

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SIGNAL TRANSDUCTION

L. Van Aelst B. Boettner R. Packer
E.-E. Govek M. Zhao
S. Newey M. Watabe

The central research interest of my laboratory is the study of signal transduction pathways involving members of the Ras and Rho GTPases and the processes that they regulate. Alterations that affect normal Ras and Rho function have been found to result in the development of several disease processes including cancer, as well as inflammatory and neuropathological disorders. Members of the Ras and Rho families encode low-molecular-weight guanine-nucleotide-binding proteins that function as binary switches by cycling between an active GTP-bound state and an inactive GDP-bound state. It is only in their GTP-bound state that they are able to interact with downstream effector molecules which mediate their effects. The ratio of the two forms is regulated by the opposing effects of guanine nucleotide exchange factors (GEFs), which promote the exchange of bound GDP for GTP, and the GTPase-activating proteins (GAPs), which stimulate hydrolysis of bound GTP. During the past year, efforts in my laboratory continued to focus on defining the role and mechanisms by which these GTPases exert their effects on specific aspects of tumorigenesis and neuronal development.

ROLE OF RAS AND RHO SIGNALING IN TUMORIGENESIS

During the development and progression of cancer, cells undergo numerous changes in morphology, adhesive properties, proliferation, and transcriptional profile. During the past couple of decades, there have been intense efforts to understand the molecular mechanisms involved, and members of the Ras and Rho GTPases have emerged as important players. Our interest lies in obtaining a better understanding of how alterations in Ras and Rho signaling affects cell growth control and the invasive behavior of cells. There are currently three projects ongoing in the lab. One project aims to gain insights into the molecular basis by which the Ras-related GTPase Rap1 and its effector AF-6 affect epithelial cell shape changes and the coordinated movements of cells, which are pivotal alterations governing tumor progression and wound-healing processes. The second project focuses on defining the mechanisms by which Rac1, a Rho family member, contributes to cell growth and invasiveness. The third project involves the functional characterization of $p62^{dok}$, a Ras-GAP-associated protein, which was found to be constitutively tyrosine-phosphorylated in chronic myelogenous leukemia (CML) progenitor cells.

RAP1 AND AF-6/CANOE FUNCTION IN EPITHELIAL MORPHOGENESIS

Several studies suggest a relationship between loss of epithelial cell polarity and loss of growth control. Studies in the laboratory using mammalian epithelial cells have suggested a role for Rap1, a member of the Ras family, and the junctional protein AF-6, which associate with GTP-bound Rap, in the regulation of epithelial cell shape changes and cell adhesion. Due to the finding that AF-6 is largely refractory to both loss- and gain-of-function analyses, we decided to explore this hypothesis in the genetically more tractable *Drosophila* system.

In *Drosophila*, a process called dorsal closure (DC) relies on elongation and migration of epithelial cells and has proved to be an excellent model system for the study of the molecular basis underlying epithelial cell shape changes. In collaboration with U. Gaul (The Rockefeller University), we recently provided biochemical and genetic evidence that Canoe (*Drosophila* orthologs of AF-6) acts as a *Drosophila* Rap1 effector in the process of dorsal closure. To date, no other protein has been reported to act as a mediator or direct effector of the small GTPase Rap1 in a physiological context. Furthermore, we obtained evidence that Canoe participates in DC through two cellular pathways: One is controlled by DRap1 and is independent of the JNK cascade, whereas the second is not activated by DRap1 and feeds into JNK signaling. We are currently setting out to identify additional Canoe-interacting proteins to obtain further insights into Canoe's mode of action in DC. To complement these studies, we are planning to assess a role for the mam-

malian counterparts of these *Drosophila* proteins in mammary epithelial polarity and invasive behavior. These studies will contribute to a better understanding of the fundamental mechanisms that drive cell shape changes and migration.

SIGNAL TRANSDUCTION PATHWAYS MEDIATING THE EFFECTS OF RHO GTPases ON CELL GROWTH CONTROL AND INVASIVENESS

In addition to their role in cell cycle control, Rho-like GTPases regulate both cell-cell and cell-matrix adhesions and can influence the motile and invasive properties of tumor cells in vitro. We continued our studies to define the signaling pathways that regulate and mediate the effects of the Rho GTPases on cell proliferation, adhesion, and invasion. Using the yeast 2-hybrid system, we recently identified a novel class of putative Rho activators, which contain a region of homology with Dock180. The latter protein has previously been shown to activate Rac1 and to have a role in phagocytosis, cell migration, and invasion of tumor cells. We are currently investigating the roles of these putative Rho activators in cell motility and invasion. As an additional approach, we employed cDNA-RDA (representational difference analysis) in combination with microarray analysis to identify target genes of Rac1 (a member of the Rho GTPases) whose expression is altered as a result of constitutively active Rac1 (Rac1V12) expression. These experiments were assisted by Dr. R. Lucito here at CSHL. Among the cDNAs isolated were cyclo-oxygenase 2 (*COX-2*) and *cyclin D1*, two genes whose expression has been previously shown to be up-regulated in many tumors. We found that the expression levels of both of these genes are up-regulated in Rac1V12 expressing epithelial and glioblastoma cells. The further characterization of the other cDNAs is presently ongoing in the lab and is likely to identify additional relevant Rac1 targets.

P62^{DOK} IS A NEGATIVE REGULATOR OF GROWTH FACTOR AND P210$^{BCR-ABL}$-INDUCED CELL PROLIFERATION

p62^{dok} is a protein initially identified as a prominent 62-kD constitutively tyrosine-phosphorylated RasGAP-associated protein in p210$^{bcr-abl}$-expressing cells. This protein was termed Dok (*d*ownstream *of k*inases), since it was also found to be a common substrate of many receptor- and membrane-associated tyrosine kinases. To investigate the role of p62^{dok}, we utilized different cell types derived from p62^{dok} null mice, generated by P.P. Pandolfi (Memorial Sloan-Kettering Cancer Center, New York). In collaboration with Pandolfi's group, we obtained evidence that p62^{dok} acts as a negative regulator of growth-factor-induced cell proliferation. We observed that p62^{dok}-deficient cells possess a higher proliferation rate in response to growth factors and that this increase in cell proliferation can be suppressed by ectopic expression of p62^{dok}. Furthermore, p62^{dok} inactivation causes a significant shortening of the latency of the fatal myeloproliferative disease induced by retrovirus-mediated transduction of p210$^{bcr-abl}$ in bone marrow cells. We have also obtained data suggesting that p62^{dok} exerts its negative effect on growth-factor-induced cell proliferation at least in part by negatively influencing the Ras/MAPK (mitogen-activated protein kinase) pathway. We are presently investigating the underlying mechanism by which p62^{dok} inhibits the Ras/MAPK pathway and growth factor and p210$^{bcr-abl}$-mediated signaling. We have already found out that association of p62^{dok} with RasGAP is not essential for its negative effect on the PDGF (platelet-derived growth factor)-triggered MAPK activation.

ROLE OF RAS AND RHO GTPases IN SYNAPTIC PLASTICITY AND NEURONAL DEVELOPMENT

Evidence provided during the past few years demonstrates that members of the Ras and Rho GTPases control important neuronal functions and have significant effects on behavior. Furthermore, several diseases causing cognitive impairment (including neurofibromatosis, autism, and mental retardation) are associated with mutations in members of the Ras and Rho GTPases or in the molecules (GEFs and GAPs) that control their activity. A major challenge remains to unravel how these molecules affect neuronal functions and behavior. We have investigated the role of Ras and Rap1 in activity-dependent synaptic plasticity and have embarked on the functional characterization of *oligophrenin-1*, a RhoGAP, that is absent in unrelated MRX (nonspecific X-linked mental retardation) families.

RAS AND RAP CONTROL AMPA-RECEPTOR TRAFFICKING DURING SYNAPTIC PLASTICITY

This work was done in collaboration with R. Malinow here at CSHL. In this study, we have investigated the role of Ras and Rap1 in activity-dependent synaptic

plasticity. The latter is believed to underlie key aspects of brain development, learning, and memory. The most widely studied examples of synaptic plasticity are long-term potentiation (LTP) and long-term depression (LTD) in the hippocampus. Recent studies have revealed that AMPA (α-amino-hydroxy-5-methyl-4-isoazole) receptor (AMPA-R) trafficking is important in synaptic plasticity; however, the signaling pathways regulating this trafficking are poorly understood. We found that Ras and Rap1 have indispensable roles in synaptic plasticity and that they signal two independent pathways at synapses. Ras mediates activity-induced synaptic enhancement by driving synaptic delivery of AMPA-Rs containing long cytoplasmic tails requiring p42/p44 MAPK activation. In contrast, Rap1 mediates activity-induced synaptic depression by removing synaptic AMPA-Rs containing short cytoplasmic tails requiring p38 MAPK activation. Taken together, these studies indicate that Ras and Rap1 serve as independent regulators for potentiating and depressing central synapses.

FUNCTIONAL CHARACTERIZATION OF THE X-LINKED MENTAL RETARDATION GENE, *OLIGOPHRENIN-1*

Nonspecific X-linked mental retardation (MRX) is characterized by mental impairment without any other distinctive clinical features. Eight genes involved in MRX have been cloned to date, and importantly, three of these genes encode regulators or effectors of the Rho GTPases. Members of this family of small GTPases, including Cdc42, Rac, and RhoA, are key regulators of the actin cytoskeleton and have been shown to affect different aspects of neuronal morphogenesis. This has led to the hypothesis that abnormal Rho GTPase signaling may be a prominent cause of MRX. However, how alterations in Rho signaling result in changes in neuronal connectivity and/or plasticity that give rise to MRX remain unknown. As yet, studies on the effects of Rho-linked MRX genes on neuronal development have not been reported. We have focused on the functional characterization of *oligophrenin-1*, which encodes a Rho-GTPase-activating protein (RhoGAP), and whose loss of function is associated with nonsyndromic or nonspecific MRX. We found that *oligophrenin-1* is highly expressed in the developing rat hippocampus and is expressed in axons, dendrites, and dendritic spines of pyramidal hippocampal cells. We also showed that *oligophrenin-1* functions as a GAP for RhoA, Rac, and Cdc42 in a cellular context. More recently, we obtained evidence that down-regulation of *oligophrenin-1* expression affects dendritic spine morphology of hippocampal neurons in brain slices. Our studies provide potential insights into the cellular bases underlying MRX associated with *oligophrenin-1* mutations.

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BIOINFORMATICS AND GENOMICS

Computers play an indispensable role in the rapidly progressing arena of biomedical research. CSHL scientists working in the allied disciplines of genomics and bioinformatics use computer science and mathematics to explore the molecular basis of life from many angles. By developing sophisticated software and novel databases, these scientists are creating tools that are vital for interpreting the volumes of information that are emerging with increasing speed from many studies of genes, genomes, proteins, and proteomes.

Lincoln Stein's lab uses computer science to give meaning to the tremendously large data sets that are resulting from a variety of genome research projects. One of several databases they have developed allows researchers to compare the genomes of rice, wheat, and corn and to identify genes that might be used to engineer hardier, more productive varieties. Another allows scientists to explore gene and genome evolution in the animal genus *Caenorhabditis*. Stein's lab is also creating a searchable database of fundamental processes in human biology called Genome Knowledgebase.

W. Richard McCombie's lab continues to contribute significantly to several genome sequencing projects. This year, McCombie and his colleagues in the International Mouse Genome Sequencing Consortium have published a high-quality draft sequence of the mouse genome, along with a comparative analysis of the mouse and human genomes. The laboratory mouse is an important animal model used widely in the study of human disease. Consequently, this landmark sequencing study has profound long-term consequences for medicine. It will help elucidate the molecular mechanisms of disease and will allow researchers to design better therapies for many illnesses. Interestingly, the genetic variety of mouse whose genome was sequenced in the study, C57BL/6, was developed during the early 20th century by Clarence Little at what is now Cold Spring Harbor Laboratory. McCombie's group is currently working with an international consortium to sequence the entire genome of the world's most important food source, rice.

Bud Mishra's lab is bringing mathematics and computer science to bear on a challenging new field known as systems biology. One of the lab's projects is to create a unified theoretical framework within which scientists can understand life's biochemical pathways and the evolutionary processes that mold them. These researchers merge math and biology to develop models of how genomes evolve, and to explore how evolution generates particular networks and modules of interacting components that are seen in biochemical pathways.

Michael Zhang's lab has created software for identifying signature sequences within DNA that reveal the presence of important genetic elements such as promoters (DNA segments that control gene activity) or exons (DNA segments likely to code for proteins). They have also compared the public and private versions of the mouse genome and have compared the human and mouse genomes. From these comparisons, they have created a database of closely related DNA sequences shared by humans and mice, and they have identified some 5000 new human genes.

Andrew Neuwald's lab has applied the power of statistical analysis to the prediction of protein structure and function. These researchers have developed a technique for identifying the most distinctive molecular features of any particular family of proteins. Once identified among closely and moderately related proteins, such features can be used to track down the most distant relatives of a protein family. As a result, the scientists can infer the properties of poorly characterized proteins by assigning them to protein families with known functions. Such analysis has revealed previously unrecognized roles for certain proteins in human disease.

GENOME SEQUENCE ANALYSIS

W.R. McCombie V. Balija, J. Baker, M. de la Bastide, N. Dedhia, S. Dike, K. Ferraro, M. Katari, F. Katzenberger, L. King, K. Kuit, B. Miller, S. Muller, L. Nascimento, A. O'Shaughnessy, L. Palmer, R. Preston, L. Santos, L. Spiegel, M. Yu, T. Zutavern

Our long-range goal is to analyze the genomes of complex organisms at the molecular level. To that end, we have been working at developing tools and strategies to obtain the salient information from these genomes and have also been applying these strategies in the analysis of several large and important genomes. That work has continued this year. The next step in genome analysis this year is the development of approaches to determine the coding information within genomes and its application at the pilot stage.

Rice Genome Sequencing

V. Balija, J. Baker, M. de la Bastide, N. Dedhia, S. Dike, K. Ferraro, F. Katzenberger, L. King, K. Kuit, B. Miller, S. Muller, L. Nascimento, A. O'Shaughnessy, L. Palmer, R. Preston, L. Santos, L. Spiegel, M. Katari, T. Zutavern [in collaboration with the University of Arizona Genome Center (Rod Wing)]

We are members of the U.S. Rice Genome Sequencing Consortium. This group in turn is a member of the International Rice Genome Sequencing Consortium (IRGSP). This year marked two major milestones by these groups. The U.S. group completed the finished sequence of rice chromosome 10. This chromosome is about 22 million bases in size. The chromosome presented considerable difficulty in sequencing due to large regions of heterochromatic DNA. The detailed analysis of rice chromosome 10 is currently under way.

The IRGSP also finished a high-quality draft sequence of the entire rice genome in 2002. This draft sequence consists of ordered and oriented sequence contigs linked to the genetic map of rice. In addition to our contribution on chromosome 10, we participated in the genome-wide draft by providing both finished and draft sequence of rice chromosome 3. We have done a total of more than 12 million bases of rice sequence. The genome draft will be an important resource for rice scientists around the world. Since it is ordered and linked to the genetic map, it will be an invaluable tool for both basic scientists and rice breeders. We, together with the IRGSP, are continuing to sequence rice with the goal of a finished sequence of the entire genome by the end of 2004.

Mouse Genome Sequencing

V. Balija, J. Baker, M. de la Bastide, N. Dedhia, S. Dike, K. Ferraro, F. Katzenberger, L. King, K. Kuit, B. Miller, S. Muller, L. Nascimento, A. O'Shaughnessy, L. Palmer, R. Preston, L. Santos, L. Spiegel, T. Zutavern

The mouse is an extremely important model organism. We have been part of an effort to sequence the mouse genome. The Mouse Genome Sequencing Consortium published the results of a draft sequence of the mouse genome. Our group sequenced about 13 million bases of the mouse genome, and most of this was finished sequence. The mouse sequence, in comparison with the human sequence, will provide an important tool in efforts to determine the workings of the mammalian genome. This will be a major goal of groups around the world including our own.

TABLE 1 Category-based Gene Count Distribution

Category	EPD	RefSeq	B	C
Gene Count	13	27	23	50

Classification of genes in test set. EPD genes come from the Eukaryotic Promoter Database and are our "gold standards." Much is known about the EPD genes and their 5′ exons have been experimentally verified. These genes have experimentally defined transcriptional start sites and serve as positive controls. RefSeq genes are a set of reference mRNAs from a genome as determined by NCBI. For our purposes, we have excluded any genes from our RefSeq category that are also in the more restrictive EPD data set. These non-EPD RefSeq genes do not have well-defined experimentally verified 5′ ends. Group B genes are gene predictions that are supported by multiple EST matches but whose structure is ambiguous. Group C genes represent a set of gene predictions that are only supported by a single EST. Group C genes are "below the radar" and are not typically annotated as genes. For each gene or gene model, the closest 5′ FirstEF prediction that also matched the gene transcription direction was chosen.

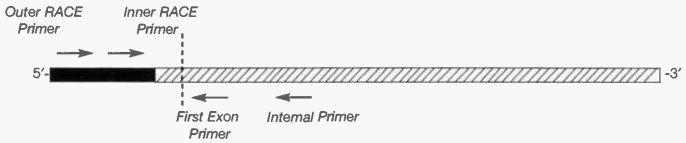

FIGURE 1 Location of nested primers used in RACE-PCR. (*Black*) RACE adapter; (*hatched*) mRNA transcript; (*dashed vertical line*) first exon/second exon boundary; (*arrows*) directionality of primers.

Mouse Transcript Detection

V. Balija, S. Dike, L. Nascimento, L. Palmer, T. Zutavern, M. Yu [in collaboration with G. Hannon and M. Zhang, Cold Spring Harbor Laboratory]

The completion of the human genome sequence is a first step toward understanding its formation. At this time, however, there is considerable uncertainty about even the number of mammalian genes. We have been collaborating with Michael Zhang and Greg Hannon here at CSHL to develop experimental tools combined with computer predictions to identify the genes. These same tools are also being used to identify the 5′ ends of genes. These regions are the most difficult to predict computationally. However, since their location is required in order to identify promoters, it is extremely important to identify these 5′ regions of transcripts.

We have chosen to use RACE-PCR (polymerase chain reaction) to identify 5′ regions of mouse transcripts. We have begun this work with a sample set of 96 genes including a number of known genes. A breakdown of these genes is shown in Table 1. Some of the genes in this test set are unknown and many have unresolved 5′ ends. These initial efforts have been focused on developing techniques to identify the 5′ ends of these genes. We have modified RACE-PCR procedures to optimize a more scaleable process. Figure 1 shows a diagram of the RACE-PCR procedure; Table 2 shows the results of testing these 96 test genes. We now have a robust approach for carrying out this work and are expanding it to include more genes. These techniques will also be very useful for a project that we will be starting in 2003, which will require us to analyze the gene content in regions of hemizygous deletions in tumors (in collaboration with the Wigler and Lucito labs here at CSHL).

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TABLE 2 Results of Identifying the 5′ Ends of Mouse Genes Using RACE-PCR, followed by Sequencing

	Number matching expected size in				Overall	Number
Category	7-day embryo	17-day embryo	testes	brain	expected size matching	sequencing confirmed by
EPD (13)	5	5	3	3	8	12
RefSeq (27)	5	9	7	5	14	18
Cat B (23)	9	12	9	10	15	19
Cat C (50)	18	15	14	15	26	8

MODELS OF CELLULAR PROCESSES AND GENOME EVOLUTION

B. Mishra M. Antoniotti, P. Barbano, W. Casey, V. Cherepinsky, R. Daruwalla, J. Feng, J. McQuown, V. Mysore, S. Paxia, M. Rejali, A. Rudra, M. Spivak, N. Ugel, Y. Zhou

Understanding biology by modeling cellular processes and genome evolution has emerged as a challenging new area: *systems biology*. Sitting at the interface of mathematics and biology, this subject aims to address many questions requiring consilience of elegant ideas and concepts from applied mathematics, theoretical computer science, logic, and physical modeling. The impulse has come from a better understanding of processes involved at the molecular level, technology at a meso- and nanoscale, the ability to perform high-throughput experiments, and the amount of genomic and proteomic data that can now be generated and made publicly available for processing. In response to these challenges and with initial funding from DARPA's BioCOMP program, scientists and mathematicians from Cold Spring Harbor Laboratory and the Courant Institute have begun to focus their collective attention on these questions.

Various projects pursued by this group are described below: (1) *Biochemical Process Theory* seeks to create a unified framework in which one can understand biochemical pathways, and how the evolutionary processes shaped them historically. (2) *Evolutionary Processes, Genomes, and Pathway Models* seeks to develop a set of genome evolution models with solid mathematical and biological foundations that allow the systematic understanding of the underlying probabilistic processes, the constraints they impose on biological systems, and deciphering the modular structure of large-scale biochemical processes. (3) *Advanced Tool Architectures* seeks to create a set of reusable, inter-operating software modules integrated within a bioinformatics language and environment. (4) *Support for Experimental Research*.

Traditionally, biology has proceeded as an observational science. Robert Hooke, whose work "Micrographia" of 1665 included the first identification of biological cells through his microscopical investigations, had said,

> The truth is, the science of Nature has already been too long made only a work of the brain and the fancy. It is now high time that it should return to the plainness and soundness of observations on material and obvious things.

Recently, we have seen an unprecedented progress in our observational/experimental abilities, thus allowing us to understand the structure of a largely unobservable transparent cell. But, much more importantly, we have also become familiar with novel "computational" approaches that rely on simultaneous progress in many fronts: (1) vast amounts of computing power (distributed or tightly coupled parallel computers); (2) accurate physical models at kinetic mass-action, stochastic, spatiotemporal, and hybrid discrete/continuum levels; (3) algorithmic efficiency through symbolic and qualitative computation; and (4) logical reasoning systems and other analysis tools at multiple resolutions. These approaches borrow ideas from computational theories and logic, systems and engineering sciences, and applied mathematics. Encouraged by this confluence of breakthroughs, we have focused on a novel research program combining mathematical theories with experimental observations with an aim of building the foundations of biological processes at many levels.

BIOCHEMICAL PROCESS THEORY

The genome of an organism is a collection of its genes, encoded by four chemical *bases* in its DNA (*d*eoxyribo *n*ucleic *a*cid), and it forms the genetic core of a cell. The genes ultimately encode the protein (a chain of amino acids), and in turn, the genes are regulated by transcription factors and other operons, many of which are proteins. The sequence of amino acids, specified by the DNA through transcription and translation processes, determine the three-dimensional structure and biochemical properties of the proteins as well as the nature of their interactions. Furthermore, mRNA stability, protein degradation, posttranslational modifications, and many other biochemical processes tightly regulate the time constants involved in the resulting biochemical machinery.

Using a system of first-order differential equations (in explicit form), one can construct a general model of a rather complex biochemical reaction involving many genes and proteins. One such model is Savageau

and Voit's S-system. The basic differential equations constituting the system take the following *power law form:*

$$\dot{X}_i = \alpha_i \prod_{j=1}^{n+m} X_j^{g_{ij}} - \beta_i \prod_{j=1}^{n+m} X_j^{h_{ij}}$$

$$C_j(X_1[t],...,X_m[t]) = \Sigma\gamma_j \prod_{k=1}^{n+m} X_k^{\beta_{jk}} = 0$$

where the α_is and β_is are called *rate constants* and govern the positive (synthesis) or negative (degradation) contributions to a given substance. The γ_is are the *rate constraints*.

Such a system of differential equations (power laws) as the above can be integrated to create "traces" either symbolically—in particularly favorable cases—or by numerical approximation using often standard and in some cases novel algorithmic techniques.

The ideas behind describing the semantics of the S-system with an automaton definition and construction are as follows: We start with snapshots of the system variables' values that constitute the possible states of the automaton. Transitions are inferred from traces of the system variables' values. Final states are those states in which the simulation reaches a recognizable end and are supposed to represent equilibrium points for the metabolic pathway to be modeled. The automaton we construct allows us to capture qualitative features of a biological system.

A simple reasoning tool, based on the notions of model checking with temporal logic, can analyze a single trace of the automaton results, where the single trace is created from only one "set-up" (e.g., initial conditions, a set of values for the parameters, a set of signaling events, etc.). To allow more than one trace, it is necessary to consider different "set-ups," e.g., many possible values for the parameters, such as rate constants and kinetic orders (see Antoniotti et al. 2002, 2003a,b; Mishra 2002).

In Figure 1, we show an example of how our systems can be used to reason about the *purine metabolism process*. For more details, see Curto et al. (*Biochem. J. 329:* 477 [1998] and *Math. Biosci. 151:* 1 [1998]) and Voit (*Computational Analysis of Biochemical Systems*, Cambridge [2000]). The main metabolite in purine biosynthesis is 5-phosphoribosyl-α-1-pyrophosphate (PRPP). A linear cascade of reactions converts PRPP into inosine monophosphate (IMP). IMP is the central branchpoint of the purine metabolism pathway. IMP is transformed into AMP and GMP. Guanosine, adenosine, and their derivatives are recycled (unless used elsewhere) into hypoxanthine (HX) and xanthine (XA). XA is finally oxidized into uric acid (UA). In addition to these processes, there appear to be two "salvage" pathways that serve to maintain IMP level and thus of adenosine and guanosine levels as well. In these pathways, adenine

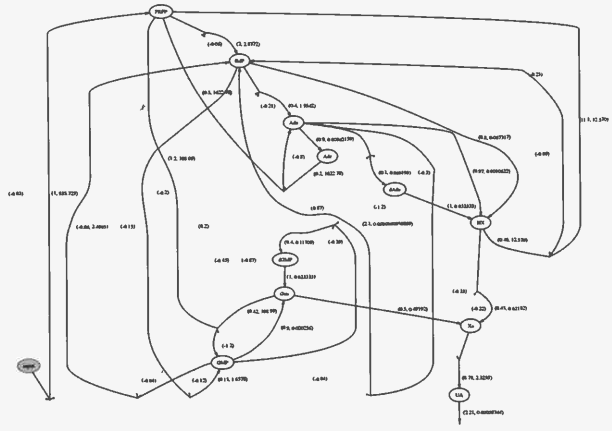

FIGURE 1 A simple (and incomplete model) of the metabolic scheme of purine metabolism in humans. The model fails to satisfy the "robustness conditions."

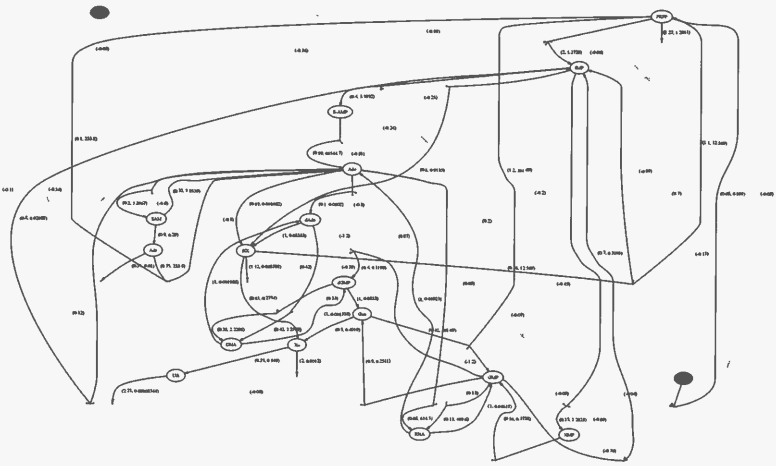

FIGURE 2 A more complete model of the same purine metabolism in humans. Our tool, Simpathica, discovered that the modified model satisfies the elusive "robustness conditions."

phosphoribosyltransferase (APRT) and hypoxanthine-guanine phosphoribosyltransferase (HGPRT) combine with PRPP to form ribonucleotides.

Using our system (Simpathica) and the approaches described earlier, one can automatically create the model shown in Figure 1 and perform model checking on the resulting qualitative automaton to show that the model fails to satisfy the following temporal logic formula (encoding robustness):

```
Eventually(Always (PRPP = 1.7 * PRPP1)
implies
steady_state()
and Eventually(Always(IMP < 2 * IMP1))
and Eventually(Always(hx_pool < 10*hx_pool1))).
```

The above formula states (in English) that "Persistent increase in the initial concentration of PRPP does not cause unwanted changes in the steady-state values of some metabolites." This reasoning process and manipulations with Simpathica ultimately lead to a more complete model shown in Figure 2 that does satisfy the robustness formula.

EVOLUTIONARY PROCESSES, GENOMES, AND PATHWAY MODELS

Genome evolution is a dynamic process. A faithful model of the evolution process can be based mainly on the changes in the genomic sequences, which can further lead to the corresponding changes in the cellular elements at higher levels (proteome, transcriptome, interactome). Of course, natural selection screens out the disadvantageous changes in the genome at the phenotype level, and thus guides the direction of genome evolution. Although different organisms may have evolved under different evolutionary history and different selection pressure, some common statistical features can be found on different levels of cellular processes and can be obtained from analyzing the vast amount of sequence data currently available. Our research has provided credence to a hypothesis first proposed in 1970 by Susumu Ohno, called "evolution by duplication," that can now be verified with the available genome sequence.

Using our models, one can explain the topology of protein interaction networks, linguistic structures of the amino acid sequences in protein domains, and even the fast protein-folding dynamics in nature. The power-law distribution with its "hubby" topology (e.g., p53 was shown to interact with an unusually large number of other proteins) can be explained if one models the new proteins emerging from the older ones, with a propensity to interact with only those proteins that its other family members already interact

with. Since the protein interaction network, as well as other higher-level cellular processes, is thus encoded in genomic sequences, the evolutionary structure, topology, and statistics of many biological objects (pathways, phylogeny, symbiotic relations, etc.) are rooted in the genomic sequence's evolution dynamics. To study the evolution processes of the kind just described, we have developed an efficient "Sequence Grammar" backend for low-level simulation tasks in genomic evolution as well as various mathematical models: an Eulerian random graph model and a model based on Polya's urn.

ADVANCED TOOL ARCHITECTURES

In the domain of most immediate interest, namely, postgenomic biology, the conventional concept of a distributed set of tools, made available through a web-browser interface, fails to adequately respond to the challenges, complexity, and exponentially growing demands. Thus, our approach focuses on creating tool architecture with following properties: (1) Allows for software modules that can be composed in a flexible manner; (2) supports multiple sets of scripting languages, large numbers of library software, and multiple computer architectures; (3) provides free-format databases that hide the idiosyncrasies of multiple formats; (4) allows for rapid prototyping for new experiments and helps in experiment design; and (5) integrates the domain-knowledge such that the tools themselves become the conduit for communication.

In particular, we have focused on enhancing and integrating the following three tools into the general framework outlined earlier:

- **VALIS:** a language-independent environment to prototype bioinformatics applications.
- **Simpathica** and the related XS-system to allow the user to describe, reason about, and interact with biochemical reactions.
- **NYU MAD:** a system to maintain and analyze biological abundance data (e.g., microarray expression levels or proteomic data).

EXPERIMENTAL RESEARCH

The most interesting data points for our study are the time-course data describing genome, transcriptome, and proteome within a single cell. In reality, we have to make do with mRNA collected from a small population of cells, where individual cells within the population may be moving through the cell cycles in an unsynchronized manner.

The required data sets can be collected using two different technologies:

- **Microarray Data:** measuring the mRNA abundance through hybridization to selected cDNAs or oligos.
- **Mass-spectrometry Data:** measuring gene-expression profiles using a mass-spectroscopy-based approach to "resequencing."

Such new technology may have many advantages: It creates less noisy data; it could potentially calibrate for mRNA stability; and finally, as more and more genes are identified, it could be easily adapted to account for the new transcriptomes.

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PREDICTION OF PROTEIN STRUCTURE AND FUNCTION

A.F. Neuwald N. Kannan
A. Poleksic

We seek to uncover clues to protein structure and function through computational and statistical analyses of available data. While analyzing specific proteins, we develop new methods, as they are required, both to identify significant features and to aid interpretation of our findings. More specifically, we are developing chemical, structural, sequence, and taxonomic routines and corresponding statistical procedures, which are being integrated into a suite of programs for comprehensive protein characterization. Getting over the necessary technological hurdles in setting up an infrastructure that can integrate these various forms of information has involved a substantial initial investment. This year, however, we have begun to reap the benefits, and we now possess tools that allow us to explore previously uncharted territory regarding various aspects of protein structure and function.

In general, our research focuses on classes of proteins for which there is abundant sequence, structural, biochemical, and genetic information from a wide range of organisms. In the course of developing our computational and statistical methods, for example, we have used as a test set the P-loop GTPases, for which there are more than 5000 sequences from all three main divisions of life, over 100 distinct structures, and tens of thousands of publications. More broadly, I am investigating the class of proteins called P-loop NTPases, while those within my group focus on other major protein classes. My current postdoc, Natarajan Kannan, is focusing on protein kinases and related kinases, of which there are currently about 12,000 sequences in the database. There are several reasons to focus on these proteins. First, the sheer volume of data available, which may otherwise mask valuable information, provides opportunities for computational approaches designed to handle large data sets. Second, large data sets make statistical approaches more powerful. Third, these classes of proteins have essential cellular roles, as is indicated by their high conservation and prevalence, and thus the aim of understanding their functions offers substantial biomedical benefit. Finally, many of these proteins are highly relevant to other research projects here at CSHL.

CHAIN ANALYSIS

This work was done in collaboration with Jun Liu, Department of Statistics, Harvard University.

This year, we completed the initial version of a major computational project called CHAIN (*c*ontrast *h*ierarchical *a*lignment and *i*nteraction *n*etwork) analysis. Proteins comprising the core of the cellular machinery are often highly conserved, presumably due to selective constraints maintaining important structural features. CHAIN analysis relies on statistical procedures to decompose these constraints into distinct categories and to quantify corresponding selective pressures, thereby pinpointing functionally critical structural features within each category (for an example, see below). Completion of this project has involved further development of (1) an important underlying statistical procedure (now called Bayesian partitioning with pattern selection), (2) automated taxonomic tools based on the NCBI taxonomy database, (3) automated tools for analysis of molecular interactions, and (4) improved multiple sequence alignment routines (via the addition of marginal probability trimming and PSI-BLAST checkpoint file routines). It also involved the incorporation of several new procedures including (1) structurally based alignment routines; (2) design of a structural visualization language and implementation of a corresponding language parser; (3) tools for identifying correlations between aligned residues, which allows us to identify key interactions involving residue pairs; (4) routines for exploring the full range of variability at specific positions in homologous proteins; and (5) a motif-based multiple alignment option that allows analysis of subtly conserved patterns missed by the default (PSI-BLAST) alignment algorithm. These new automated features allow us to more rapidly analyze large sets of proteins and thereby speed up comprehensive analysis of large sequence sets.

SURVEY OF PROTEINS AMENABLE TO CHAIN ANALYSIS

Using these CHAIN analysis tools, we have initiated a broad search of the protein database in order to locate

those protein domains most amenable to CHAIN analysis. Ideally, candidate protein families should be relatively highly conserved across diverse taxa and should be a subset of a much larger protein class. Automated routines for finding such proteins have been implemented. Another valuable strategy is to examine all such families in a class, which thereby helps us interpret analyses of individual families by leveraging information from other families. This survey has yielded various candidate protein domains of particular current interest, including, for example, members of the AAA+ and actin/hsp70 classes of chaperones, protein and histidine kinases, and helicase superfamilies 1 and 2. While we are now focusing our efforts on specific P-loop NTPases and kinases, limited further analysis of these other groups has been helpful, as it has revealed the nature of various conserved structural motifs, thereby aiding interpretation of analyses for specific families of interest.

PROTEIN STRUCTURAL PRINCIPLES

Structural information gathered from this broad survey more generally aids our understanding of proteins by revealing basic structural principles, which are manifested as recurring conserved features. This information is being interpreted in light of basic principles gathered from the chemical and structural literature. Published principles have been and are being incorporated into computer routines that, for example, allow us to exhaustively search the structural database for all proteins exhibiting a given feature. Several such routines were merged into the CHAIN analysis suite of programs. The utility of this approach is illustrated, for example, in our description below of the role of amino acid torsion angle strain in protein kinase mechanisms.

CHAIN ANALYSIS OF "FY-PIVOT" GTPASES

We have completed our analysis of a subclass of Ras-like proteins, which we term "FY-pivot" GTPases. In particular, this analysis has revealed within Rab, Rho, Ras, and Ran a canonical network of molecular interactions centered on bound guanine nucleotide (see Fig. 1). This network presumably performs a crucial structural and/or mechanistic role, considering that it has persisted for more than a billion years after the divergence of these families. We call these "FY-pivot" GTPases after their most distinguishing feature, a phenylalanine or tyrosine that functions as a structural pivot within this network. We found that specific families deviate somewhat from canonical features in interesting ways, presumably reflecting their functional specialization during evolution. In Ran, for example, two conserved histidines that strikingly diverge from their canonical counterparts appear to facilitate alternative protein conformations coupling Ran's "basic patch" and "carboxy-terminal switch" (two prominent structural features of Ran) to guanine

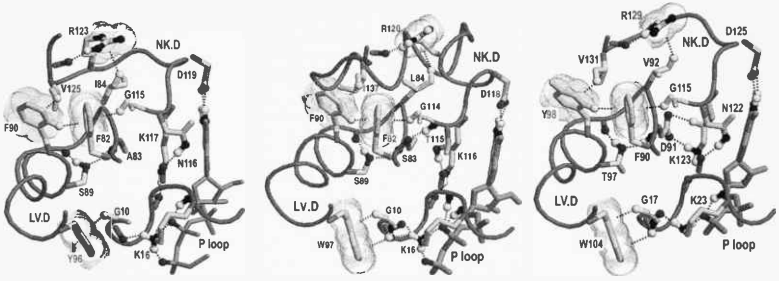

FIGURE 1 Canonical structural features shared by Ras, Rac1, and Ran as revealed through CHAIN analysis.

nucleotide exchange and effector binding. Ran and these related proteins, which have served as a test set for development of CHAIN analysis procedures, have very critical roles in cellular signaling events. Publication of these findings thus has broad biological implications and at the same time illustrates the utility of CHAIN analysis.

PROTEIN KINASE STRUCTURAL MECHANISMS

CHAIN analysis of P+1-directed protein kinases has revealed, as their most distinguishing feature, an arginine that, by interacting with the backbone of a substrate-binding residue, induces a strained conformation facilitating substrate recognition. This interaction is initiated upon phosphorylation of the activation loop via propagated conformational changes involving other distinctively conserved residues. We predict similar mechanisms for the glycogen synthase kinase Gsk3β and the SR kinases, which processively phosphorylate tandem recognition sites and in which a previously phosphorylated substrate site functions as a surrogate activation site, and for autophosphorylation of dual-specificity tyrosine-phosphorylated and -activated kinases.

In a related analysis of atypical kinases, we found (using a recently improved motif-based search procedure) several new kinase families possessing the protein kinase structural fold. Moreover, this analysis strongly suggests that four residues in this protein class (two aspartates, one asparagine, and a histidine or tyrosine) constitute the minimum requirement for kinase activity. It appears that an essential role for this histidine or tyrosine residue in the kinase mechanism has not been proposed previously. Kinase mechanisms are classified into two categories: those with associative modes of phosphoryl transfer and those with dissociative modes of phosphoryl transfer. We found a correlation between an associative versus a dissociative mechanism and the occurrence of tyrosine versus histidine, respectively, at this site. A search of the chemical literature revealed principles explaining this correlation.

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Andy Neuwald

COMMUNITY ACCESS TO GENOME RESOURCES

L.D. Stein S. Avraham, S. Cain, N. Chen, K. Clark, F. Cunningham, P. D'enstachio, T. Harris, G. Joshi, A. Kanani, X. Pan, L. Ren, S. Schmidt, L. Tegtelman, G. Thorisson, P. Van Buren, D. Ware, W. Zhao

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 non-rice crop they are studying. Hence, the resource allows researchers studying traits, for example, in maize and barley, 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 900,000 monocot expressed sequence tags (ESTs) to the rice genome, allowing gene predictions to be further refined based on cross-species comparisons. During this year, we developed a new integrated map of the rice and maize genomes that allows researchers to move back and forth between various genetic and physical maps of these species in order to apply the knowledge developed in one organism to finding functionally significant genes in the other.

Curation is an important component of Gramene. In collaboration with Susan McCouch's laboratory at Cornell, we have curated more than 12,000 rice proteins, assigning them classifications in the Gene Ontology (www.geneontology.org). In addition, we have classified more than 700 rice mutants using a trait ontology that we have developed. We are currently curating the rice biological literature, much of which is in non-English languages, and making this information available via Gramene as well.

Version 1.00 of Gramene went live in December 2001 and has been followed by a new release every 3 months. Much of the software developed for Gramene is novel and includes such useful software modules as a comparative map viewer (Fig. 1), an ontology browser, and a physical map viewer. The work on Gramene has resulted in three publications in prestigious scientific journals.

WORMBASE: A RESOURCE FOR *C. ELEGANS* GENOME AND BIOLOGY

During the past year, our lab received a major grant to develop WormBase, an on-line 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 with 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 2002, we added the following key features to Wormbase:

- A synteny viewer (Fig. 2), which allows researchers to compare the *C. elegans* genome to orthologous regions in *Caenorhabditis briggsae*, a worm that diverged over 100 million years ago. This comparison allows researchers to refine the structures of genes and to discover putative regulatory regions.
- A redesigned gene page that gives researchers an "at-a-glance" summary of the functional significance of a confirmed or predicted gene.
- Interfaces to the BLAST and BLAT sequence similarity engines.

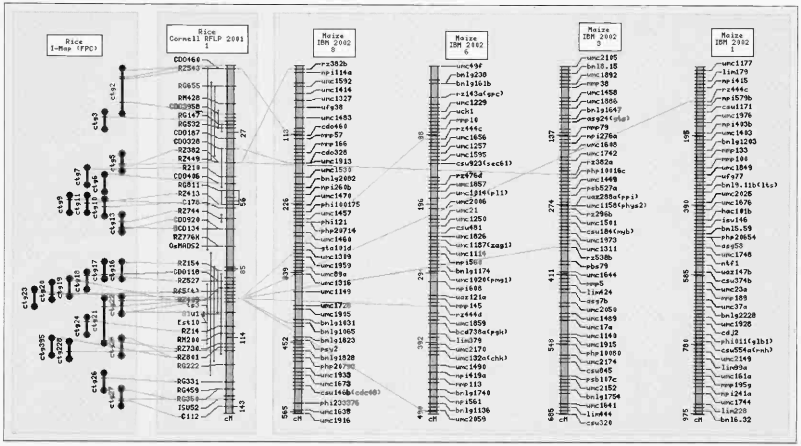

FIGURE 1 The Gramene comparative map viewer interrelates the genetic and physical maps of maize and rice.

DISTRIBUTED SEQUENCE ANNOTATION SYSTEM

DAS, the distributed sequence annotation system, is a software architecture that allows researchers from around the world to share and exchange annotations on the genome without the need for error-prone and time-consuming reformatting currently required. This year saw the widespread adoption of DAS. It is now in use as the data exchange format by the EBI/Sanger EnsEMBL project, the UCSC Genome Browser, TIGR, FlyBase, WormBase, JGI at OakRidge, and the Whitehead Institute.

GENOME KNOWLEDGEBASE

The Genome KnowledgeBase (GK) is a collaboration with Ewan Birney of the EBI and Suzanna Lewis of the Gene Ontology Consortium, to develop a Web-accessible resource for curated information about biological processes.

The GK is organized like a review journal. Practicing biologists are invited to create "summations" that summarize a particular aspect of their field. Summations currently include "DNA Replication," "Protein Translation," and the basic pathways of intermediate metabolism, such as the TCA cycle. New modules for "RNA Splicing" and "Cycle Checkpoints" are in progress. Summations 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. The Web Site can be browsed like a textbook, or searched with queries to discover pathways and connections. The second major release of GK occurred on February 1, 2003, and is available at www.genomeknowledge.org.

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. The hope is to significantly reduce the time and expense required to create new databases to curate genomic information coming from various model organism system sequencing projects (e.g., rat,

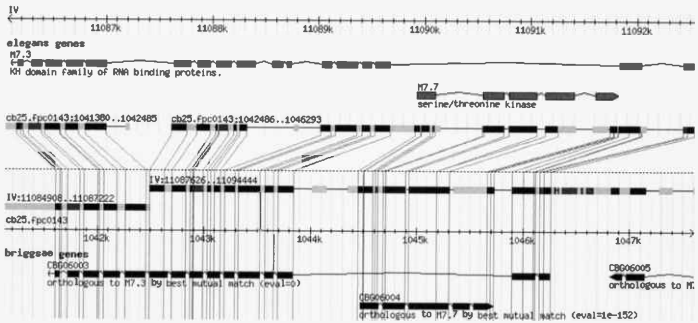

FIGURE 2 WormBase displays the relationship between a region of the C. *elegans* genome (*upper panel*) and the corresponding region of the C. *briggsae* genome (*lower panel*).

Dictyostelium, and *Plasmodium*). As of the end of 2002, we released the following modules:

- GBrowse, a genome browser and annotation tool.
- CMap, a comparative mapping tool.
- LabDoc, a system for creating and maintaining standard operating procedure documents.

THE HUMAN HAPLOTYPE MAP

Following our successful participation in The SNP Consortium, which culminated in the publication of a map of 1.6 million human single-nucleotide polymorphisms (SNPs), our lab was selected to coordinate the next SNP-based project, the HapMap. The HapMap project is an international effort to find and define regions of high linkage disequilibrium in the human genome. These are regions in which genetic recombination is rare and across which most individuals in the general population carry a small number of common variants. The goal of the HapMap project is to characterize more than 2 million SNPs in order to define linkage disequilibrium blocks covering 85% of the human genome. The outcome is called a "haplotype map."

The significance of this map is that it will dramatically reduce the time and cost necessary to perform genetic screens to identify genes that are responsible for human disease. It will also provide valuable insights into human evolutionary history.

Our lab will be supporting the project by coordinating the efforts of the various research groups and by running the main database in which intermediate results are stored.

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COMPUTATIONAL GENOMICS

M.Q. Zhang Z.Y. Xuan, J.H. Wang, N. Banerjee, L.H. Liu, I. Grosse, F. Wang, J. Ou, N. Hata, G.X. Chen, K. Mamoru

Our research interest continues to be the identification and characterization of the genetic elements in nucleic acid sequences by computational means. As the Human Genome Project is close to the finishing the sequencing of the human genome, developing efficient computational methods for identification of genes and their control/regulatory elements has become extremely important. Knowing the organization of a gene often becomes the prerequisite for further functional studies. In the past, we studied statistical characters of exons and introns in protein-coding regions and developed coding-exon prediction programs by applying multivariate statistical pattern recognition techniques. Recently, we started looking into the more difficult problems of finding regulatory *cis*-elements in noncoding regions by taking advantage of new resources, such as the mouse draft genome and large-scale chromatin immunoprecipitation (ChIP)-chip data. Our main goal was to carry out a whole-genome comparison between human and mouse as well as between the public and Celera mouse genome assemblies. As a result, we have developed the Conserved Sequence Element database (CSEdb). Furthermore, we have developed an annotation pipeline, which enabled us to combine FirstEF and mRNA/EST data to make a first-pass human genome annotation of promoters and first exons. For our collaborative works, please see the Krainer, Tully, Lowe, McCombie, and Hannon reports in this Annual Report.

We (J. Ou, N. Hata, Z.Y. Xuan, and M.Q. Zhang) are also developing high-throughput experimental validation technologies for first exon predictions in collaboration with the McCombie and Hannon labs.

First-pass Annotation of Promoters and First Exons in the Human Genome

I. Grosse, M.Q. Zhang [in collaboration with R. Davuluri, Ohio State University]

The human genome contains a vast amount of *cis*-regulatory regions responsible for directing spatial and temporal patterns of gene expression, and delineating their locations is of paramount importance for our understanding of gene expression and regulation. However, due to the absence of full-length mRNA transcripts, the identification and annotation of these functional regions are behind that of coding regions. Here, we present an initial computational annotation of promoters and first exons for the 24 chromosomes of the human genome, which we will make publicly available at http://www.cshl.edu/mzhanglab/ once our paper is accepted. We are currently working on the mouse promoter and first exon annotation problem by taking into account human-mouse comparison.

Computational Comparison of Two Mouse Draft Genomes and the Human Goldenpath

Z.Y. Xuan, J.H. Wang, M.Q. Zhang

The availability of both mouse and human draft genomes has marked the beginning of a new era of comparative mammalian genomics. The two available mouse genome assemblies, namely, those from the public mouse genome sequencing consortium and Celera Genomics, were obtained by using different clone libraries, as well as different assembling methods. By taking advantage of both public and private mouse genome sequencing efforts, we were able to present a critical comparison of the two latest mouse genome assemblies. The utility of the combined mouse genomes is further demonstrated by comparing them with the human goldenpath and through a subsequent analysis of the resulting CSE database. In particular, the CSE information allows us to identify more than 6000 potential novel genes and to derive independent estimates of the number of human protein-coding genes. Although the Celera and public mouse assemblies agree to a great extent, they differ in about 10% of the mouse genome. Each assembly has advantages over the other. Among the advantages, the Celera assembly has higher accuracy in base pairs and overall higher coverage of the genome. The public mouse assembly, however, has higher sequence quali-

ty in some newly finished BAC (bacterial artificial chromosome) regions and the data are freely accessible. Perhaps most importantly, by combining both assemblies, we can get a better annotation for the human genome. In particular, we can obtain the most complete set of CSEs. One third of these CSEs are related to known genes. Some CSEs are related to other functional regions in the genome, and more than half of the CSEs are still functionally unknown. CSEs allow us to estimate the total number of the human protein-coding genes to be about 40,000. Since CSEs can shed additional light on the functional regions of the genome as we have demonstrated, making this searchable CSEdb publicly available on-line will expedite new discoveries through comparative genomics. We are continuing on with comparative genomics with more species, such as fish and rat.

The Argonaute Family: Tentacles That Reach into RNAi, Developmental Control, Stem Cell Maintenance, and Tumorigenesis

Z.Y. Xuan, M.Q. Zhang [in collaboration with G. Hannon, Cold Spring Harbor Laboratory]

The Argonaute proteins are related to RNA interference (RNAi), developmental control, and other biological functions. Using a protein motif of the family members (PIWI and PAZ), together with the expressed sequence tags (ESTs) and the mouse genome of Celera Genomics, we discovered five novel mouse *argonaute* gene candidates and one novel human homolog. The phylogenetic analysis of all seven human proteins and eight mouse Argonaute proteins, together with other family members in *Drosophila, Arabidopsis*, rabbit, etc., have revealed potentially important functions of these novel human and mouse genes.

Characterization and Prediction of the Nanos Response Element with Sequence Analysis in *Drosophila*

G.X. Chen, M.Q. Zhang [in collaboration with T. Tully, Cold Spring Harbor Laboratory]

The Nanos response element (NRE) in the 3′-untranslated repeat (3′UTR) of *Drosophila hunchback* (*hb*) mRNA has been shown to be critical to normal abdominal segmentation during *Drosophila* development. NRE in *hb* mRNA is bound by Pumilio, which further recruits Nanos and another factor, Brain Tumor (Brat). The formed complex represses the translation of *hb* mRNA, possibly via a deadenylation mechanism. Another protein Bicoid (Bcd), which establishes head and thoracic development in *Drosophila*, is also shown to be regulated by Nanos and contains NRE. We are interested in characterizing the sequence pattern of NRE and using this pattern to search for other potential targets of Pumilio and Nanos. Fifteen 3′UTR sequences were retrieved from GenBank based on annotation, including *bcd* mRNA from seven different *Drosophila* species and *hb* mRNA from five *Drosophila* species as well as one *hb* mRNA from *Musca domestica*. First, Gibbs motif sampling was used to identify common motif elements among the 15 3′UTR sequences. Since Gibbs sampling is a stochastic method, multiple runs were performed with different parameters. The most stable motif found included one element from every *bcd* 3′UTR and two elements from every *hb* 3′UTR. To further improve the alignment obtained from Gibbs, we realigned the motif elements with T-Coffee. The realignment revealed a motif with two highly conserved cores separated by five or six bases, as regular expression GTTGT.{5,6}ATTGTA. To verify our local alignment result based on Gibbs sampling, we also carried out global alignments (ClustalW) with *bcd* and *hb* 3′UTR sequences, respectively. All of the elements identified with Gibbs were aligned in conserved regions except one of the elements in the *M. domestica* 3′UTR. Next, we downloaded all *Drosophila* 3′UTR sequences in the UTRdb (8998 in total, 6063 nonredundant) and searched for potential NREs with the regular expression defined previously. Besides *bcd* and *hb*, six other genes were found to contain the pattern in the 3′UTR. Little is known about the function of those genes except one, *Serrate* (*Ser*). *Ser* is shown to be involved in wing development and is expressed in tissues including the central nervous system and dorsal mesothoracic disc, during specific development stages. Interestingly, it is reported that misexpression of Brat suppresses growth of the wing, suggesting that *Ser* may also be a target of regulation by the Pumilio-Nanos-Brat complex.

Developing ChIP-chip and Promoter Data Analysis Algorithm Package

N. Hata, M.Q. Zhang [in part in collaboration with the Ren Lab at the University of California, San Diego]

We have been creating a comprehensive software library for discovering transcription binding sites in

promoter sequences. The package includes a new motif-finding algorithm, promoter database for mouse and human, and incorporation of microarray gene expression data and ChIP data. Our binding-site search is based on motif counting by comparing promoters identified by the expression or ChIP data with respect to control (background) promoter sequences. Our algorithm is robust, exhaustive, and quick enough for genome-wide analysis. Our collection of mouse and human promoter sequences based on the RefSeq database is extremely useful in analyzing general microarray/ChIP experiments. We are producing our package to become the standard sequence analysis tool for microarray/ChIP experimentalists. We have applied our algorithm to mouse tissue-specific microarray data from Riken and c-Myc-binding promoter data obtained from the ChIP experiment by the Ren lab. We found known as well as novel binding sites for tissue-specific (liver, kidney, bone, brain, etc.) promoters and obtained discrimination functions that can be used in genome-wide search of tissue-specific *cis*-elements. The results were presented in an invited talk at the Genome Informatics meeting in Cambridge. The details and technical description of the algorithm will be published separately. We have obtained even cleaner and convincing motif candidates from the c-Myc ChIP data. The predicted binding sites are new and have distinct spatial distribution. There are also new results for co-occurring motifs. Once the package is complete, there will be wide applications. Our prediction of novel binding sites and tissue specificity of genes should be validated by experiments, and further feedback as well as new data from biologists will improve our algorithm.

Genome Level Analysis of Alternative Splicing, and Cross-species Comparison between Human and Mouse

J.H. Wang, M.Q. Zhang [in collaboration with the Fu Lab at the University of California, San Diego]

We are in the process of developing alternative transcript databases for the human and mouse and have compared the alternative spliced transcripts of the human and mouse. It is a great challenge to keep these results in sync with the constant updating of the genome assemblies. The growing amount of full-length RefSeq mRNAs/cDNAs have turned out to be most useful and provide us more insight into the variety of alternative splicing patterns.

We are classifying genes into two groups: One has conserved splicing patterns and another has potentially distinct splicing patterns.

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NEUROSCIENCE

With its 100 billion cells of many different types forming 100 trillion specific connections or "synapses" among themselves via 300 million feet of biological wiring, few things are more mysterious or hold more fascination than the human brain, and nothing is more biologically complex. CSHL neuroscientists are tackling the epic task of sorting out how the brain develops, learns, remembers, experiences, and recovers from injury or disease, and ultimately makes us who we are.

The brain is able to solve certain computations that remain far beyond today's fastest and most sophisticated computers. Anthony Zador's lab is probing the brain's unique and powerful computational style by examining how the brain processes complicated auditory input we call sound. Zador's lab has uncovered a code that neurons use to represent sound and is working to sort out the different excitatory and inhibitory components that generate the code. These studies are helping us to understand the unique and powerful but largely unexplored computational properties of the brain.

Dmitri Chklovskii's lab uses mathematics and physics to examine constraints on brain structure and function. By uncovering such limits, they hope to delineate the "engineering" principles that govern brain evolution. The scientists ask how neurons are connected and are working to understand how neurons organize themselves into circuits and how those circuits produce behavior. By comparing the patterns of brain structure between animals, they have begun to pinpoint features that are fixed and characteristics that can vary.

By focusing on a part of the brain that is variable, or plastic, Hollis Cline's lab examines how sensory experience shapes the ways neurons connect during development. Cline and her colleagues study the development of the visual system in amphibian tadpoles. They address how sensory activity stimulates mechanisms within brain neurons and thereby guides visual system development. Many animals share mechanisms of brain development. Therefore, Cline's research is revealing fundamental processes that govern brain development in a wide range of species, including humans.

Brain plasticity comes into play not only during brain development, but also during learning and memory formation. Karel Svoboda's lab investigates how sensory experience triggers the long-term changes in brain architecture believed to underlie memory. Using custom-made, state-of-the-art imaging technology, the scientists have taken pictures of synaptic connections between neurons in the brains of adult mice. From the images, they detailed the first measurements of the stability of synapses, measuring how often the connections form and disappear in a part of the adult brain called the barrel cortex. They found that some connections are stable for months while others disappear within a day and that synapse turnover was influenced by experience. Their research suggests that the new connections encode memories.

Roberto Malinow's lab studies how the molecular composition of synapses changes in response to sensory experience. Perhaps their most exciting finding this year was their demonstration that experience drives neurotransmitter receptor proteins called AMPA receptors into synapses. This is the first experience-induced specific molecular modification of a synapse to be discovered. Malinow's findings indicate that the dynamics of AMPA receptor replacement at synapses is likely to be a principal mechanism of learning and memory.

Josh Huang's lab also studies molecular responses to sensory experience and has focused on a specific type of inhibitory synapse or "off switch" defined by a molecule that it releases (GABA). They have developed techniques for studying these special synapses in animals and cell cultures, which allow them to investigate how a myriad of crucial "off" switches are built into complex brain circuitry during development.

Because the same proteins implicated in human Alzheimer's disease are present in the fruit fly brain, Yi Zhong is using the power of fruit fly genetics to model and unravel the causes of Alzheimer's disease in humans. Zhong's group has found that buildup of one particular protein (Aβ42) is sufficient to cause nerve cell death and memory loss in flies. Zhong's "Alzheimer's Flies" can be used to identify

new molecular targets for treating the disease, or to screen large numbers of drugs for those that might slow or prevent nerve cell death in the brain of Alzheimer's patients.

Tim Tully's lab studies memory formation by using genetics to isolate fruit fly mutants ("Pavlov's Flies") that display defects in memory formation. They have identified 42 genes that may work to form memories in flies. Interestingly, many of these genes have counterparts in mice and humans. Therefore, Tully has tapped into the genetic basis of a fundamental memory mechanism.

Jerry Yin's lab investigates a process believed to underlie memory called synaptic tagging. Yin's lab is interested in how the interaction of particular proteins during synaptic tagging drives the formation and maintenance of memory. They have characterized several proteins that might control memory formation and have investigated how neurons might confine proteins to particular synapses via synaptic tagging. Once memories are formed, Yin and others theorize that sleep plays a role in memory consolidation, perhaps by providing time for memories to be "replayed" and thereby strengthened. In one test of this idea, Yin's lab has devised a genetic method for manipulating how much sleep flies get.

While several CSHL researchers study long-term memory, Carlos Brody explores short-term memory, which is essential for carrying on a conversation and many other vital functions. In collaboration with neurophysiologists at several institutions, Brody and others in his lab have studied the electrical impulses of neurons that support short-term memory. They have also created mathematical models of neural networks that recapitulate the patterns of real-world neural activity they observe. Such models explain the mechanism of short-term memory in a rigorous, testable fashion and may also predict unexpected properties of short-term memory.

Zachary Mainen's lab studies rodent behavior that is influenced by smell to explore awareness, motivation, and decision-making. The group studies small- and large-scale events, from how single olfactory neurons send out signals to how large ensembles of neurons enable rats to make decisions based on what they smell. Last year, Mainen's group found that rats get a complete take on their olfactory world with each 150-millisecond sniff, a discovery that guides how we interpret the brain activity that underlies decision making.

Part of what makes the brain so powerful is the extraordinary array of diverse cell types it comprises. In studying adult stem cells, or unspecialized cells that can develop into many different cell types, Grigori Enikolopov's lab has found that the compound nitric oxide can guide stem cells to become neurons. In related research, the lab has found that the stem cells in the mouse brain are remarkably plastic, capable of adopting any one of several fates. Enikolopov's studies support the notion that coaxing stem cells down particular developmental pathways might be an effective strategy for treating brain disease or injury.

Koichi Iijima

COMPUTATIONAL SYSTEMS

C.D. Brody S. Chakraborty
C. Machens
A. Penel

During 2002, we continued to pursue our investigation of mechanisms of short-term memory. Here, we use "short-term" to mean the type of memory that lasts a few seconds, for example, the type of memory one uses to hold a telephone number actively "in mind," in between seeing it on a page and dialing it. In collaboration with Ranulfo Romo (National University of Mexico), we published a paper on the role of monkey secondary somatosensory cortex in the short-term memory and decision-making components of a vibrotactile stimuli discrimination task. Santanu Chakraborty (Watson School graduate student) began a collaboration with David Tank (Princeton) to develop methods to study the anatomy of the neuronal circuits in the goldfish brain that support short-term memory of eye position. Amandine Penel (postdoc) joined our lab in September to work on human psychophysics of short-term memory of temporal patterns. In separate projects, Christian Machens (postdoc), collaborating with the Zador lab here at CSHL, submitted a paper on using natural sounds to discover the structure of receptive fields of cortical auditory neurons; and collaborating with John Hopfield (Princeton), we submitted a paper on using spike timing synchronization to robustly recognize odors.

Timing Effects in the Representation of Short-term Memories in the Prefrontal Cortex

C.D. Brody [in collaboration with R. Romo, National University of Mexico]

Collaborating with the awake monkey laboratory of Ranulfo Romo, we have studied the time-dependence of short-term memory neural activity in the prefrontal cortex. A central idea behind current models of short-term memory activity is that neurons involved in it represent memories through activity patterns that persist long after the stimulus that induced them is gone. For example, the neuron with responses illustrated in Figure 1 has a firing rate that, even after the stimulus is over, depends on f1, the remembered stimulus. Current models of such activity posit that this f1-dependent firing rate should be constant in time during the delay period—the lines in the lower part of Figure 1a should be flat. If this were so, then for each firing rate, there would be a single corresponding value of f1, and knowing the firing rate would tell us the value of f1. But we have found that in fact most neurons have responses like those illustrated in Figure 1a: The lines are most decidedly not flat. One cannot straightforwardly decode what 11 spikes/sec means, because it means quite different things during the first vs. middle vs. last seconds of the delay period (see Fig. 1b). However, if we knew which part of the delay period we were in, *then* we could correctly decode the f1 value that corresponds to 11 spikes/sec. The brain faces precisely the same problem. We hypothesize that some time-dependent neurons in the prefrontal cortex have an explicit timekeeping role, thus enabling accurate decoding of short-term memory firing rates, and enabling completion of the sequential discrimination task. We are constructing models of mechanisms behind such time-keeping neurons, and how they could interact with f1-dependent neurons.

Perception and Short-term Memory of Temporal Patterns

A. Penel, C.D. Brody

Recordings such as the one illustrated in Figure 1 suggest that timing has an important role in short-term memory. We will manipulate timing in human psychophysics experiments, using tasks similar to the one illustrated at the top of Figure 1. First, we will investigate in detail how short-term memory decays as a function of delay period length. We are now asking whether timekeeping is important for decoding on an ongoing basis during the delay period, or whether decoding of

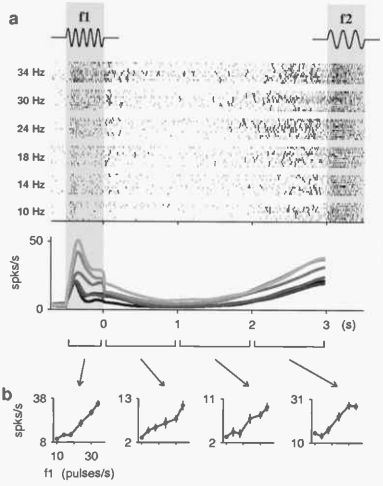

FIGURE 1 Time dependence of the firing rate of a short-term memory neuron in monkey prefrontal cortex. The monkey has been trained to perceive the frequency of two mechanical vibrations sequentially applied to a fingertip, and to report which of the two was the higher (see task schematic at top of figure; PB indicates the time at which the monkey makes its report). To carry out this task, the subject must remember stimulus f1 during the 3-second-long delay period. (*a*) Responses of a neuron in the prefrontal cortex during the task. Each row represents a trial, and each tick mark represents the time of an action potential. Trials are grouped according to the value of stimulus f1, indicated at the left. The lines below indicate smoothed estimates of firing rate as a function of time for each f1 value. Although firing rates are a function of f1 throughout the delay period, they also change markedly during the delay period. (*b*) Firing rate of the neuron as a function of f1 for four different nonoverlapping periods, as indicated.

short-term memory firing rates is carried out only at the end of the delay period. In the latter case, delay periods terminated at unexpected points in time should lead to significantly higher errors than those of trials with a similar, but expected, delay period length. In the former case, there should be no significant difference. Requisite experiments will be carried out to distinguish between these two possibilities. We will also develop a discrimination task similar to the one of Figure 1 but based on discriminating temporal patterns of pulses. This task will be used to investigate the properties of short-term memory of temporal patterns.

Computational Modeling of the Olfactory Bulb

C.D. Brody [in collaboration with J. Hopfield, Princeton University]

Collaborating with John Hopfield, we have studied models of how synchronization of cells in different glomeruli of the mammalian olfactory bulb can lead to odor identification in a manner that is invariant to changes in concentration of the target odor, and also invariant to the presence of background odors. Our model makes specific predictions regarding both synchronization properties of mitral cells, and the conditions under which synchronization and odor recognition should break down. In a model in the computer, the designers can carefully specify each connection. But in a biological system, connections are specified through a combination of genetics and experience. We are using our olfactory bulb model to investigate unsupervised connection learning rules that would allow the model to automatically self-wire, based on the experience of being exposed to odors.

Structure of Auditory Neuron Receptive Fields

C. Machens

Although it is widely agreed that the primary visual cortex decomposes images into components such as

oriented edges, the corresponding decomposition of acoustic sounds in the primary auditory cortex remains somewhat obscure. In the last few years, a candidate approach for characterizing the function of auditory neurons by describing their responses in terms of a spectrotemporal receptive field (STRF) has been explored. The appeal of the STRF rests, in part, on its conceptual simplicity: Like its successful visual counterpart, the *spatio*temporal receptive field, the STRF offers a straightforward linear description of a neuron's behavior.

In a collaboration with Mike Wehr and Anthony Zador here at CSHL, however, we showed that the STRF can describe only part of what cortical neurons do. Using in vivo whole-cell recordings, we tested the ability of the STRF to predict the responses of cortical neurons to natural sounds. In contrast to the simple, artificial sounds usually employed in neurophysiological experiments (such as sine tones, ripple stimuli, or random chord stimuli), natural sounds such as animal communication calls are far more complex and thus probe a greater range of the system's capabilities. Our results show that a few of the sounds lead to quite reasonable predictions, whereas most responses could only partially be estimated and some not at all. Hence, in a more natural mode of operation, the responses of neurons in the auditory cortex are mostly dominated by nonlinearities.

The estimation of STRFs from natural sounds is severely complicated due to the fact that natural sounds do not sample all directions in stimulus space in a natural manner. The methods that neurophysiologists usually employ for the computation of STRFs from the actual data are therefore no longer suited in this specific case. Hence, we developed an improved estimation procedure using regularization methods specifically tailored toward the STRF estimation task. These tools allowed us to overcome the severe overfitting problems associated with the estimation of parameters from natural sounds.

Our next step is to explicitly model the encountered nonlinearities in order to gain a greater understanding of the neural code in the auditory cortex.

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PRINCIPLES OF BRAIN DESIGN

D.B. Chklovskii A. Stepanyants S. Song
M. Reigl

The following are two main questions in neuroscience: How do neurons organize themselves into highly interconnected circuits? How do these circuits function to produce behavior? Neither of these questions can be answered without thoroughly understanding neuronal connectivity. Therefore, our immediate goal is to build a theoretical framework that would help establish neuronal connectivity. We work toward this goal from several different directions. First, we analyze physical constraints on brain design and function in order to determine "engineering" principles that govern brain evolution. These principles, like the laws of conservation in physics, can narrow down the set of plausible neuronal circuits and help focus our efforts in the search for the models of brain function. Second, we determine which features of neuronal connectivity are conserved and which can vary from animal to animal and within the same animal over time. Understanding these different features exposes biological substrates for individual variability and neural plasticity. Third, we analyze wiring diagrams of neuronal circuits in an attempt to find parsimonious descriptions of connectivity. This should allow us to make a connection between the wiring diagram of the brain and its function. In 2002, we made excellent progress on all of these research directions.

Relationship between Neurite Diameters and Branching Angles in Cultured Neurons

D.B. Chklovskii [in collaboration with A. Ayali, Tel-Aviv University]

The wiring economy principle allows one to derive a relationship between branching angles of neurites and their diameters. The derivation is based on balancing virtual forces on neurite branches. Although this relationship makes interesting experimental predictions, testing these predictions in vivo is problematic because angles are modified due to high density of entangled neurite branches. Therefore, we decided to perform the test of this theory in a low-density invertebrate neuronal culture. We measured neurite diameters and angles with optical microscopy and compared them with our theoretical predictions. Preliminary results show that the predicted relationship seems to hold, although the measurements are obscured by the adhesion of neurite branches to the substrate.

Search for Repeating Motifs in Biological Networks

D.B. Chklovskii, M. Reigl [in collaboration with U. Alon, Weizmann Institute]

The functioning of living organisms relies on numerous complex networks: from gene transcription regulation to neuronal circuits in the brain. Although these networks have been mapped out in simpler cases—*Escherichia coli* (transcription regulation) and *Caenorhabditis elegans* (synaptic connectivity)—their function is not understood. Identifying functional modules out of subsets of the whole network may help our understanding of these networks. The goal of this project is to search for functional modules, including several neurons in *C. elegans*. The hope is that the modules which have a stereotypically functional role are present more often than at chance. To find them, we perform a statistical analysis on a real network and compare the outcome with the randomized network. We found several overrepresented motifs that may perform a stereotypical function.

Correlation Analysis of the Spatial Locations of Dendritic and Axonal Branches

D.B. Chklovskii, A. Stepanyants [in collaboration with G. Tamas, University of Szeged, Hungary]

Potential connectivity between neuronal pairs is often evaluated by calculating the overlap between their axonal and dendritic arbors. This procedure is justified only if the spatial locations of dendritic and axonal

branches in the arbors are independent. We determine whether this is the case by performing a statistical analysis of three-dimensional reconstructions of neuronal pairs. If we find that the locations of dendritic and axonal branches are independent, arbor overlap can be used to predict potential connectivity. If the locations of dendritic and axonal branches are found to be correlated, we will modify this procedure. In addition, detecting correlations in branch locations would indicate the existence of developmental mechanisms that need to be investigated experimentally.

Plasticity Potential due to Rearrangement of Synapses on GABAergic Interneuron

D.B. Chklovskii, A. Stepanyants [In collaboration with Z.J. Huang, Cold Spring Harbor Laboratory]

Changing synaptic strength of existing synapses (through LTP or LTD) is a commonly studied plasticity mechanism, but it is not the only one. Another plasticity mechanism is formation and elimination of synapses. Knowing whether this plasticity mechanism can reorganize neuronal circuits by connecting previously unconnected neurons (in addition to changing connection strength) is important for the study of learning and memory. We will evaluate the plasticity potential of such a mechanism for GABAergic interneurons by analyzing the geometry of cortical circuits. In addition to evaluating plasticity potential, this analysis will aid in inferring actual connectivity from potential connectivity.

Optimal Stimulus Design

D.B. Chklovskii

Neurons in the brain respond to sensory stimuli by firing action potentials. The firing rate of a particular neuron depends on which stimulus is presented to the animal. Stimulus to which a neuron responds with the highest firing rate is called the optimal stimulus. Therefore, a neuron can be thought of as a detector for an optimal stimulus. Although we know what the optimal stimulus is for some neurons, it remains unknown for the majority of neurons. Our goal is to design a computer algorithm that would perform a search for an optimal stimulus automatically. This is a very difficult task because the dimensionality of the stimulus space is extremely high. Currently, we are designing an appropriate algorithm and testing it against a computer model of a neuron with a known optimal stimulus.

Statistical Analysis of Spine Growth and Retraction

D.B. Chklovskii, S. Song [In collaboration with K. Svoboda, Cold Spring Harbor Laboratory]

Previously, we have shown that the reorganization of the pyramidal neuron circuit through growth and retraction of spines is a potential plasticity mechanism with high information storage capacity. Subsequently, 2-photon in vivo imaging experiments have confirmed the existence of this plasticity mechanism in adult animals. Next, we need to determine what rules govern spine growth and retraction. We will start addressing this question by analyzing the statistics of spine growth and retraction from chronic in vivo 2-photon imaging in transgenic mice.

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BRAIN DEVELOPMENT AND PLASTICITY

H.T. Cline M. Agathocleous, C. Aizenman, C. Akerman, J. Bestman, K. Bronson, I. Cantallops, A. Carlberg, R. Ewald, K. Haas, A. Javaherian, K. Jensen, W.C. Sin, V. Thirumalai, E. Rial Verde, E. Ruthazer

Activity that arises from sensory input is known to have a prominent role in organizing brain connectivity and circuit function during development. We have only a limited understanding of the cellular and molecular mechanisms that are called into play by sensory activity, or how they regulate brain development and plasticity. The goal of the work in our laboratory is to determine the effect of activity on brain development and plasticity. We address this issue by examining the structural and functional development of the visual system in amphibian tadpoles. We collect time-lapse imaging of neurons in vivo to assess structural plasticity. In addition, we assess neuronal function using electrophysiological assays of synaptic connectivity and synaptic plasticity. We combine these studies with gene transfer methods, which allow us to test the function of genes of interest in brain development.

Visual Stimulation Regulates Dendritic Arbor Development and Visual System Responses

C. Aizenman, C. Akerman, W.C. Sin, K. Haas, E. Ruthazer, H.T. Cline

One of the highlights of our research this year was the direct observation that visual stimulation increases the structural development of neurons and strengthens the synaptic connections in the visual system in such a way as to increase the signal to noise of visual inputs. Although the role of sensory experience in the development of neuronal circuits has been firmly established, most conclusions have been based on long-term deprivation experiments. Our recent work uses a novel approach in which freely swimming tadpoles are presented with a simulated motion stimulus for 1–8 hours. This allows us to draw conclusions about the positive effects of visual experience over a relatively short timescale.

W.C. Sin and K. Haas demonstrated by in vivo time-lapse imaging of optic tectal neurons that enhanced visual activity driven by a light stimulus promotes optic tectal cell dendritic arbor growth. The stimulus-induced dendritic arbor growth requires glutamatergic synaptic transmission. Increasing synaptic activity with optic nerve stimulation down-regulates endogenous RhoA activity and increases endogenous Rac activity (Li et al. 2002). These same changes in Rho GTPases are required for light-induced elaboration of dendritic arbors. The results delineate a role for Rho GTPases in the structural plasticity driven by visual stimulus in vivo.

One central question is how neurons maintain a constant level of activity when faced with an enhanced sensory environment, and thus prevent the system from overloading. C. Aizenman explored the role of the polyamine-mediated modulation of AMPA (α-2-hydroxy-5-methyl-4-isoazole) receptor-mediated responses (Aizenman et al. 2002). Intracellular polyamine levels are increased by increased levels of visual activity. The intracellular polyamines decrease conductance through calcium-permeable AMPA receptors. This may function to compensate for increased levels of activity experienced during visual stimulation and thus maintain a constant level of excitation. More recently, we found that visual system activity changes two other properties of neurons in the optic tectum: One is an increased responsiveness to bursts of synaptic input and the second is an increase in the intrinsic excitability of neurons. Together, these results indicate a multifaceted and coordinated homeostatic response of the brain to periods of increased sensory input, which ensures optimal operation of the brain under a wide range of experiential conditions.

The Function of Activity-regulated Genes in Brain Development and Plasticity

I. Cantallops, A. Javaherian, K. Jensen, E. Rial Verde, K. Bronson, H.T. Cline [in collaboration with R. Malinow, Cold Spring Harbor Laboratory, E. Nedivi, Massachusetts Institute of Technology, and P. Worley, Johns Hopkins]

Neural activity changes the expression of several genes, including *Arc*, *CPG15*, and *Homer*. We study

the function of these activity-regulated genes in the *Xenopus* central nervous system and spinal cord and in hippocampal slice cultures.

arc (*a*ctivity-*r*egulated *c*ytoskeleton-associated protein) is among a small set of activity-responsive genes whose mRNA is targeted to active dendrites. Despite the potential for *arc* to act as a local regulator of synaptic function in response to activity, there is little information about its function. E. Rial Verde finds that expression of green fluorescent protein (GFP)-tagged ARC in CA1 hippocampal pyramidal cells resulted in a significant depression of AMPA-receptor-mediated synaptic transmission. This effect of ARC relies on activation of protein phosphatases to induce depression. We postulate that upon activity-dependent mRNA localization to dendritic sites, ARC protein is translated and exerts its depressing action. This mechanism can operate to maintain the synapses in an operational range as a way to maximize the signal-to-noise ratio among potentiated and nonpotentiated neighboring synapses.

CPG15 (also known as Neuritin) is an activity-induced GPI-anchored axonal protein. Previous work in our lab has shown that CPG15 promotes dendritic and axonal growth and accelerates synaptic maturation in vivo. More recently, I. Cantallops has shown that intracellular CPG15 is localized to synaptic vesicles and endosomes, is trafficked to the axonal surface by a depolarization- and calcium-dependent mechanism, and is endocytosed in an activity-regulated manner. To assess CPG15 trafficking in vivo, she expressed an ecliptic pHluorin (EP)-CPG15 fusion protein in retinal ganglion cells of intact *Xenopus* tadpoles. Intraocular kainic acid (KA) injection rapidly increased cell surface EP-CPG15 in retinotectal axons, but coinjection of TTX and KA did not. The results indicate that the delivery of the neurotrophic protein CPG15 to the axon surface can be regulated on a rapid time scale by activity-dependent mechanisms in vivo.

A. Javaherian has found that CPG15 is heavily expressed in the developing spinal cord of *Xenopus* tadpoles. He has examined the function of CPG15 in the morphological development of motor neuron axons by collecting time-lapse in vivo images of axons from neurons expressing GFP or GFP plus CPG15. These studies are the first to characterize *Xenopus* motor neuron axon development in vivo over a period of several days. CPG15 expression causes a significant increase in elaboration of motor neuron axon arbors and corresponding postsynaptic specializations.

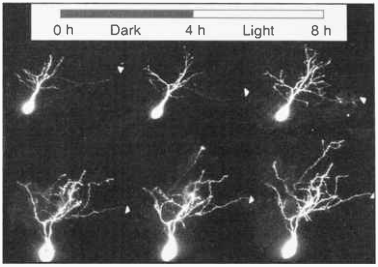

FIGURE 1 Time-lapse images of GFP-expressing neurons were collected on a 2-photon microscope three times over an 8-hour period in anesthetized *Xenopus* tadpoles. Animals were brought out of anesthesia after imaging sessions and either placed in a dark chamber, for the first 4-hour period, or placed in a chamber with a simulated motion stimulus, for the second 4-hour period. The dendritic arbor of the neurons grows significantly more during the 4-hour period with the visual stimulation than during the 4-hour period without visual stimulation.

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SIGNAL TRANSDUCTION AND DIFFERENTIATION

G. Enikolopov J. Hemish, P. Krasnov, B. Kuzin, M. Maletic-Savatic, T. Michurina, J. Mignone, B. Mish, N. Nakaya, M. Packer, N. Peunova, V. Scheinker, Y. Stasiv, A. Vaahtokari, X. Wang, D. Herman

Our laboratory is interested in stem cells in the adult organism and the signaling pathways that control their division and maturation. Among many signals that guide the cells along pathways of differentiation, we focus on the signals initiated by a versatile messenger molecule, nitric oxide (NO). We found that NO regulates the transitions of adult stem cells through the differentiation cascades in several settings, including hematopoiesis and neurogenesis. This year, we have been focusing on the genetic and molecular interactions of NO with major signaling pathways, especially those that control cell division. In our most significant advances, we have demonstrated an essential role for NO in regulating the generation of new neurons in the adult brain using two independent lines of inquiry. We have also demonstrated a role of NO in regulating hematopoiesis. Furthermore, we have identified a large number of genes that are activated by NO, and we are studying how some of them may link NO signals with cell division. We also used *Drosophila* as a model to reveal the link between NO and genes that control cell cycle progression. Finally, we have found a novel form of a NO synthase gene that acts as an endogenous negative regulator of NO production.

Several other projects in our lab make use of transgenic mouse models in which selected classes of cells in the adult brain are marked. By investigating these animals, we have been able to isolate stem cells from the adult brain as well as other tissues and have found that they are able to adopt an unexpectedly wide range of cell fates.

Negative Regulation of Adult Neurogenesis

M. Packer, Y. Stasiv [in collaboration with S. Goldman, Cornell Medical School, and H. Westphal, National Institutes of Health]

Negative regulators of neurogenesis have been postulated on the basis of in vivo and in vitro data. Indeed, progenitor cells are widespread throughout the adult central nervous system but only give rise to neurons in specific loci. However, the mechanisms for suppression of adult neurogenesis are unclear. Our experiments demonstrate that NO acts as an important negative regulator of cell proliferation in the adult mammalian brain. We used two independent approaches to examine the function of NO in adult neurogenesis. In a pharmacological approach, we suppressed NO production in the rat brain in vivo by intraventricular infusion of a nitric oxide synthase (NOS) inhibitor. In a genetic approach, we generated a null mutant neuronal NOS (nNOS) mouse line by targeting the active center of the enzyme. In both models, the number of new cells generated in neurogenic areas of the adult brain, the olfactory subependyma and the dentate gyrus, was strongly augmented. This indicates that division of neural stem cells in the adult brain is controlled by NO and suggests a strategy for enhancing neurogenesis in the adult central nervous system.

Neural Stem Cells in the Adult Nervous System

J. Mignone, A. Vaahtokari, M. Maletic-Savatic, B. Mish

To follow the transitions of neural stem and progenitor cells in the developing and adult nervous system along the differentiation cascade, we generated a series of transgenic reporter mouse lines. In these animals, different classes of neural cells are labeled with different fluorescent proteins. We used those animals that express fluorescent proteins under the control of the regulatory elements of the *nestin* gene to visualize and isolate neural stem cells. We characterized the expression of a panel of differentiation markers in nestin-GFP (green fluorescent protein) cells and found that they can be divided into groups, based on the intensity of their fluorescence. These groups represent distinct classes of neuronal precursors in the adult mammalian brain and may reflect different stages of neu-

ronal differentiation. Furthermore, by isolating nestin-GFP cells using a fluorescence-activated cell sorter (FACS) and testing their ability to form multipotential neurospheres, we found that these cells encompass the majority of neural stem cells in the adult brain. We are now studying the ability of nestin-expressing cells from nonneural tissues to reveal their neural potential in in vitro and in vivo settings.

NO and Hematopoiesis

T. Michurina, P. Krasnov

We examined whether NO may be involved in the control of hematopoiesis. We found that in a model of bone marrow transplantation, inhibition of NO synthase activity increases the number of early hematopoietic progenitor cells, as revealed by several in vitro and in vivo assays. We are now testing human hematopoietic progenitor cells to determine whether they can be similarly expanded through the use of NOS inhibitors. Furthermore, we are studying the isoforms of NO synthases that may be responsible for the regulatory action of NO in the bone marrow.

NO and *Xenopus* Development

N. Peunova, V. Scheinker

We have recently cloned the neuronal NOS gene XNOS from the *Xenopus* genome. XNOS transcripts are present in unfertilized oocytes and thus are stored as maternal RNAs. The complex changes in the patterns of XNOS expression following fertilization suggest its involvement in the early development and organogenesis of the tadpole. We found that the appearance of some of the transcripts marks specific stages of the embryo development; for instance, transcription of some isoforms marks the zygotic induction of the genome, and the appearance of others specifically mark gastrulation, neurulation, and organogenesis. We used the information about the structure of XNOS to generate recombinant constructs that code for potent dominant-negative inhibitors of NOS action. These recombinant inhibitors suppress XNOS activity, probably by preventing the formation of the enzymatically active homodimers of XNOS. We used these reagents as well as recombinant XNOS, in parallel with chemical NOS inhibitors and NO donors, to investigate the early development of the nervous system in *Xenopus*. We found that NO suppresses neural induction so that deficit of NO results in an enlarged nervous system of the tadpole. Furthermore, deficit of NO leads to profound defects in gastrulation movement and forebrain patterning. We are now investigating the components of the major development pathways affected by the NO signals.

NO and *Drosophila* Development

Y. Stasiv, B. Kuzin [in collaboration with M. Regulski and T. Tully, Cold Spring Harbor Laboratory]

There is only one NOS gene in the *Drosophila* genome, but it generates a large family of mRNAs and potential protein products through the use of alternative promoters and splice sites. We focused on one of the short isoforms, DNOS4, to show that it is expressed in vivo and that it acts as a negative regulator of NOS activity in the developing *Drosophila* by forming inactive heteromers with the active enzyme DNOS1 in vitro and in vivo. The ectopic expression of DNOS4 resulted in increased cell proliferation in the imaginal discs and an increased cell number in the pupae and adults, suggesting that DNOS4 suppresses the antiproliferative activity of DNOS1 during fly development. This inhibitory role of DNOS4 was supported by the analysis of genetic interactions of DNOS4 with various components of the *Drosophila* cell cycle machinery, such as RBF and dE2F. We are now trying to define the stages in *Drosophila* development when selective activation and suppression of DNOS activity regulates the progression through the cell cycle.

Mechanisms of NO Action

N. Nakaya, J. Hemish [in collaboration with V. Mittal, Cold Spring Harbor Laboratory]

To understand the mechanisms of long-term action of NO, one approach is to identify the genes that are controlled by NO. We employed transcriptional profiling

using microarrays to identify some of the direct and indirect targets of NO-related signaling cascades. We determined the temporal expression profiles of NO action in cells and verified the hits using cytokine-induced NO production. We next used specific chemical inhibitors and cells from mutant mice to probe the signaling pathways that are used by NO to induce gene activity. Our results demonstrate that NO uses multiple signaling pathways to regulate gene expression.

Among the identified NO-inducible genes, we focused on a novel gene, which we called *noxin*. Expression of *noxin* is strongly up-regulated by NO and is related to cell cycle progression. We are now investigating whether *noxin* serves as link between NO signaling and the cell cycle control.

As another approach to understanding the signaling events that mediate the action of NO in the cell, we used bivariate fluorescent-activated sorting of cells from various mutant mice to determine the stages in the cell cycle that are affected by NO. Our results suggest that NO affects several independent checkpoints within the cell cycle machinery.

Vladimir Scheinker

Tatyana Michurina

CONSTRUCTION AND PLASTICITY OF GABAERGIC CIRCUITS

Z.J. Huang F. Ango, B. Chattopadhyaya, C. Chiariello, G. diCristo, S. Kulhman, Q. Wang, T. Pal, C. Wu

The appropriate wiring of neuronal circuits in the mammalian neocortex forms a basis to generate and process patterns of neuronal activity which underlie perception and cognition. GABAergic inhibitory circuits consist of an array of interneuron cell types with distinct morphology, physiological properties, and synaptic connectivity patterns and may constitute a scaffold to organize local cortical connectivity and activity. A defining feature of GABAergic innervation is their specific targeting to restricted subcellular compartments (i.e., soma, dendrite, axon initial segment) of pyramidal neurons, which allow precise spatial and temporal control of neuronal signaling, dynamics, and plasticity.

Although GABAergic connectivity is essential for the construction of functional cortical circuits, the formation and maturation of GABAergic synapses in general are poorly characterized. The mechanisms underlying the subcellular domain-specific targeting of distinct classes of GABAergic synapses are almost entirely unknown. To a large extent, this is due to the extraordinary complexity of neocortical tissue and the heterogeneity of GABAergic neurons. In the past year, we have made great strides in establishing both in vivo and in vitro experimental systems which allow visualization and genetic manipulation of identified GABAergic cell types and their postsynaptic pyramidal neurons with synaptic resolution. Using cell-type-specific promoters, bacterial artificial chromosome (BAC) engineering, and 2-photon imaging, we have shown that the development of GABAergic synaptic connectivity proceeds in a cortical slice culture system, in the absence of experience- and afferent-driven activity. The recapitulation of GABAergic circuits in vitro opens unprecedented opportunity to characterize its developmental process at synaptic resolution, in real time, and to dissect the underlying molecular mechanisms.

Development of Somatic Innervation by Basket Interneurons in Visual Cortex

G. diCristo, Z.J. Huang [in collaboration with G. Knott, University of Laussane, Switzerland]

We have generated BAC transgenic mice in which basket interneurons in neocortex express green fluorescent protein (GFP) from the *GAD67* gene promoter. In these *GAD67*-GFP mice, GFP expression labels soma, dendrite, axon, and synaptic boutons of basket interneurons in visual cortex. In mature visual cortex, these basket interneurons form perisomatic bouton rings around pyramidal cell soma. The identification of green boutons as GABAergic synaptic terminals was confirmed by colocalization with the GABAergic presynaptic enzyme GAD65 and by immunoelectron microscopy. All GFP-positive boutons were presynaptic components of symmetric synapses located on soma or proximal dendrites. At 2 weeks of age (P14), rather few somatic boutons were observed. The maturation of perisomatic bouton rings in visual cortex is a prolonged process, which continues well into the fourth postnatal week. Our results provide the first description of the development of somatic synapses in visual cortex. These mice allow reliable characterization of the development of somatic synapses and examination of whether visual experience influences the development of somatic innervations at ultrastructural resolution.

Experience and Afferent-independent Development of Subcellular GABAergic Synapse Targeting in Neocortex

B. Chattopadhyaya, G. diCristo, Z.J. Huang

We have established an in vitro brain slice culture system that recapitulates the development of GABAergic circuits. This was made possible by the cloning, through BAC engineering, of a segment of the *GAD67* gene promoter that conferred basket-cell-specific gene expression in vitro. We have also identified a transcriptional promoter that conferred bitufted cell-specific gene expression in slice culture. Using these reagents, we have demonstrated that the development of dendrite and axonal arbors of basket, chandelier, and bitufted interneurons, and the subcellular-domain-specific targeting of GABAergic synapses, proceeded in cortical slice cultures in vitro, in the absence of experience and afferent activity. These results suggest that subcellular-domain-specific targeting of GABAergic synapses is

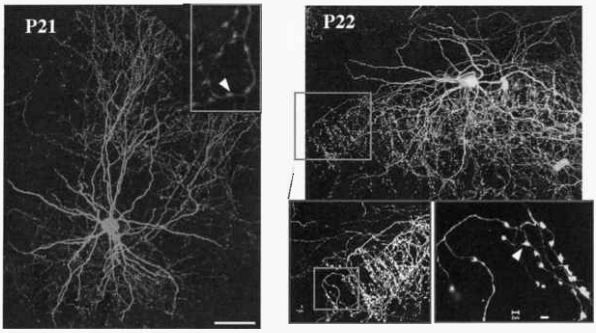

FIGURE 1 A region of the GAD67 promoter, subcloned through BAC engineering, confers basket and chandelier-cell-specific gene expression in slice culture. Pgad67 directs red fluorescent protein (RFP) (*left*) and GFP (*right*) expression only in parvalbumin-positive GABAergic neurons but not in somatostatin, calretinin, VIP GABAergic neurons, and pyramidal neurons (data not shown). Basket and chandelier cells develop their elaborate axon arbors and highly distinct synapses (*insets*) in slice culture, in the absence of experience and afferent activity. Bars: Basket cell, 50 μm; (*lower right*) chandelier cell, 2 μm. P21 = postnatal day 5 + 16 days in vitro.

likely driven by interactions between genetically determined cell surface labels displayed on the axons of distinct GABAergic cell types and on appropriate subcellular compartments of pyramidal neurons. We have initiated experiments to identify and test putative subcellular-domain-specific molecular labels.

Activity-dependent GABAergic Synapse Formation and Maintenance in Neocortex

G. diCristo, B. Chattopadhyaya, Z.J. Huang

We have shown that the time course of the development of somatic innervation in slice culture was remarkably similar, if not identical, to that observed in visual cortex in vivo. Using 2-photon microscopy, we have succeeded in chronically imaging the dynamics and developmental process of somatic innervation in slice culture for up to 4 days. We have also demonstrated that the formation and maintenance of GABAergic synapses, although they do not require experience and afferent activity, are critically dependent on spontaneous activity within the slice culture. Because we can selectively label and manipulate GABAergic interneurons and their postsynaptic pyramidal neurons, we are in a position to pinpoint the precise nature of neuronal activity required for GABAergic synapse formation and maintenance. For example, we are testing (1) whether neuronal firing in presynaptic GABAergic or postsynaptic pyramidal neurons are required; (2) whether GABAergic transmission is required; and (3) whether bone-derived neurotrophic factor (BDNF)/*trkB* signaling is required.

Role of Basket Interneurons in Critical Period Plasticity in Visual Cortex

B. Chattopadhyaya, Z.J. Huang [in collaboration with L. Maffei, Institute of Neurophysiology, Italy]

GABAergic inhibitory circuits have been implicated in the regulation of experience-dependent development of visual cortex. For example, ocular dominance (OD) plasticity, the capacity of visual cortical neurons

to modify their eye preferences following visual deprivation to either eye, was abolished in mice deficient in an isoform of the GABA synthetic enzyme (GAD65). Because GAD65$^{-/-}$ mice show no critical period, yet are normal in other aspects of visual physiology and function, this mutant strain provides an ideal genetic background to systematically study the role of specific classes of interneurons in OD plasticity using a transgenic "rescue" approach. We have generated BAC transgenic mice expressing GAD65 under the control of the parvalbumin promoter. We are introducing the BAC transgene expressing GAD65 in basket interneurons into the GAD65$^{-/-}$ mice by breeding. We will then test whether expression of GAD65 in a specific class of interneurons can restore critical period plasticity in GAD65$^{-/-}$ mice. In addition, this strategy will allow us to correlate the specific types of inhibitory mechanisms that are restored by cell-type-restricted expression of GAD65 with the rescue of critical period plasticity.

Cellular Mechanisms of Critical Period Plasticity in Visual Cortex

S. Kuhlman, Z.J. Huang

The cellular mechanisms by which GABAergic circuits regulate ocular dominance plasticity and the critical period are unknown. We hypothesize that during critical period plasticity, maturation of synaptic inhibition allows cortical circuits to detect the difference between the temporal patterns of open- and closed-eye inputs, thus engaging spike-timing-dependent plasticity rules to drive synaptic competition and OD plasticity. This hypothesis makes several predictions. First, maturation of GABAergic transmission should correlate with the time course of the onset of critical period. Second, before the onset of the critical period, the cortical circuit is incapable of distinguishing closed-eye input from that of open-eye, due to the immature state of GABA transmission, and therefore cannot subject closed-eye input to synaptic depression. Third, at the peak of the critical period, maturation of synaptic inhibition allows cortical circuits to distinguish closed-eye inputs from open-eye inputs, and readily subject closed-eye inputs to synaptic depression through spike-timing-dependent mechanisms. We are testing these predictions using in vitro brain slice preparations.

Our parvalbumin-GFP BAC transgenic mice allow reliable and efficient identification of basket interneurons in acute brain slice. We have shown that in response to somatic current injection over a range of intensities, there is a dramatic developmental increase in the firing rate of FS interneurons which corresponds to the onset of OD plasticity. There was a twofold increase in maximal firing rate between 2 weeks (149 ± 31 Hz) and 3 weeks (302 ± 25 Hz, $p > 0.05$), and a further 1.7-fold increase between 3 weeks and 5 weeks (507 ± 37 Hz, $p > 0.05$). The first interspike interval of a train of spikes induced by current injection decreased with age (ANOVA on ranks, $p > 0.01$) by an average of 30% between 2 and 3 weeks of age across a range of current intensities, and decreased further by 25% between week 3 and 5. There was a concomitant decrease in action potential spike width and decay rate. Both the spike width at threshold and falling phase decreased by 75% between 2 and 5 weeks of age. These changes are likely due to the compliment of ionic conductances that these neurons express, and promote rapid, high-fidelity spike transmission important for feedforward inhibition. Current studies are examining the functional consequence of increased inhibition on the input integration window of pyramids.

We are also developing a "two-pathway synaptic competition" preparation in a visual cortical brain slice, which captures properties of OD plasticity in vivo. Our goal is to demonstrate that GABAergic inhibition promotes synaptic competition during experience-dependent synaptic refinement via precise and rapid regulation of a fundamental parameter of neuronal excitability, the membrane time constant. We will then test whether the regulation of input discrimination by a specific cell type, fast-spiking basket interneuron, is a prerequisite for induction and expression of OD plasticity.

Molecular Mechanisms of Subcellular Domain-specific Synapse Targeting in Cerebellum

F. Ango, Z.J. Huang

Another good example of subcellular domain-specific synaptic innervation comes from the cerebellum.

Cerebellar basket and stellate interneurons selectively innervate Purkinje cell soma/axon initial segments and dendrites, respectively. The cellular and molecular mechanisms underlying such subcellular synaptic targeting are unknown. In our *GAD67*-GFP and parvalbumin-GFP BAC transgenic mice, cerebellar interneurons can be visualized at unprecedented resolution. These mice have allowed us to systematically describe, for the first time, the process of the innervation of the Purkinje cell axon initial segment by cerebellar basket interneurons. We found that basket interneuron axons first contacted Purkinje cell soma about postnatal day 12. These contacts were then guided toward axon initial segments and concentrated there at P18. Because of their striking subcellular localization, we hypothesized that an ankyrin-based localization of L1 immunoglobulin-type cell adhesion molecules at the axon initial segment of Purkinje neurons may be necessary for its somatic and axon initial segment (AIS) innervation by cerebellar basket interneurons.

We tested this hypothesis using ankyrin G knockout mice in which AIS localization of L1CAMs was abolished. We found that basket cell innervation at AIS of Purkinje cells in *ankG*$^{-/-}$ mice was either greatly reduced or extended far beyond their usual boarder. This result provides the first evident that L1 immunoglobulin-type cell adhesion molecules may serve as subcellular-domain-specific molecular labels that guide GABAergic synapse targeting. We are in the process of testing the role of specific L1CAMs in subcellular-domain-specific synapse targeting in the cerebellum.

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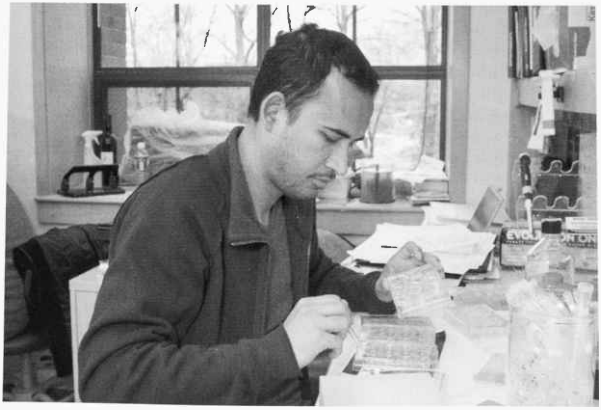

Jean-Raymond Ango

THE NEURAL ORGANIZATION OF OLFACTORY BEHAVIOR

Z.F. Mainen N. Uchida A. Kepecs C. Feierstein
V. Egger S. Ranade E. Friedman
M. Quirk H. Zariwala

Our laboratory is interested in how brains make decisions. For several years, we have been developing new paradigms for tackling this problem using rodent olfactory-guided behavior as a model system. In 2002, we set up technology to make multiple single-neuron recordings from behaving animals. This brings a new and powerful physiological approach to CSHL that, in conjunction with tightly controlled psychophysical paradigms pioneered in our lab, yields a powerful model system for understanding the biological substrates of cognitive function. Our approach encompasses multiple levels of analysis ranging from single spines to behavior, and the past year has produced breakthroughs on both ends of this spectrum. In the olfactory bulb slice, we have completed a study of the signaling properties of the granule cell, a type of neuron responsible for network computations in the olfactory bulb. Using 2-photon calcium imaging, we discovered that these cells regulate their output using the same voltage-sensitive T-type calcium channel that underlies the gating of sensory information by the thalamus. This is the first evidence that T channels underlie action-potential-mediated synaptic release and suggests novel regulatory mechanisms for computations in the olfactory system. At the behavioral end of the spectrum, in the last year, we completed a detailed study of an olfactory discrimination in the rat. The surprising result of this study is that each sniff, an event lasting only 150 msec, is a complete and independent picture of the olfactory world. This result establishes important constraints on the timing of both sensory-coding and decision-making processes and has strong implications for understanding the organization of the neural activity underlying decisions.

Temporal Constraints on Olfactory Coding and Decisions

N. Uchida

Olfaction is sometimes thought of as a slow sense, and thus it has been proposed that processing neuronal activity over time might have a role in odor coding. In insects and zebrafish, population activity in the olfactory bulb evolves such that neuronal firing patterns to similar odorants are decorrelated in the course of 1–2 sec, facilitating discrimination of similar odors. This year, we tested this idea using an odor mixture discrimination task in rats (Fig. 1). Rats discriminated odors very rapidly, with a median odor sampling duration of <300 msec. Moreover, accuracy of performance saturated with just 200 msec of odor sampling, regardless of the difficulty of the discrimination. More surprisingly, measurement of the rats' sniffing revealed that optimal performance was achieved with only one sniff at theta frequency (~7 Hz). The fact that odor information fails to be integrated over cycles of sniffing rules out, as a potential olfactory code, slow temporal modulation occurring over periods longer than about 100–200 msec and suggests that the sniffing (theta) cycle is a fundamental feature component of the neural mechanisms of olfactory perceptual decisions in the rat.

Concentration Invariant Odor Quality Discrimination

N. Uchida, S. Ranade, E. Friedman

Any odor activates a specific combination of odorant receptors, and it has been postulated that combinations of activated receptors define odor quality ("combinatorial receptor coding"). However, changes in concentration also lead to changes in activated receptors, yet perceived odor quality usually remains constant over a range of concentrations for a given odorant ("concentration-invariance"). This animal could recognize an odor as belonging to the same source despite variable amounts of dilution that will occur depending on distance.

Using an odor mixture discrimination task (Fig. 1), we tested explicitly the idea that a rat perceives mixture qualities to be invariant to the ratios of their com-

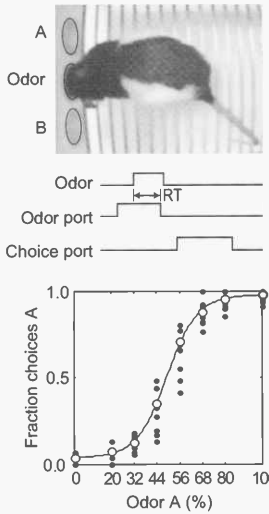

FIGURE 1 Odor mixture discrimination task. (*Top*) Still frame from a video of a rat performing an odor discrimination task. The rat is shown performing a "nose poke" at the odor port. This triggers the delivery of an air stream containing a mixture of two pure odorants (*A* and *B*) in one of eight different ratios (e.g., 0/100%, 80/20%, etc.). Responses at the choice port corresponding to the dominant odorant are rewarded as correct. (*Middle*) Timing of task events. The odor sampling or response time (RT) is the period between odor onset and odor poke withdrawal. Median observed RT was 280 ± 30 msec (n = 10 rats, 52,760 correct and error trials; mean ± sd). (*Bottom*) Performance of one rat in discriminating mixtures of caproic acid and hexanol. The fraction of caproic acid (Odor A) is indicated on the abscissa. (*Closed circles*) Performance from single sessions (~250 trials); (*open circles*) average performance (10 sessions). The data are well-fit using a logistic function.

ponents. After training, rats were tested using probe stimuli with ratios identical to the training set but at different absolute concentrations. Performance was predicted by a ratio-based discrimination strategy but not by alternatives such as an intensity-based discrimination strategy. This result indicates that molar ratios of the components of a mixture are the critical determinants of choice in this task. We propose that odor quality is coded by the ratios of ligand coverage of odorant receptors, "ratio coding." To understand how ratio coding might arise from ensemble activity in the olfactory bulb, we are currently measuring the population response of receptors using both electro-olfactogram (EOG) and intrinsic signal imaging.

Using Categories to Guide Behavior

C. Feierstein, M. Quirk

To better understand the function of olfactory cortical areas in guiding behavioral decisions, we are making multineuron extracellular recordings using tetrodes implanted in the orbitofrontal and piriform cortices of rats performing an odor mixture discrimination task (Fig. 1). Neurons representing purely sensory information should show graded responses varying with the smoothly varying stimulus parameter. On the other hand, neurons representing a motor response should show categorical (binary) responses reflecting the assignment of two stimulus groups. By comparing neuronal responses with behavioral choices on a trial-by-trial basis for correct and error trials, we will be able to assess the relationship between neuronal activity in different cortical areas and the formation of an olfactory-guided decision. We are also interested in tracking the changes in neuronal activity that result from shifting the boundary that defines categories ("recategorization"). Adaptation to changes in task contingencies is thought to depend on orbitofrontal activity, and therefore, relearning of relevant categories should be reflected in the activity of this cortical area and possibly in the piriform cortex as well. With this experimental design, we expect to learn about the roles of both piriform and orbitofrontal cortices in the processing and learning of task-relevant information, as well as to clarify the contribution of these brain areas to decision making.

Serotonin Activity during Goal-directed Behavior

M. Quirk

Although numerous behavioral, pharmacological, and genetic studies have demonstrated that serotonin has a critical role both in the processing of behaviorally rel-

evant stimuli and in the execution of appropriate actions, little is known about the functional role of serotonin in the neural processes underlying goal-directed behaviors. The problem stems in large part from our lack of knowledge about the conditions under which serotonin neurons are activated in a behavioral context. To address this issue, we are recording from serotonin-producing cells located within the dorsal raphe nucleus of freely behaving rats during the performance of an olfactory-based two-choice discrimination task (Fig. 1). By recording from serotonin neurons during this well-controlled task, we will be able to directly relate the moment-by-moment activation of these cells to specific sensory, motor, and motivational task variables. We are particularly interested in characterizing how raphe cells respond to the anticipation of reward, as well as the failure of an animal to receive a reward following an incorrect behavioral response. Furthermore, during the odor-sampling portion of the task, we are interested in determining whether fluctuations in raphe activity correlate significantly with an animal's behavioral choices.

Role of the Theta Cycle in Perceptual Decisions

A. Kepecs

Rhythmic oscillations in the theta frequency band (4–10 Hz) are observed in local field potentials recorded from many different cortical and subcortical brain regions in rodents. The presence and amplitude of theta oscillations are correlated with specific behavioral states, being particularly prominent during active, exploratory behaviors. In addition, the phase of theta oscillations can be reset by behaviorally relevant stimuli. Although theta oscillations may have different functional roles in different brain regions and behavioral contexts, anatomical and physiological evidence suggests that theta oscillations may coordinate information flow between brain regions and thereby instruct or facilitate sensorimotor integration. We will examine the role of theta rhythm in an olfactory discrimination task (Fig. 1). In this task, we have found that a simple perceptual decision (from sensory input through decision and motor execution) requires only a single respiration cycle taking only 200 msec, which corresponds to one cycle of theta. To elucidate the neural mechanisms underlying sensory-motor integration in this task, we will record local field potentials (LFPs) in behaving animals in several sensory and motor regions from olfactory bulb to motor cortex. We will analyze the spectral characteristics of LFPs to determine the correlation of the theta rhythm across brain regions and between sensory and motor behavioral events as a function of task contingencies.

Interaction of Reward and Sensory Information in Decision Making

H. Zariwala, A. Kepecs, N. Uchida

Decisions are determined not only by sensory cues, but also by the consequences of actions taken in the presence of those cues. In the context of simple behavioral choice tasks, learning theory suggests that animals make decisions so as to maximize reward over some time horizon. To understand even a simple perceptual decision, it is therefore crucial to consider how sensory information interacts with the reward structure of the task. With the eventual goal of elucidating the neural basis of this interaction, we have begun to characterize the effects of manipulating the reward contingencies in the odor mixture discrimination task (Fig. 1). Whereas under standard conditions, the rat always receives a water reward for a correct choice, in this series of experiments, we are systematically varying the probability of reward. By changing the ratio of reward probability at the two choice ports, we expect to introduce a bias toward responding to the port with a higher reward, as shown in classical "matching" experiments. By changing reward probability of both choice ports equally, we expect to manipulate the motivation to complete task problems, which may increase error rates. The use of a gradient of mixture stimuli should allow the detection of small effects of these kinds.

Spike-evoked Calcium Dynamics in Olfactory Bulb Granule Cells

V. Egger [in collaboration with K. Svoboda, Cold Spring Harbor Laboratory]

Granule cells are axonless GABAergic interneurons that mediate recurrent coupling between the principal

neurons (mitral cells) in the olfactory bulb. To examine the properties of action potential propagation within the granule cell dendritic tree, we imaged AP-evoked dendritic Ca^{++} transients using 2-photon imaging in rat olfactory bulb slices (Egger et al. 2002). We determined that action-potential-mediated calcium influx in the dendritic output region is robust and, hence, will contribute to synaptic release via the dendrodendritic reciprocal mitral-granule cell connection. We call this pathway global lateral inhibition. Interestingly, we found that action-potential-evoked Ca^{++} transients were dramatically modulated by small changes in the resting membrane potential. Blockers of the low-threshold, fast-inactivating T-type Ca^{++} channel reduced the Ca^{++} transient amplitudes and blocked their voltage dependence.

In the past year, we have further corroborated this finding with additional experiments and explored its implications for signaling in the olfactory bulb. Thus, we have demonstrated that the blockade of T-type channels reduces synaptic transmission from granule to mitral cells, which is the first example of a role for T-type channels in neurotransmitter release. We have also observed that voltage dependence evolves with a time constant of about 300 msec, making it likely that the prominent theta-frequency rhythmic activity in the olfactory bulb will interact with global lateral inhibition.

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Eugenia Friedman and Claudia Feierstein

TRANSMISSION AND PLASTICITY IN MAMMALIAN CENTRAL SYNAPSES

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Our laboratory is directed toward an understanding of learning and memory by studying the physiology of synapses. We study synaptic transmission and plasticity in rodent brain slices. This preparation is sufficiently complex to show glimpses of emergent properties as well as simple enough to allow hard-nosed biophysical scrutiny. To monitor and perturb the function of synapses, we use a combination of electrophysiology, microscopic imaging, and molecular transfection techniques, which allows us to examine the cellular and molecular basis for changes in electrophysiological function. This year, we added a new preparation, in which we acutely deliver genes of interest into living animals with viruses and examine the impact of experience on the subcellular distribution of these gene products. With this preparation, we have been able to show that experience strengthens transmission by driving AMPA (α-amino-3-hydroxy-5-methyl-4-isoazole) receptors (AMPA-Rs) into synapses. Such experiments provide support to our philosophy that synapses have key properties whose understanding will provide insight into phenomena at higher levels of complexity. Below are examples of projects recently published, and their subsequent progress.

INCREASING MOLECULAR REQUIREMENTS FOR SYNAPTIC PLASTICITY WITH AGE

Synaptic plasticity has an important but distinct role throughout life. Early in development, synaptic plasticity controls establishment of neural connections. Later in life, plasticity controls learning and memory. Here, we compare molecular mechanisms controlling synaptic plasticity at different ages. We find that PKA phosphorylation of AMPA-Rs has a key role throughout. Activity-driven PKA phosphorylation of GluR4 is necessary and sufficient for delivery of receptors to synapses during early development, making synapses functional. Phosphorylation relieves a retention interaction that in the absence of synaptic activity, maintains GluR4-containing receptors away from synapses. In contrast, phosphorylation of GluR1 by PKA is necessary but not sufficient for delivery of receptors to synapses in older animals; CaMKII (calcium/calmodulin-dependent protein kinase II) activity is also required. Thus, a mechanism that mediates plasticity early in development becomes a gate for plasticity later in life. Increasing requirements may be one way that plasticity becomes more specific and also recalcitrant with age.

We are currently identifying additional phosphorylation sites on AMPA-Rs that control plasticity. Our current view is that multiple phosphorylation steps are involved in various aspects of receptor trafficking, such as relief from extrasynaptic retention, integration into the dendritic surface, access to the perisynaptic region, integration into synapse, anchoring to the synapse, and removal from the synapse. For instance, by mimicking phosphorylation at four sites on the AMPA-R cytoplasmic tail, we can drive receptors into synapses. We are also taking a proteomic approach to determine what protein-protein interactions are enhanced/removed by phosphorylation events.

MONITORING AMPA-R TRAFFICKING DURING PLASTICITY WITH FLUORESCENCE AND ELECTRON MICROSCOPY

Interactions between AMPA-R subunits and PDZ domains are critical for the proper delivery of receptors to synapses. Synaptic delivery of GluR1-containing AMPA-Rs can be driven by CaMKII activity or long-term potentiation (LTP) and requires an interaction between GluR1 and a type-I PDZ domain. Synaptic delivery of AMPA-Rs with only GluR2

occurs continuously, and this requires an interaction between GluR2 and a type-II PDZ domain. We now show that mutations on GluR1 or GluR2 that perturb these critical PDZ domain interactions lead to the accumulation of these receptors at different subcellular sites. GluR1 mutants accumulate in the dendrite, whereas GluR2 mutants accumulate in dendritic spines. This suggests that the critical PDZ-domain interactions are required for entry into spines, for GluR1, and into synapses, for GluR2.

We are currently using dual emission two-photon scanning laser microscopy to monitor AMPA-R redistribution as well as structural changes simultaneously during plasticity-inducing stimuli (LTP and LTD). We find that during LTP-inducing stimuli, GluR1 AMPA-Rs are primarily driven to existing synapses and enhance their size; newly formed synapses have few (if any) synaptic AMPA-Rs. Long-term depression (LTD)-inducing stimuli remove GluR2-containing receptors, leading to a decrease in size; some synapses are lost completely. Interestingly, LTP-inducing stimuli also lead to removal of some GluR2-containing receptors, but not GluR1-containing receptors. This indicates that LTP-inducing stimuli likely induce LTD at some synapses.

We are also developing tagging methods that will allow us to detect the distribution of AMPA-Rs with electron microscopic resolution. Such techniques will allow us to determine where receptors containing specific mutations stop trafficking and thereby associate phosphorylation as well as protein-protein interactions with particular trafficking functions.

RAS AND RAP CONTROL AMPA-R TRAFFICKING DURING SYNAPTIC PLASTICITY

Recent studies have shown that AMPA-R trafficking has an important role in synaptic plasticity. However, the intracellular signaling pathways controlling this trafficking remain largely unknown. Small GTPases can signal diverse neuronal processes, and their perturbation is responsible for several mental disorders. Here, we have examined the role of small GTPases Ras and Rap in the postsynaptic signaling that mediates synaptic plasticity. We show that Ras is the downstream effector for NMDA (*N*-methyl-D-aspartate) receptor (NMDA-R) and CaMKII signaling during LTP. Ras activity drives synaptic delivery of AMPA-Rs. In contrast, postsynaptic activation of Rap mediates NMDA-R-dependent removal of synaptic AMPA-Rs that occurs during LTP. Ras and Rap exert their effects on AMPA-Rs that contain different subunit compositions. Thus, Ras and Rap, whose activity can be controlled by postsynaptic enzymes, serve as independent regulators for potentiating and depressing central synapses. Our studies may have general implications as Ras and Rap have opposing functions in synaptic plasticity as well as oncogenesis and T-cell activation.

We are currently identifying the connection between Ras activity and phosphorylation events on AMPA-Rs.

CONTROL OF NMDA-R TRAFFICKING TO SYNAPSES BY THE NR2 SUBUNIT

To elucidate the mechanisms controlling the number and subunit composition of synaptic NMDA-Rs in hippocampal slice neurons, the NR1, NR2A, and NR2B subunits were optically and electrophysiologically tagged. The NR2 subunit directs delivery of receptors to synapses with different rules controlling NR2A and NR2B. NR2B-containing receptors incorporate into synapses in a manner that is not limited by synaptic transmission nor enhanced by increased subunit expression. NR2A-containing receptors, whose expression normally increases with age, replace synaptic NR2B-containing receptors. Replacement is enhanced by increased NR2A expression, requires synaptic activity, and leads to reduced NMDA-R responses. Surprisingly, a spontaneously released transmitter acting on synaptic NMDA-Rs is sufficient for replacement. Thus, as with AMPA-Rs, synaptic trafficking of NMDA-Rs is tightly regulated and has unique rules.

We are currently testing the impact of the NR2B to NR2A switch on synaptic plasticity. Our preliminary results indicate that driving this switch reduces the generation of LTP. We will test what differences between NR2B and NR2A are responsible for this change in plasticity.

HOMEOSTATIC FEEDBACK BETWEEN NEURONAL ACTIVITY AND APP PROCESSING

A large body of work implicates the accumulation of Aβ peptides and other derivatives of the amyloid precursor protein (APP) as central to the pathogenesis of Alzheimer's disease. However, little is known regarding the relationship between neuronal electrophysio-

logical function and APP processing. Here, we find that neuronal activity controls the formation of Aβ. Furthermore, increased formation of Aβ reversibly depresses synaptic function. These results suggest that APP cleavage products act as negative feedback homeostatic regulators that keep neuronal hyperactivity in check. Pathology that disrupts this homeostatic system could increase the production of Aβ and/or produce neuronal hyperactivity, events that may contribute to Alzheimer's disease.

We are currently determining the impact of longer-term (1–2 weeks, rather than the 1–2 days used to determine the feedback described above) overexpression of Aβ. Our preliminary evidence indicates structural as well as physiological aberrations. Our plan is to determine the mechanisms underlying the short- and long-term effects of increased Aβ production.

EXPERIENCE STRENGTHENS TRANSMISSION BY DRIVING AMPA-R INTO SYNAPSES

To elucidate the mechanisms underlying experience-dependent plasticity in the brain, the trafficking of the AMPA subclass of glutamate receptors into synapses was examined in the developing rat barrel cortex. In vivo gene delivery was combined with in vitro recordings to show that experience drives recombinant GluR1, an AMPA-R subunit, into synapses formed between layer 4 and layer 2/3 neurons. Furthermore, expression of the GluR1 cytoplasmic tail, a construct that inhibits synaptic delivery of endogenous AMPA-Rs during LTP, blocked experience-driven synaptic potentiation. In general, synaptic incorporation of AMPA-Rs in vivo conforms to rules identified in vitro and contributes to plasticity driven by natural stimuli in the mammalian brain. We are currently testing whether a similar process occurs during learning paradigms.

PSD-95 AS A CRITICAL SYNAPTIC SCAFFOLD REQUIRED FOR LTP

The regulated delivery of AMPA-type glutamate receptors (AMPA-Rs) to synapses is one mechanism underlying synaptic plasticity. Here, we investigate how the synaptic scaffolding protein PSD-95 participates in AMPA-R trafficking to synapses during plasticity. Overexpression of PSD-95 specifically increases AMPA-R-mediated transmission by delivering GluR1-containing receptors to synapses, thus mimicking LTP. Consistent with a role in LTP, wild-type PSD-95 occludes LTP, and dominant-negative forms block LTP. The action of PSD-95 on GluR1 is independent of LTP signaling downstream from NMDA-R activation, although it appears to require ligand binding to NMDA-Rs. The assembly of scaffolding complexes containing PSD-95 may be an important step in recruiting AMPA-Rs to synapses during plasticity.

Currently, we are examining the role of PSD-95 during experience-driven plasticity. Our preliminary results indicate that this protein is required for experience to drive GluR1 into synapses.

In conclusion, we are continuing to elucidate the basic mechanisms involved in central synaptic transmission and plasticity and are testing how these mechanisms participate in experience-driven plasticity.

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PLASTICITY OF CORTICAL NEURONS AND THEIR CIRCUITS

K. Svoboda B. Burbach, I. Bureau, B. Chen, A. Karpova, M. Maravall, E. Nimchinsky, T. Oertner, T. Pologruto, V. Scheuss, G. Shepherd, J. Trachtenberg, R. Yasuda, C. Zhang, K. Zito

The cortex underlies most cognitive functions in mammals. Cortical tissue is dauntingly complex: 1 mm^3 contains nearly a million neurons, each of which connects to thousands of other neurons. To begin to unravel neocortical function, we are studying how the basic units of the network, neurons, and their synapses work within the intact network and how they change in response to experience. We build and use sensitive optical tools. 2-photon laser-scanning microscopy (2PLSM) allows us to detect the excitation of single synapses in brain slices with single-receptor sensitivity. Excitation of neuronal elements by focal uncaging of neurotransmitters allows us to efficiently probe the connectivity of neural networks. 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. Similar to other sensory areas in the mammalian cortex, the barrel cortex is arranged in maps. Each whisker is represented by a small cortical region (barrel). Whisker maps are shaped by experience during development and reshaped in the adult. Plasticity in these maps can be induced by trimming specific whiskers for hours to days. The cellular mechanisms underlying sensory map plasticity are likely to share mechanisms with those underlying learning and memory. Because of the precision of the barrel map, it is possible to predict where in the brain experience-dependent changes are likely to occur, simplifying the search for the cellular, synaptic, and molecular basis for plasticity.

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

R. Yasuda, V. Scheuss, E. Nimchinsky

Long-term changes in synaptic efficacy are triggered by increases in [Ca^{++}] in the postsynaptic neuron. We have used whole-cell patch-clamp recordings and 2PLSM to measure [Ca^{++}] signals that are evoked in spines and small dendrites of CA1 pyramidal neurons by back-propagating action potentials and synaptic stimuli. By contrasting the properties of action-potential-evoked [Ca^{++}] transients in the main apical dendrite with those of distal dendrites and spines, we were able to define functionally distinct neuronal compartments for Ca^{++} signaling. We now have a complete understanding of the life cycle of Ca^{++} ions in dendritic spines. We have developed a variety of assays to learn about the molecular pathways of Ca^{++} influx, including the counting of individual Ca^{++} permeable channels and receptors in spines. Recently, we have discovered that Ca^{++} extrusion is inhibited by long-duration Ca^{++} elevations, which could be an important factor underlying the induction of synaptic plasticity.

We have also probed the plasticity of voltage-gated Ca^{++} channels in single spines. The activation voltage of spines can be shifted for long times by neuronal activity in a Ca^{++}-dependent manner. This modulation is produced stochastically and requires the activity of calcium/calmodulin protein kinase (CaMKII). All of the channels in a spine (~10) are modulated together, suggesting that we are witnessing either the output of a single enzyme or the result of an autophosphorylation cascade involving several copies of CaMKII. Finally, we find that calcium entering the same synapse through different types of channels is able to activate different signal transduction pathways. This discovery may help to explain how one second-messenger system, calcium, can have a large variety of functions in neurons.

Optical Studies of Single Synapses

E. Nimchinsky, T. Oertner, R. Yasuda, T. Pologruto

Central nervous system (CNS) synapses release vesicles of glutamate stochastically in an all-or-none fashion. We can detect these successes and failures at single synapses by imaging Ca^{++} in postsynaptic spines. We can also measure the amount of glutamate released during a success as follows: *N*-methyl-D-aspartate recep-

tors (NMDA-Rs) have high affinities for glutamate and are therefore perfect detectors for synaptic glutamate. We have previously shown that NMDA-Rs sense glutamate in a roughly linear manner. Furthermore, NMDA-Rs admit Ca^{++} which can be measured in spines. Thus, the amount of glutamate released can be inferred from the Ca^{++} accumulations mediated by NMDA-Rs.

The ability to image transmission at single synapses allows us to address old questions in synaptic physiology that have important implications for the nature of information transmission in the brain and the mechanisms of plasticity. Our studies show that synaptic glutamate receptors are not saturated by the release of a single vesicle. We counted the number of receptors opened by synaptic transmission and found that this number is surprisingly small, ~1. Thus, only a small fraction of receptors open during low-frequency synaptic transmission, and the dynamic range of synapses is large. Furthermore, stochastic interactions between ligand and receptor and molecular fluctuations of synaptic channels contribute to noise in synaptic transmission. We analyzed transmitter release at single synapses during synaptic transmission showing that the number of vesicles released at single active zones is variable, with a larger number of vesicles released in potentiated synapses. In conclusion, we find that synaptic transmission couples action potentials into graded and noisy postsynaptic responses. The magnitude of postsynaptic responses, not only their probability, depends on the history of the synapse.

Do synapses in fact function as independent information transmission channels? Can mixing of signals occur by diffusion of glutamate released at one synapse to postsynaptic receptors of a neighboring synapse? To investigate cornstalk synapses, we measured Ca^{++} responses in individual spines and monitored signals in their neighbors less than 1 μm away. We find that under a wide range of conditions, spillover of glutamate to neighboring synapses does not occur. However, during intense bursts of stimuli, glutamate escapes the cleft and activates extrasynaptic receptors. Using genetic and molecular tools, we have evidence that astrocytic glutamate transporters are significant players in limiting the spread of glutamate in the extracellular space.

Experience-dependent Plasticity in Adult Cortex In Vivo

A. Holtmaat (sabbatical visitor from the University of Amsterdam), B. Chen, J. Trachtenberg

The rodent somatosensory cortex contains a map of the facial whiskers (barrel cortex) where the neuronal responses in each barrel-column are dominated by a particular principal whisker. Whisker deprivation changes the responses in the whisker map. It has long been debated whether changes in neuronal structure underlie such experience-dependent cortical plasticity. We used time-lapse 2PLSM of layer-5 pyramidal neurons in adult mouse barrel cortex to image the structural dynamics of dendritic spines. We are using transgenic mice that express green fluorescent protein (GFP) and other fluorescent proteins in specific subpopulations of mouse neurons. These animals allow us to look at the structure of neurons and their synapses over chronic time scales. We made the surprising discovery that even in the adult brain, some synapses turn over. Other synapses are stable over months. We also analyzed the ultrastructure of previously imaged synapses at the electron microsocopy level. Induction of experience-dependent plasticity is associated with synaptic growth, suggesting that memories are encoded in new synaptic connections. We are currently investigating the differences between stable and transient synapses.

Cellular Basis of Experience-dependent Plasticity in the Developing Cortex In Vivo

I. Bureau

How do the ordered connections of the brain form? How does sensory experience early in life shape the development of topographic maps in the barrel cortex?

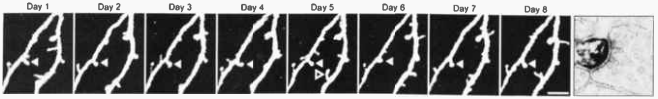

FIGURE 1 Experience-dependent synaptic plasticity in the adult neocortex in vivo. (*Left*) Images collected 24 hours apart. Note stable (*closed arrows*) and transient (*open arrow*) spines. (*Right*) Electron micrograph of new spine indicating that it participates in synaptic transmission.

To address these issues in layer 2/3, we performed whole-cell recordings of sensory-evoked synaptic potentials at the earliest ages possible. Whole-cell recordings allow us to perform voltage-clamp experiments and to figure out the identity of the synaptic receptors underlying the sensory response. To our surprise, we found that at PND 8, highly ordered sensory maps already exist and that these are mostly supported by synapses dominated by NMDA-Rs. Thus, highly ordered sensory maps form much earlier than previously thought.

Cellular Basis of Experience-dependent Plasticity in the Developing Cortex In Vitro

G. Shepherd, M. Maravall [in collaboration with T. Takahashi and R. Malinow, Cold Spring Harbor Laboratory]

Some subtle experience-dependent changes in cellular properties are difficult to study in the intact brain. For example, the sizes of quantal synaptic currents cannot be measured in vivo but they can be measured in brain slices. We are comparing the properties of neocortical pyramidal neurons in brain slices derived from deprived and control animals. To discover possibly diverse cell-autonomous modes of plasticity, we are using a barrage of electrophysiological and imaging approaches to look for effects of sensory experience on neuronal Ca^{++} dynamics, excitability, and synaptic currents. We found that sensory experience remodels the dendritic structure of cortical neurons and shapes the membrane conductances that produce mature firing properties in cortical neurons, during a sharp critical period. We have also found that sensory experience drives glutamate receptors into synapses.

In addition, we have developed tools that allow us to look at circuit-wide changes produced by sensory experience. We adopted and improved laser-scanning uncaging to rapidly and efficiently probe the structure of neural circuits. To determine the locus of plasticity, we used laser-scanning photostimulation, which allows us to rapidly map intracortical synaptic connectivity in brain slices. Layer-2/3 neurons differed in their spatial distributions of presynaptic partners: Neurons directly above barrels received, on average, significantly more layer-4 input than those above the septa separating barrels. Complementary connectivity was found in deprived cortex: Neurons above septa were now strongly coupled to septal regions, whereas connectivity between barrel regions and layer 2/3 was reduced. These results indicate competitive interactions between two thalamocortical circuits, the VPM-barrel and POm-septal pathways, in the establishment of precise intracortical circuits.

Experience-dependent Gene Expression in the Neocortex

K. Zito, B. Burbach [in collaboration with F. Naef and M. Magnasco, The Rockefeller University]

Progress in sequencing the mouse genome and the availability of cDNA and oligonucleotide expression arrays offer an opportunity to discover genes ("plasticity genes," *pgs*) regulated by sensory experience. We trim mouse whiskers to induce receptive field plasticity in particular neocortical barrels. To isolate a population of neurons that has undergone plasticity, we have developed protocols to rapidly isolate and freeze tissue from particular barrels of the living mouse. The procedure is brief (20 minutes per animal), allowing large quantities of tissue (hundreds of milligrams) and mRNA (micrograms) to be harvested, sufficient for expression array studies. mRNA is isolated from deprived and control brains at various times after deprivation and used to make a fluorescent probe. The probe is then hybridized to microarrays. Presently, we are using Affymetrix oligonucleotide arrays. Fluorescence images of the arrays are analyzed for patterns of expression.

Cellular and Molecular Mechanisms of Dendritic Spine Morphogenesis

K. Zito

How are synaptic connections formed between neurons in the mammalian brain? Most of the excitatory synapses in the cortex occur on dendritic spines, small protrusions extending from neuronal dendrites. Spines are highly motile during development, suggesting a role for spine dynamics in synapse formation. We searched for molecules that altered the dynamics of spine morphogenesis by examining the effects of overexpression of wild-type, activated, and dominant-negative versions of candidate genes on spine number, density, morphology, and dynamics. We are studying Neurabin-I (NrbI), a *neur*onal-specific *a*ctin-*bin*ding

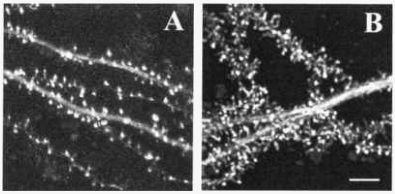

FIGURE 2 Dendrites and spines from neurons transfected with GFP-Neurabin (*A*) or truncated GFP-Nrbl (version1) (*B*).

protein. Nrbl is a 122-kD protein that contains an amino-terminal actin-binding domain, a protein phosphatase 1 (PP1)-binding site, a PDZ domain that binds p70S6K, and a carboxy-terminal coiled-coil dimerization domain. GFP-tagged Nrbl localized specifically to dendritic spines in neurons in slice culture, and this localization required an intact actin-binding domain. Remarkably, a severely truncated GFP-Nrbl caused a dramatic increase (~5-fold) in the number of spine-like protrusions. We are presently characterizing the ultrastructural and physiological properties of these cells to determine whether the observed morphological changes are reflected in altered numbers of functional synapses.

Molecular Methods to Reversibly Inactivate Synapses In Vivo

A. Karpova, K. Svoboda [in collaboration with J. Huang, Cold Spring Harbor Laboratory]

A major roadblock in gaining an understanding of neural networks is our inability to modulate selected circuit elements in vivo with temporal control and specificity. We have begun a research program to harness recent insights about the molecules that regulate neurotransmitter secretion to design genetically deliverable systems that can interfere with synaptic function in a rapidly inducible manner. Interference is achieved with dimerization induced by rapamycin-like compounds. The first two systems, based on synaptophysin and VAMP, are currently being tested in in vitro systems. These systems will be introduced into specific cell populations using specific promoters or BAC (bacterial artificial chromosome) transgenic technology.

Instrumentation

K. Svoboda, T. Pologruto, G. Shepherd, R. Yasuda [in collaboration with R. Eifert, Cold Spring Harbor Laboratory]

Our instrumentation efforts during the last year have focused on developing two new approaches to studying signaling in neurons and their networks. To measure intracellular signal transduction in single synapses, we developed a microscope that measures fluorescence lifetime. The fluorescence lifetime can be used as a sensitive and robust read-out of fluorescence energy transfer and thus allows measurements of protein-protein interactions inside neuronal microcompartments, such as synapses. We have also set up experimental stations that allow us to efficiently probe neural circuits with laser scanning photostimulation. This has advanced our analysis of experience-dependent changes in neural circuits.

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NEUROBIOLOGY OF MEMORY IN *DROSOPHILA*

T. Tully J. Barditch, S. Gossweiler, M. Regulski, F. Bolduc, L. Grady, P. Smith, J. Dubnau, J. McNeil, S. Xia, A.-S. Chiang

From DNA microarray experiments using wild-type (normal) flies, we have (1) developed a novel statistical method to analyze these data and (2) confirmed 42 candidate memory genes (CMGs) that are transcriptionally regulated during long-term memory formation. One pathway implicated from this analysis involves microtubule-dependent transport of mRNA to subcellular compartments and local regulation of translation. The genetic components are *pumilio, staufen, orb, moesin*, and *eIF2G*. We have provided biological validation that *staufen* is involved in memory formation by showing defective one-day memory after spaced training for a conditional *staufen* mutant.

From a traditional mutagenesis, we have identified 60 new memory mutants. Molecular lesions have been characterized for 58 of these mutants, defining 49 new candidate memory genes. Notably, three of these behavioral mutants also carry defects in the *pumilio/staufen* pathway: *pumilio, oskar*, and *eIF5C*. Taken together with recent studies from other labs, these convergent data from complementary forward- and reverse-genetic approaches have succeeded in identifing several molecular components of the cellular mechanism likely responsible for synapse-specific modification during long-term memory formation.

Identification of New Genes Involved with Olfactory Memory

T. Tully, J. Dubnau, L. Grady, J. Barditch, S. Gossweiler, P. Smith

A behavioral screen (forward genetics) for defects in olfactory memory has yielded 60 new mutant lines. The molecular lesion has been identified for 58 of these, defining 49 new genes: 25 carry P-element transposon insertions (molecular lesions) within identified transcripts, and 24 carry transposon insertions between identified transcripts. Thus, the data suggest 73 candidate memory genes, which appear to be involved in several cellular processes including gene regulation, RNA processing, translational control, trafficking, signal transduction, and cell adhesion.

Two independent transposon insertions were identified in the large intron of *pumilio*, a "developmental" gene involved in regulation of translation. Genetic complementation with an extant allele of *pumilio* and *milord-2* demonstrates that *pumilio* is involved in memory formation (Fig. 1).

We also have used DNA microarrays to detect transcriptional responses during memory formation in normal flies. By comparing mRNA from flies subjected to spaced training versus massed training, nonspecific transcriptional changes are minimized. Full-genome analyses have detected more than 3900 statistically significant candidate memory genes at 0, 6, or 24 hours after training. To date, 49 candidate memory genes have been confirmed with follow-up assays. Here again, several candidate memory genes are involved in gene regulation, RNA processing, translational control, trafficking, signal transduction, and cell adhesion.

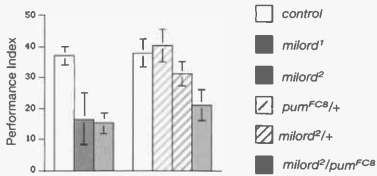

FIGURE 1 The *milord-2* memory mutation fails to complement an extant allele of *pumilio* (pum^{FC8}). One-day memory after spaced training in wild-type flies (control), *milord-1* homozygotes, *milord-2* homozygotes, pum^{FC8}/+ and *milord-2*/+ heterozygotes, and *milord-2*/pum^{FC8} heteroallelics. These data also show that the pum^{FC8} allele is recessive to wild-type (+), and *milord-2* is semi-dominant to +. Together, these results demonstrate that *pumilio* is involved in *Drosophila* memory formation.

Of these, temperature-sensitive mutants exist for *staufen*. Behavioral experiments on these mutants have demonstrated defective long-term memory formation after spaced training only when flies were stored at restrictive temperature during the retention interval. These results provide biological validation of an acute role for *staufen* during long-term memory formation.

Anatomical Studies

T. Tully, A.-S. Chiang, F. Bolduc, J. Dubnau, J. McNeil, L. Grady

Twenty nine of the mutant strains carry a PGAL4 P-element insertion, which allows "enhancer-trap" reporter-gene expression of green fluorescent protein (GFP). Three-dimensional reconstructions of confocal images through whole-mount brains of adult flies has revealed a "circuit" for olfactory memory, which extends beyond mushroom bodies, one well-established anatomical site involved in olfactory learning. A subset of these enhancer-trap strains suggest inputs to, and outputs from, mushroom bodies.

Thirty one of the mutant strains carry a PlacW P-element insertion, which allows enhancer-trap reporter-gene expression of β-galactosidase. These allow cell-body expression patterns but not neuronal processes. Nonetheless, these 60 enhancer-trap lines reveal 70% with expression that includes mushroom bodies. These observations support the notion that mushroom bodies participate in memory formation but not exclusively.

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LONG-TERM MEMORY FORMATION IN *DROSOPHILA*

J. Yin E. Drier K. Iijima C. Margulies L. Moy E. Paniagua M.Tello P. Wu

DaPKC and Synaptic Tagging

E. Drier, P. Wu, J. Yin [in collaboration with P. Schedl, Princeton University; A. Wodarz, Duesseldorf; J. Knoblich, IMP, Vienna, Austria]

In all systems where it has been tested, long-term memory requires the synthesis of new proteins around the time of training. This acute requirement for transcription and translation in the cell body is necessary for long-lasting increases in the strength of synaptic connectivity. However, the universal requirement for de novo gene expression begs the question of synaptic specificity: How do neurons that participate in behavioral circuits only strengthen the synapses that are behaviorally relevant? How does an individual neuron only strengthen the few synapses that participate in the behavior, when it has thousands of neurons? The synaptic tag hypothesis has been proposed, and it is hypothesized that tagging enables neurons to confine the products of transcription and translation to particular synapses, thereby maintaining synaptic specificity when neuron-wide events such as gene expression occur. The cellular processes and molecular participants in tagging remain undescribed.

We have shown that *DaPKM*, a truncated isoform of the *DaPKC* (*Drosophila* atypical protein kinase C) gene, is sufficient to enhance memory formation (under certain circumstances) and is necessary for its formation. The Sacktor lab (Drier et al. 2002) has shown that aPKMζ is necessary and sufficient for the maintenance of long-term potentiation (LTP) in hippocampal slices. What functions do atypical proteins perform when they participate in memory formation and LTP? Based on a wealth of information from developmental biology, we postulate that aPKC, the full-length protein, and aPKM participate in tagging recently active synapses. The hypothetical tag allows recently active synapses to uniquely utilize macromolecules made and transported ubiquitously.

The aPKC protein, and its partners Par3 (Bazooka) and Par6, have been extensively studied in the process of asymmetric cell division in the *Drosophila* embryonic neuroblast. In this process, aPKC/Par3/Par6 are *localized* on the apical side of the neuroblast and *scaffold* a large number of other proteins and translationally silent mRNAs. Among these are Staufen, an mRNA-binding protein that has been shown to be important in neuronal plasticity. In the process of establishing cell polarity, the aPKC/Par3/Par6 complex is localized on the apical/lateral border of *Drosophila* epithelial cells. These cells contain a number of other localized protein complexes, including the cadherin/catenin complex (located more basally along the lateral membrane) and the Dlg/Lgl/Scribble complex, which is even further down (basally) on the lateral face. All of these proteins are also located in synapses of neurons. The *dlg* gene can produce at least two different protein isoforms, one of which is the fly homolog of PSD-95, the other being the homolog of SAP97. These are important scaffolding proteins at the mammalian synapse that bind NMDA (*N*-methyl-D-aspartate), and AMPA (α-amino-3-hydroxy-5-methyl-4-isoazole) subtypes of glutamate receptors. These large complexes all mutually *interact* with each other in setting "boundaries" to their localization. Finally, in oogenesis, a stem cell undergoes four rounds of cell division with incomplete cytokinesis, producing a 16-cell cytocyst where the cells are interconnected by ring canals. Macromolecules can be transferred between the 16 cells through these channels. Early on, one of the 16 cells is fated to become the oocyte, and the other 15 cells begin to traffic proteins and translationally silent mRNAs into the 16th cell. The repressed mRNAs traffic to a particular location in the 16th cell, where aPKC/Par3/Par6 are localized, and are translated there. Mutations in any of the three genes (*DaPKC, par3* or *bazooka*, or *par6*) do not affect mRNA trafficking, but lead to ectopic translation of the mRNAs all around the 16th cell, and the future oocyte loses its fate. Among the proteins that are important in this process are the RNA-binding protein dFMR1 (the fly Fragile X protein) and Orb, the fly cytoplasmic polyadenylation enhancer-binding protein (CPEB). These have been shown to be critical synaptic plasticity and activity-dependent translation. Therefore, in oogenesis,

aPKC/Par3/Par6 are required for the *localized translation* of silent mRNAs. Our conceptualization of tagging involves the subcellular processes in which aPKC/Par3/Par6 participate, namely, localization, scaffolding, interacting with other localized complexes, and localization of translation.

We have begun purifying the DaPKM p60 isoform from adult heads. Using column chromatography and an immunoaffinity column, we have partially purified the protein. In the eluted fraction from the antibody column, we detect Dlg, the SAP97 form of Dlg, and truncated forms of Par3, Par6, and dFMR1. We are currently trying to detect some of these interactions in the other direction and carrying out further biochemical steps to increase the stringency of our interaction data. In our cleanest fraction, we detect about 50 discrete protein bands using the most sensitive silver stain technique that we can find. We have cut out 25 of these bands and will analyze them using mass spectrometry. The presumed DaPKM p60 band is very prominent, and we hope to positively identify it by sequencing and peptide analysis. Future work will focus on detecting phosphorylation, further biochemical purification of discrete subcomplexes, and functional tests in development and behavior.

Molecular Analysis of the *DaPKC* gene

M. Tello, E. Drier, E. Paniagua, P. Wu, J. Yin

We have continued our characterization of the *DaPKC* gene. There are at least three major mRNA transcripts of approximately 6.5, 5.5, and 4.5 kb in length. These three transcripts initiate from three different locations in the gene. The largest transcript (A) starts from the 5′ end of the internal intron located between coding exons 2 and 3. Transcript B initiates from the same 12-kb intron, but in the last 3′ third of the intron. Transcript C initiates from upstream of the entire coding region, but it is the shortest of the transcripts. Because of these locations, transcripts A and B are likely to have extensive 5′- and 3′-untranslated regions (UTRs). At the 3′ end of the gene are two short exons that are alternatively spliced into mRNAs in a mutually exclusive manner. These exons are almost identical in the number of amino acids that they encode, and nothing is known about their functional significance. One of the exons is exclusively connected with transcript C, whereas the other one is found with all three classes of transcripts. We have not characterized alternative splicing, or alternative polyadenylation, of possible 3′UTRs.

The protein-coding capacity of the different transcripts is not entirely clear. Three protein bands are visualized on a western blot using a mammalian-specific, atypical PKC antibody raised against a peptide at the very carboxy-terminal end of the rat atypical PKCζ protein. These three bands of apparent molecular weights p73 (aPKC or full-length), p60 (aPKMp60), and p50–55 (aPKMp50) are detected in adult head and embryonic extracts. We are using a P-element insertion in the 12-kb internal intron, and precise and imprecise excisions of this element, to assign transcript coding capacity to the different protein bands. It is probable that the p73 protein is made exclusively off transcript C, whereas the p60 isoform is internally initiated from transcript B. We hypothesize that the p50 isoform is a proteolytic product of p73.

Imprecise excisions of the P element that remove sequence distal to the insertion site result in a loss of transcript B. Together with 5′ RACE analysis, RNase protection, and northern analysis using probes made from across the intronic region, we have localized the region where transcript B probably initiates transcription. Interestingly, there is a perfect CRE sequence and TATA box nearby the cluster of RACE start sites. This transcript has the potential to encode many different internally initiated proteins, since there are many in-frame methionine codons prior to the point where the transcript becomes identical to the full-length transcript. Our preliminary evidence (see below) suggests, though, that the last one or two in-frame methionines are used for internal translation initiation.

DaPKC and Neuronal Dysfunction

P. Wu, L. Moy, J. Yin [in collaboration with B. Ganetzky, University of Wisconsin]

We have screened 29 different temperature-sensitive paralytic mutants (obtained from Barry Ganetzky). These mutants, all in different genes, result in hyperneuronal activity and seizure-like behavior when flies are shifted to the nonpermissive temperature. Of the 29 mutants, 14 vastly overproduce the aPKMp60 isoform, without appreciably changing the levels of p73. Northern analysis of eight of these mutant lines does not show any dramatic changes in the relative ratios of the three transcripts, nor in their absolute amounts.

Two of the mutants, though, have altered patterns of transcripts. One is missing transcript A, whereas the other has an insertion similar to that of all three transcripts. Collectively, all of these data suggest that overproduction of p60 occurs posttranscriptionally and, at least in the case of the last two mutants, probably at the level of translation. These results suggest that a major step in the regulation of p60 occurs at the level of translation.

The correlation of overproduction of p60 with seizure-like behavior is particularly interesting. Given our hypotheses about synaptic tagging, it is easy to imagine how overproduction of p60 might participate in seizures, whether in the ectopic spread of neuronal activity through different circuits, or in the prolonged synaptic transmission at various synapses, or both. Since it is likely that many genes, when mutant, predispose to seizure-like behavior, it is potentially very useful to identify genes and signaling pathways that are commonly recruited to seizures, since these are potentially useful drug targets for therapeutic intervention.

Sleep and Memory Formation

P. Wu, J. Yin [in collaboration with J. Hendricks and A. Sehgal, University of Pennsylvania]

We have shown that dCREB2, and the PKA pathway, is important for rest homeostasis in flies. We have also investigated the participation of various genes known to be involved in the circadian system for their effects on sleep homeostasis. Loss-of-function mutations in *clock* and *cycle*, or induced disruption of their activities, interfere with normal homeostasis, shortening the average amount of rest that flies get. We are currently investigating the role of disruption and overexpression of these proteins on sleep homeostasis and memory formation.

Anatomy of Long-term Memory Formation

C. Margulies

The standard methodology used in *Drosophila* to investigate anatomical requirements for a gene is to use the Gal4/UAS system. When the dCREB2-b (blocker) isoform is expressed pan-neuronally, depending on the driver, we either detect no effect on memory after spaced training or an effect that is larger than what is seen with a heat shock transgene. This latter result suggests that expression of the blocker is partially affecting memory after massed training (ARM), hinting at a developmental effect. Therefore, it is necessary to use the spatially restricted, temporally regulated Tet-On system to define anatomical locations where dCREB2 is needed during adult memory formation.

dCREB2 and Neuronal Dysfunction

K. Iijima

Overexpression of activator or blocker isoforms of the dCREB2 in a pan-neuronal pattern during development has negative effects on eclosion and viability. When the dCREB2-b (blocker) isoform is overproduced, there is a decrease in the expected number of mutant progeny. These adults have shorter longevity, and show deficits in learning, without effects on the peripheral behaviors (olfactory acuity and shock reactivity). When the dCREB2-a isoform is overproduced, almost no adult flies eclose. When these isoforms are overproduced in the adult eye, they do not result in a "rough" eye phenotype, suggesting that developmental effects are specific to the nervous system. When crossed to genetic models of neuronal degeneration, perturbations of dCREB2 can enhance the phenotypes of certain dysfunctions, suggesting that part of the dysfunctional effects occurs through the dCREB2-responsive transcription pathway.

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NEURAL SUBSTRATE OF SELECTIVE AUDITORY ATTENTION IN THE CORTEX

A. Zador M. DeWeese G. Otazu L.-H. Tai
T. Hromadka S. Rumpel M. Wehr
M. Moita

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 our 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—in awake behaving rats by the demands imposed by attentional tasks.

Binary Coding in the Auditory Cortex

M. DeWeese

Cortical neurons have been reported to use both rate and temporal codes. We have discovered a novel mode, in which each neuron generates exactly zero or one action potential, but not more, in response to a stimulus. Cortical responses recorded using conventional extracellular techniques typically show responses to be highly variable. However, in our studies, we have been using a technique adapted from in vitro studies, cell-attached recording, which has the advantage of ensuring that the recorded responses all arise from a single neuron. Surprisingly, we have found that most neurons exhibit binary behavior, with few multispike responses; some responses consist of exactly one spike on 100% of trials, with absolutely no trial-to-trial variability. We are currently investigating the computational implications of this previously undescribed coding scheme.

Variability of Coding in the Auditory Cortex

M. DeWeese

Computers rely on extremely precise, low-noise components to compute, whereas the components that make up neural circuits appear to be very noisy. Nevertheless, brains outperform computers on the kinds of hard computational problems required for survival in the real world. To understand how brains compute in the presence of such high levels of apparent noise, we are characterizing the sources of variability (i.e., noise) in single neurons. Using in vivo whole-cell patch-clamp recording techniques, we are examining the trial-to-trial variability of the postsynaptic potential (PSP) elicited by brief tone pips. In some neurons, trial-to-trial variability in the PSP is small, consistent with "private" sources limited to only the neuron under study; but for other neurons, "shared" sources of variability produce circuit-wide fluctuations in the synaptic drive to the neuron and its neighbors, greatly increasing the apparent noise in the PSP. These stimulus-independent correlations could provide a substrate for feedback underlying cognitive processes, such as attention and motivation.

In Vivo Whole-cell Patch-clamp Recordings of Sound-evoked Synaptic Responses in Auditory Cortex

M. DeWeese, L.-H. Tai

Neurons in the auditory cortex respond to some sounds but not to others. What determines this selectivity? We are using whole-cell patch-clamp recording methods in vivo to measure the synaptic currents elicited by simple and complex auditory stimuli. Patch-clamp recordings provide a much richer source of information than do conventional single-unit extracellular recordings because they allow us to monitor not just the *output* of the neuron—the spike train—but the input as well. These data provide clues about the representations with which the cortex solves hard problems in auditory processing.

Optimal Stimulus for Auditory Cortex

T. Hromadka [in collaboration with M. Chklovskii, Cold Spring Harbor Laboratory]

Single neurons in auditory cortex respond differently to different sounds. What are the sound features that evoke the strongest response? To answer these questions, we use in vivo cell-attached recordings in awake Mongolian gerbils in combination with evolutionary algorithms to search for the most appropriate stimulus for the individual neurons. In addition, we are characterizing the responses of single neurons to a wide variety of conventional stimuli, including pure tone pips, sweeps, and moving ripples.

Role of Auditory Cortex in Frequency Discrimination

M. Moita

Cells in the primary auditory cortex respond selectively to pure tone frequencies. However, most studies on the effect auditory cortex lesions have on frequency discrimination show that frequency discrimination is intact after auditory cortex lesions. These studies normally test discrimination between frequencies far apart. Thus, failure to observe an effect of auditory cortex lesions in frequency discrimination might arise from the possibility that auditory cortex is only necessary for fine-grained frequency discriminations. We are developing a task that tests frequency discrimination between a wide range of frequency distances in rats. We will then use this paradigm to test the role of auditory cortex in frequency discrimination, using both gain-of-function and loss-of-function approaches. In the first case, we will use microstimulation of auditory cortex cells, and in the second case, we will temporarily inactivate the auditory cortex during performance of this task.

Separation of Sound Sources by Awake Behaving Animals

G. Otazu, M. Moita

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 selectively attend 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.

Analysis of AMPA Receptor Trafficking during Learning-induced Reorganization of the Auditory Cortex

S. Rumpel [in collaboration with R. Malinow, Cold Spring Harbor Laboratory]

Does learning change the strength of neuronal connections in the brain? Insertion of new postsynaptic AMPA (α-2-hydroxy-5-methyl-4-isoazole) receptors

has been identified as a major process leading to increased synaptic strength. However, these results have been obtained primarily in cultured neurons, and the relationship of these processes to learning in the intact animal has remained unclear. We have been investigating whether reorganization of the auditory cortex induced by classical conditioning leads to increased insertion of AMPA receptors. We are using virus-mediated overexpression of specially engineered recombinant AMPA receptors as a marker of this in vivo plasticity. These properties will enable us to detect added recombinant receptors after a learning protocol and thereby establish the role of AMPA insertion in vivo. If the molecular rules of learning identified in cultured neurons should apply in vivo, we will also have developed a valuable tool for identification of synaptic circuits involved in memory formation.

Responses to Complex Stimuli

M. Wehr [in collaboration with C. Machens and C. Brody, Cold Spring Harbor Laboratory]

How do neurons in the auditory cortex encode complex stimuli, such as animal calls or the sound of rain falling on leaves? Sensory physiologists often ignore this question, dismissing it as too difficult to approach in a systematic fashion. Instead, they often limit their inquiries to simple stimuli, such as (in the auditory system) brief tone pips or sweeps. We have instead tackled this problem head-on, by measuring the responses of neurons in the auditory cortex to a wide variety of natural sounds. We then use these responses to construct and test models of how cortical neurons encode natural sounds.

Inhibitory and Excitatory Contributions Underlying Synaptic Responses

M. Wehr

The cortical responses to sensory stimuli such as sounds consist of a mix of excitation and inhibition. Conventional extracellular recording techniques using tungsten electrodes provide information only about excitatory responses. We have developed an approach using whole-cell patch-clamp recording in vivo in voltage-clamp mode to tease apart the temporal dynamics of the excitatory and inhibitory drive underlying responses to auditory stimuli.

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NEURAL BASIS OF LEARNING AND MEMORY IN *DROSOPHILA*

Y. Zhong H.-F. Guo K. Iijima
I. Hakker J. Tong
F. Hannan Y. Wang
I. Ho

We combine genetic manipulation and functional analyses in the study of neural basis of learning and memory in *Drosophila*. Two approaches are being taken to identify the players and events that are critical to the learning and memory process. One approach is modeling human pathological conditions in *Drosophila* that impair learning and memory. Specifically, we focus on neurofibromatosis 1 (NF1) and Alzheimer's disease (AD). Mutations in the NF1 tumor suppressor gene in humans cause neurofibromas and other symptoms including learning defects. We are investigating the hypothesis that NF1 not only acts as a Ras-specific GTPase-activating protein (GAP), but also is involved in mediating adenylyl cyclase (AC) activation, and this NF1-dependent AC pathway is required for learning. AD is a neurodegenerative disorder accompanied by deterioration of memory. A major hypothesis concerning the pathogenesis of AD is that accumulation of toxic fragments of the amyloid precursor protein (APP), $A\beta_{42}$, leads to pathologic progression. We have tested the hypothesis by introducing into *Drosophila* human minigenes that encode the $A\beta_{42}$ and $A\beta_{40}$, the less toxic APP fragment. The other approach is through examining physiological processes and activities that may underlie learning and memory at the synaptic and neuronal network levels.

At the synapse level, we continue our tradition of examining functions of genes in regulating synaptic plasticity at the *Drosophila* neuromuscular junction (NMJ) and find that a serine/threonine kinase is involved in the long-term depression of NMJ activities. At the neuronal network level, our newly constructed transgenic flies expressing a green fluorescent protein (GFP)-based Ca^{++} probe in combination of 2-photon imaging have allowed us to examine with single-cell resolution odor-evoked neuronal responses in a large number of neurons in a higher olfactory center of the fly brain, the mushroom body (MB). Currently, the effort is focused on odor representation in the MB. Since the MB is involved in associative olfactory learning and memory, the next step is to examine how such a process is achieved in the MB.

Requirement of Protein Kinase B (Akt) for Long-term Depression at the *Drosophila* Neuromuscular Junction

H.-F. Guo

Several forms of short-term synaptic plasticity have been reported at the *Drosophila* NMJ, including paired-pulse facilitation, short-term facilitation, augmentation, and post-titanic potentiation. Genetic dissection has provided insights into the mechanism of synaptic transmission and plasticity, and conversely, analyses of these forms of synaptic plasticity have helped to elucidate the function of learning and memory-related genes in synaptic transmission and plasticity.

We have discovered a new form of long-term synaptic plasticity at the *Drosophila* NMJ. A titanic stimulation (30 Hz, 20 sec) induced a long-term depression (LTD) of evoked-synaptic currents (EJC), which lasts at least for 1 hour. The amplitude of EJC was reduced to 30–50% after the titanic stimulation. The induction of LTD is calcium-concentration-dependent, which can be induced at 0.35–0.4 mM Ca^{++}, but not at 0.7 mM (or higher concentration) Ca^{++}, and it is expressed on specific muscle fibers (e.g., #13 but not on #6). Quantal analyses revealed that the quantal size was not changed, whereas the quantal content was reduced to less than 50%. In addition, direct application of glutamate-induced synaptic currents did not change significantly after the titanic stimulation, suggesting a presynaptic mechanism of this plasticity.

Genetic analyses indicate that protein kinase B (Akt), which is a major target of the phosphoinositol-3 (PI-3) kinase, is required for this LTD. Mutations of the *akt* gene largely attenuated the depression. We are currently conducting more detailed analyses of the role of *akt* in this LTD. This long-term plasticity can be used as a model for studying gene function and sig-

nal transduction underlying synaptic plasticity and learning and memory mechanism.

Site-directed Mutagenesis in Human NF1

F. Hannan, I. Hakker

The NF1 tumor suppressor gene encodes a protein with a centrally located GTPase-activating protein (GAP)-related domain (GRD) that negatively regulates Ras activity. Studies of NF1 have focused on its role in the Ras pathway, but we have demonstrated that NF1 also regulates AC activity in *Drosophila* and that the NF1-regulated AC pathway is essential for learning in *Drosophila*. Recently, we have shown that NF1 affects neuropeptide- and G-protein-stimulated AC activity in mice and that expression of a human NF1 transgene in NF1 mutant flies rescued small body size and G-protein-stimulated AC activity defects. We have since found that normal human NF1 can also rescue the learning defect seen in NF1 mutant flies. These phenotypes are all cAMP-dependent, thus demonstrating that vertebrate neurofibromin can regulate AC activity in both mammals and flies.

The neurofibromin protein is very large (~250 kD), and regions outside the central GRD domain are also critical for its function since clinically observed missense mutations resulting in single-amino-acid changes occur throughout the entire length of the protein. Our current studies show that human NF1s with a number of different missense mutations, both within and outside the GRD, allow partial rescue of some cAMP-dependent phenotypes, including body size and learning defects, when expressed in transgenic NF1 mutant flies. This suggests that none of the mutations assayed to date affect a region critical for cAMP-dependent phenotypes.

Preliminary results have shown that NF1 mutant flies expressing human NF1 with a mutation in the GRD domain have reduced memory compared to those expressing normal human NF1. This suggests that the GAP activity of NF1 may be important for long-term memory formation. Normal human NF1 is also able to fully rescue a circadian rhythm defect in NF1 mutant flies. This phenotype has been shown to be Ras-dependent; thus, we predict that mutations in the GRD will not allow rescue of the circadian defect.

We have begun an analysis of NF1-dependent gene expression profiles in *Drosophila*, using oligonucleotide-based microarrays. Preliminary data indicate several genes whose expression is altered consistently in NF1 mutant heads. The microarray results are being confirmed by quantitative reverse transcriptase–polymerase chain reaction (RT-PCR) and northern analysis. The effect of expressing wild-type and mutant human NF1 transgenes on NF1 mutant flies is also being assessed using microarrays.

A Novel Ras- and NF1-mediated Pathway Regulating Adenylyl Cyclase in *Drosophila*

I.S. Ho, J. Tong

Ras is essential in various cellular signaling pathways, being the molecular switches in governing cell survival, cell death, proliferation, and differentiation. We found that in *Drosophila* head extract, human Ha-Ras protein can stimulate AC activity. This stimulation requires the presence of neurofibromin, a GAP-related domain (GRD)-containing protein encoded by the NF1 gene. Previous studies showed that NF1 regulates AC activity in *Drosophila,* and this regulation is important for learning.

To substantiate that the Ras-stimulated AC activity is of physiological significance, we tested the effect of epidermal growth factor (EGF) on stimulating AC activity. EGF activates receptor tyrosine kinases (RTKs), which leads to Ras activation. It has been shown that an EGF receptor homolog, DER, is present in *Drosophila*. Since there is no known EGF homolog in *Drosophila*, we used mouse EGF (mEGF). Third-instar larval brains were treated with mEGF and tested in an AC assay. We found that mEGF is able to consistently stimulate AC activity approximately 150% of the basal level. This stimulation was largely abolished in DER, Ras, and NF1 mutants, indicating that DER, Ras, and NF1 are essential for regulating EGF-stimulated AC activity.

Our study revealed a novel EGF receptor (EGFR) signaling pathway that leads to the activation of AC. Since cAMP is a secondary messenger involved in various neuronal functions such as synaptic plasticity, and learning and memory, we are interested in examining the role of this EGFR signaling pathway in neuronal functions.

A *Drosophila* Model of Alzheimer's Disease: Dissecting the Pathological Roles of $A\beta_{42}$ and $A\beta_{40}$

K. Iijima [in collaboration with H.-P. Liu and A.-S. Chiang, National Tsing Hua University, Taiwan, and M. Konsolaki, Novartis Institute for Biomedical Research]

AD is a neurodegenerative disorder characterized clinically by progressive decline in memory accompanied by histological changes, including neuronal loss and the formation of neurofibrillary tangles and senile plaques. It has been proposed that accumulation of Aβ peptides, the major component in the senile plaques, is the primary event in the AD pathogenesis. The strongest support for this hypothesis comes from genetic analysis of familial AD (FAD). All FAD mutations identified appear to cause excessive accumulation of Aβ. The hypothesis, however, remains controversial because of a number of concerns, including a weak correlation between Aβ deposits and decline in cognitive functions, mild neurodegeneration in mice with excessive accumulation of Aβ and abundant senile plaques, and difficulties in defining in vivo the specific species of Aβ and the nature of its neuronal effects. To address these concerns, we examined the effects of expression of two transgenic minigenes that encode the secreted form of either human $A\beta_{42}$ or $A\beta_{40}$ in the *Drosophila* brain. It was revealed that accumulation of $A\beta_{42}$ led to age-dependent learning defects and extensive neurodegeneration. In contrast, expression of $A\beta_{40}$ did not induce degeneration, although it affected learning when expressed at a high level. This study demonstrates that accumulation of $A\beta_{42}$ is sufficient to cause progressive memory loss and neurodegeneration in an organism with a very short life span.

Imaging of Stereotyped Odor Representation in the Mushroom Body of *Drosophila* with a GFP-based Ca^{++} Sensor

Y. Wang [in collaboration with T. Pologruto and K. Svoboda, Cold Spring Harbor Laboratory]

Recent development in understanding odor receptors and anatomic organization of the olfactory nervous system has strongly implicated a spatial mechanism for olfactory coding. This prompted us to investigate odor representation in the *Drosophila* mushroom body (MB), a higher olfactory center that is required for formation of olfactory-related memory. For this purpose, we have generated transgenic flies that express GFP-based Ca^{++} sensor specifically in the MB. Combined with 2-photon microscopy, this has allowed us to map odor-evoked neuronal responses with single-cell resolution throughout the MB of a living fly. We find that each odor evokes large-amplitude fluorescence transients in the somata of distinct subsets of MB neurons (Kenyon cells [KCs]) and in subregions of the calyx, the neuropil of the MB. The spatial distribution of odor-evoked activities are similar from fly to fly, indicating a stereotyped representation of odors in the MB. Our data suggest that olfactory information is represented by sparse population coding and that spatial patterns of activity encode olfactory information in MBs.

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PLANT DEVELOPMENT AND GENETICS

Like animals, plants use unspecialized stem cells to create new organs and tissues. One question fundamental to all multicellular life is how organisms trigger stem cells to proliferate and subsequently adopt special properties characteristic of a particular organ or tissue, a process called stem cell patterning. David Jackson and Marja Timmermans are investigating the signals that plants use to guide stem cell patterning. Their findings are shedding light on some processes that are common to plant and animal development, and on others—unique to plants—that could ultimately have significant impacts on agriculture, horticulture, and forestry.

One mechanism that appears to be involved in stem cell patterning, and that is unique to plants, is the movement of proteins between cells through structures called plasmodesmata. Jackson and his colleagues have created several methods for investigating how proteins move through plasmodesmata to carry out stem cell patterning. That understanding could also help block the spread of devastating plant viruses, which travel along the same intercellular highways.

Genes and proteins studied by Timmermans's lab are also emerging as key players in guiding stem cell patterning and organ development. She has identified several proteins that act in a complex way to turn key genes on or off at the correct time and in the correct location as plants develop, a process that is required, for example, for the proper outgrowth of leaves and for differentiating the top layers versus the bottom layers of leaves. Timmermans's lab has also uncovered evidence that leaf patterning is controlled by RNA interference.

Part of Rob Martienssen's interest in exploring plant genetics and development has focused on a specialized type of DNA structure called heterochromatin. By virtue of the fact that heterochromatin is typically rich in "transposable elements" (segments of DNA that usually stay put but can move to other chromosomal locations), most often stays tightly packaged, and has few canonical protein-coding genes, heterochromatin has a unique impact on chromosome structure and function. Martienssen's lab is exploring the activity of genes located within heterochromatin and how such genes are silenced through the influence of transposable elements (or ancient remnants of such elements). In both plants and yeast, the lab has found that RNA molecules transcribed from remnants of transposable elements trigger the formation of heterochromatin, and hence establish gene silencing, through RNA interference.

PLANT DEVELOPMENTAL GENETICS AND FUNCTIONAL GENOMICS

R. Martienssen J.-M. Arroyo, M. Byrne, J. Fontana, A. Groover, C. Kidner, Z. Lippman, B. May, M. Ronemus, P.D. Rabinowicz, D. Roh, J. Simorowski, R. Shen, A. Tang, G. Teng, U. Umamaheswari, M. Vaughn, E. Vollbrecht, T. Volpe, C. Yordan

We are using genomics and developmental genetics to investigate epigenetic mechanisms of gene regulation, transposon silencing, and stem cell function in plants. *Arabidopsis* chromosome-4 tiling microarrays are being used to investigate transposons and heterochromatin. We are investigating the role of a*symmetric leaves1, bell-ringer, ramosa1*, and *argonaute* in stem cell function and leaf patterning. *Argonaute* has an important role in RNA interference (RNAi), and our studies in fission yeast have revealed a role for centromeric transcripts and RNAi in histone modification and chromosome segregation. We have demonstrated parallel mechanisms in plants. During the summer, we were joined by our URP, Grace Teng, and guest postdoc Andreas Madlung from Seattle, as well as Ruth Bastow from the John Innes Institute. We said goodbye to Cristy Yordan, who was a research associate in the lab for almost 10 years and is greatly missed. She moved to Harvard Medical School in the summer and we wish her well in her new appointment.

Role of *ramosa1* in Inflorescence Architecture

E. Vollbrecht, R. Martienssen

In the domesticated cereals and other grasses, the presence or absence of long inflorescence branches defines panicle and spike architectures, respectively. For example, the maize tassel bears both long and short (spikelet pair) branches, whereas the ear bears only short branches. Mutations in the *ramosa1* (*ra1*) and *ramosa2* (*ra2*) genes transform most spikelet pair branches from short to long. Thus, *ra1*, which encodes a small zinc finger protein, imposes short branch identity. *ra1* expression defines a boundary between nascent spikelet pair meristems and the primary inflorescence. *ra2* mutants are phenotypically similar to weak *ra1*, although *ra2* also represses internode elongation. In the spike, *ra2* positively regulates *ra1* expression levels, and *ra2 ra1* double mutants resemble *ra1*, indicating that a single pathway assigns spikelet pair identity. Molecular population genetic tests indicate that *ra1* was a target of selection during maize evolution or domestication from the teosintes and *Tripsacum*. Within the sugar cane tribe, which diverged some 20 million years ago, *Miscanthus sinensis* and *Sorghum bicolor* have more extensive branching, and delayed onset of *ra1* expression. Moreover, *Sorghum* BAC (bacterial artificial chromosome) sequencing demonstrated that rice lacks a *ra1* ortholog, consistent with the branched architecture of rice. These findings implicate the *ramosa* pathway in the evolution of grass inflorescence diversity.

ASYMMETRIC LEAVES1 Reveals Homeobox Gene Redundancy in *Arabidopsis*

M. Byrne, J. Simorowski, R. Martienssen

The shoot apical meristem comprises undifferentiated stem cells and their derivatives, which include founder cells for lateral organs such as leaves. Meristem maintenance and lateral organ specifications are regulated in part by negative interactions between the Myb domain transcription factor *ASYMMETRIC LEAVES1* (*AS1*), which is expressed in lateral organ primordia, and homeobox transcription factors, which are expressed in the shoot apical meristem (*knox* genes). The *knox* gene *SHOOT MERISTEMLESS* (*STM*) negatively regulates *AS1* which, in turn, negatively regulates other *knox* genes, including *KNAT1* and *KNAT2*, and positively regulates the novel gene *LATERAL ORGAN BOUNDARIES* (*LOB*) identified through enhancer-trap analysis. Genetic interactions with a *LOB*-related gene *AS2* indicate that it acts at the same position in this hierarchy as *AS1*. We have used a second-site suppressor screen to isolate mutations in *KNAT1*, and we have shown that *KNAT1* is partially redundant with *STM* in regulating stem cell function. Mutations in *KNAT2* show no such interaction.

BELLRINGER Regulates Phyllotactic Patterns and Stem Cell Fate

M. Byrne, A. Groover, J. Fontana, R. Martienssen

Lateral organs in plants arise from the meristem in a stereotypical pattern known as phyllotaxy (Greek for *leaf order*). In most plants, such patterns comprise individual leaves and flowers at spiral positions which are related to each other by consecutive terms in the Fibonacci series. Stem cell lineages also expand according to the Fibonacci series when daughter cells are delayed from acquiring stem cell fate, raising the possibility that stem cells are responsible for phyllotactic patterns (Klar, *Nature 417:* 595 [2002]). We demonstrate that a homeodomain protein related to the *BELL1* gene, which we have named *BELLRINGER*, is required to maintain the phyllotactic pattern in *Arabidopsis*. In the absence of *BELLRINGER*, the regular pattern is disturbed and lateral organs are initiated more frequently. *BELLRINGER* is also required for maintenance of stem cell fate in the absence of *STM* and *AS1*. We propose a model whereby *BELLRINGER* coordinates the maintenance of stem cells with differentiation of daughter cells in stem cell lineages.

ARGONAUTE Regulates Leaf Polarity in *Arabidopsis* via a Small RNA Signal

C. Kidner, M. Ronemus, R. Martienssen

argonaute1 (*ago1*) is a pleiotropic mutation in *Arabidopsis* defective in gene silencing via RNAi. We have collected an allelic series of *ago1* with defects in meristem function, organ polarity, and organ identity. Altered reporter gene expression and double-mutant analysis suggest that *AGO1* controls meristem function mediated by *STM*, and floral organ identity mediated by *LEAFY*. Weak *ago1-11* resembles and strongly enhances weak *carpel factory* (*caf*), a homolog of *Drosophila DICER*, indicating that phenotypic effects of *ago1* are due to its role in RNAi. The partial rescue of *ago1* by loss-of-function mutations in *CURLY LEAF* (*CLF*), a SET domain gene, suggests a link between RNAi and polycomb-mediated gene silencing. Weak *ago1-12* resembles dominant *PHABULOSA1-D* (*PHB*) and is enhanced by *revoluta* (*rev*), indicating a central role for RNAi in leaf polarity. A 21-nucleotide microRNA (miRNA) matches a contiguous 19-bp sequence of *PHB/REV* homologous mRNA and has been shown to guide cleavage. Dominant *PHB* alleles disrupt this homology, and result in ectopic *PHB* expression. We have shown that this miRNA is expressed in the abaxial domain of early primordia and over-accumulates in *ago1*. Thus, miRNA acts as a signal to specify leaf polarity. We have used Affymetrix microarray analysis of *ago* and *caf* mutants to detect targets of RNAi. Up-regulated genes include several that have unique regions of homology with miRNA sequences, which have been recently obtained in *Arabidopsis*. We are investigating the basis for increased transcript accumulation.

Regulation of Histone H3 Lys-9 Methylation, Centromeric Silencing, and Centromere Function by RNAi

T. Volpe, C. Kidner, G. Teng, R. Martienssen
[in collaboration with R. Allshire, Wellcome Institute, and S. Grewal, Cold Spring Harbor Laboratory]

Eukaryotic heterochromatin is characterized by a high density of repeats and transposons, as well as by modified histones, and influences both gene expression and chromosome segregation. In the fission yeast *Schizosaccharomyces pombe*, we deleted the *argonaute, dicer*, and RNA-dependent RNA polymerase gene (RdRP) homologs, which encode part of the machinery responsible for RNAi. Deletion results in the aberrant accumulation of complementary transcripts from centromeric heterochromatic repeats. This is accompanied by transcriptional de-repression of transgenes integrated at the centromere, and loss of histone H3 Lys-9 methylation. We propose that double-stranded RNA arising from centromeric repeats targets formation and maintenance of heterochromatin through RNAi. A subfragment of the centromeric repeat (*dg*) is known to induce silencing of marker genes at euchromatic sites and is required for centromere formation. We have shown that the RNAi components, Argonaute (Ago1), Dicer (Dcr1), and RNA-dependent RNA polymerase (Rdp1), are required to maintain silencing, Lys-9 methylation of histone H3, and association of Swi6 at the *dg* ectopic silencer. Deletion of Ago1, Dcr1, or Rdp1 disrupts chromosome segregation, leading to a high incidence of lagging chromosomes on late anaphase spindles and sensitivity to a microtubule poison. Analysis of *dg* transcripts at the ectopic site indicates that *csp* mutants, which are defective in centromere silencing

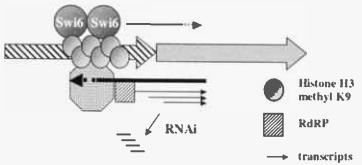

FIGURE 1 A model for maintenance of centromeric silencing by RNAi. Centromeric repeats (block arrows) are constitutively transcribed from one strand, but the transcripts are rapidly turned over by RNAi. RdRP binds the centromeric repeats and somehow recruits the histone modification (octagon). Histone H3 methyl-Lys-9 then recruits Swi6, which silences the other strand.

and chromosome segregation, are also defective in the regulation of noncoding centromeric RNAs. In addition, recruitment of Swi6 and cohesin to centromeric repeats is disrupted in these mutants. Thus, the formation of silent chromatin on *dg* repeats and the development of a fully functional centromere depend on RNAi (Fig. 1).

Role of RNAi, Chromatin Remodeling, DNA, and Histone Modification in Transposon Silencing and Inheritance

Z. Lippman, B. May, C. Yordan, R.A. Martienssen
[in collaboration with V. Colot, IGRV, Evry France]

The *Arabidopsis* gene *DDM1* is required to maintain DNA methylation levels and is responsible for transposon and transgene silencing. *DDM1* has similarity to the SWI/SNF family of adenosine triphosphate-dependent chromatin remodeling genes, and we have shown that *DDM1* is also required to maintain histone H3 methylation patterns. In wild-type heterochromatin, transposons and silent genes are associated with histone H3 methylated at Lys-9, whereas known genes are preferentially associated with methylated Lys-4. In *ddm1* heterochromatin, DNA methylation is lost, and methylation of Lys-9 is largely replaced by methylation of Lys-4. Because DNA methylation depends on histone H3 Lys-9 methylation, our results suggest that transposon methylation may be guided by histone H3 methylation, potentially accounting for the epigenetic inheritance of hypomethylated DNA once histone H3 methylation patterns are altered. Both DNA methylation and chromatin remodeling mutants strongly reactivated transposons, and in most cases, this correlated with a loss of H3mK9 methylation and a gain of methylation on histone H3 Lys-4. When these mutants were crossed to wild type, the transposons were inherited as dominant epialleles, retaining chromatin modifications characteristic of transcribed genes. In contrast, transposon activation in histone modification mutants was typically not inherited. Although RNAi mutants exhibited changes at a subset of the transposons analyzed, they did not reveal chromatin defects to the extent observed in other mutants. Thus, plants have redundant mechanisms that maintain heterochromatic silencing, which involve chromatin remodeling and DNA methylation. These mechanisms may be responsible for presetting the heritable alteration of transposon activation first described by B. McClintock in maize. We have found that strand-specific centromeric repeat transcripts accumulate in mutant but not wild-type *Arabidopsis*. Accumulation is not always accompanied by loss of DNA and/or histone methylation, which may indicate that only a subfraction of the repeats are transcribed.

Chromatin Profiling of the Chromosome 4 Heterochromatic Knob in Wild-type and *ddm1* *Arabidopsis*

Z. Lippman, M. Vaughn, R. Martienssen [in collaboration with V. Colot, IGRV, France, and W.R. McCombie and V. Mittal, Cold Spring Harbor Laboratory].

Arabidopsis chromosome 4 carries an interstitial heterochromatic knob on the short arm, which was completely sequenced by the CSHL/Washington University consortium during the *Arabidopsis* genome sequencing project. We have designed and printed a genomic tiling microarray that covers this knob and flanking euchromatic sequences. Our results show that transposons within the knob, as well as numerous hypothetical genes and some unique genes, are dramatically up-regulated in *ddm1* plants, indicating that *DDM1* has a global role in maintaining silent epigenetic states. Using chromatin immunoprecipitation (ChIP) and a genomic DNA fractionation method based on the methylation-sensitive enzyme McrBC, we have used these microarrays to show that DNA methylation and Lys-9 methylation of histone H3 are enriched at repetitive sequences in wild-type plants, but erased in *ddm1* mutants. We are presently expanding our array to cover all of chromosome IV.

Genes and Transposons Are Differentially Methylated in Plants but Not in Mammalian Cells

P.D. Rabinowicz, B. May, R. Martienssen [in collaboration with W.R. McCombie and S. Lowe, Cold Spring Harbor Laboratory]

In plants, transposable elemcnts are typically methylated, but methylation in genes is restricted to the 5′- and 3′-flanking regions. In animals, there are two alternative views concerning the role of DNA methylation. One suggests that DNA methylation silcnces transposons as it does in plants, and retrotransposon transcripts accumulate in methylation-defective mutant mice. The second view is that methylation targets both genes and repetitive DNA to decrease transcriptional "noise." Only promoters arc protected from methylation to allow gene expression. We have studied the methylation status of plant and animal genes using a sensitive, high-throughput PCR (polymerase chain reaction) method we call McrPCR. We have shown that most mammalian exons arc methylated while plant exons are not. Repetitive elements are methylated in both organisms. We spcculate that the mechanistic basis of this difference may involve RNAi, as mammalian genomes lack RdRP, which targets heterochromatin formation in yeast (see above).

Functional Genomics Using Transposon Populations in Maize and *Arabidopsis*

B. May, M. Vaughn, D. Roh, J. Simorowski, J.-M. Arroyo, R. Shen, A. Tang, U. Umamaheswari, R. Martienssen [in collaboration with W.R. McCombie and L. Stein, Cold Spring Harbor Laboratory]

MTM (maize-targetcd mutagcnesis) is an efficient system for site-selected transposon mutagenesis. As a scrvice to the maize community, MTM sclects insertions in genes of intcrest from a library of 45,000 plants using the PCR. Pedigree, knockout, sequence, phenotype, and other information arc storcd in a powerful interactive database (mtmDB) that enablcs analysis of the entirc population as well as handling knockout rcquests. By monitoring *Mutator* activity, we can demonstratc that seed phcnotypes depcnd on it. We conclude that morc than one half of all mutations arising in this population arc suppressed on losing *Mutator* activity. We also maintain and expand a collection of approximately 20,000 *Arabidopsis* gene-trap and enhancer-trap transposon lines, about half of which have been mapped to the genome by PCR and sequencing. The resulting knockouts, along with phenotypic and expression data, are made available to the public via TRAPPER, an interactive database and ordering system. More than 200 orders wcre filled for maize and *Arabidopsis* lines in 2002.

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PLANT CELL SIGNALING AND GENETICS

D. Jackson K. Aliano, U. Au, S. Aw, S. Bangaru, M. Cilia, W. Deleu, A. Giulini, L.A. Haller, J. Hsieh, R. Jain, J.Y. Kim, C. Kopec, A. Mohanty, N. Satoh, J. Wang, Z. Yuan

We are attempting to understand the genetic programming of plant morphology. This is a fundamental question in developmental biology, with obvious applications to crop yields. Specifically, we aim to define the molecular genetic pathways that control development. In recent years, we have been studying how cells signal each other to coordinate their development and fate. We are also developing new projects to extend what we learn from model systems to other cereal crop plants such as wheat, barley, and rice, to learn how developmental genes have contributed to the selection of these different crop species.

A Novel Assay for Intercellular Protein Trafficking

J.Y. Kim, J. Hsieh

We previously reported the specific intercellular trafficking of GFP (green fluorescent protein) fusions to the maize KNOTTED1 (KN1) protein, and to viral movement proteins (MP). These proteins presumably traffic through plasmodesmata (PD), small channels that connect plant cells, to control development and virus spread, respectively. Plasmodesmata are still very poorly characterized, and we envisage that a genetic approach will allow us to identify regulatory or structural components of these elusive channels. We therefore developed a novel intercellular trafficking assay system using trichomes (hairs) on the *Arabidopsis* leaf as a visual output. The *GLABROUS1* (*GL1*) gene, which encodes a *MYB*-related transcription factor, is normally expressed in epidermal cells that will make trichomes. When we expressed GL1 in mesophyll cells in *gl1* mutant plants, these plants failed to make trichomes, presumably because GL1 protein was in the wrong cell type. However, fusions of GL1 to KN1 were able to complement trichome production, because the fusion protein could traffic from mesophyll to epidermal cells and initiate trichomes. This provided us with a sensitive screen to define regions of KN1 involved in intercellular targeting, and we have also initiated screens to identify other loci required for trafficking of KN1. These screens should identify genes required for intercellular targeting of KN1.

Genetic Screens for Protein Trafficking Mutants

M. Cilia, S. Aw, K. Aliano

Cytoplasmic GFP can freely diffuse between plant cells in the *Arabidopsis* epidermis when driven by the *Glabra2* promoter (Fig. 1A), and unloads out of the phloem into the root tip when driven by the Sucrose H^+ symporter promoter (Fig. 1C), because it is smaller than the size exclusion limit of the plasmodesmata on these tissue boundaries. In contrast, the endoplasmic reticulum (ER)-localized version of GFP driven by the *Glabra2* (Fig. 1B) or Sucrose H^+ symporter (Fig. 1D) promoters does not move between cells, presumably because the ER lumen in the plasmodesmata becomes appressed during formation of the pore. We designed genetic screens for mutants defective in regulating the size exclusion limit of the plasmodesmata using transgenic *Arabidopsis* lines expressing GFP under the control of the *Glabra2* or Sucrose H^+ symporter promoters. We have identified several putative mutants that do not permit the intercellular trafficking of cytoplasmic GFP. Preliminary analysis of the mutants indicates that regulation of the plasmodesmata size exclusion limit may be essential for development, because these mutants display a range of developmental phenotypes. We are presently making mapping populations with the goal of isolating these genes that regulate plasmodesmata permeability. The mutants will also be characterized for other related phenotypes such as plasmodesmata structure and viral transport.

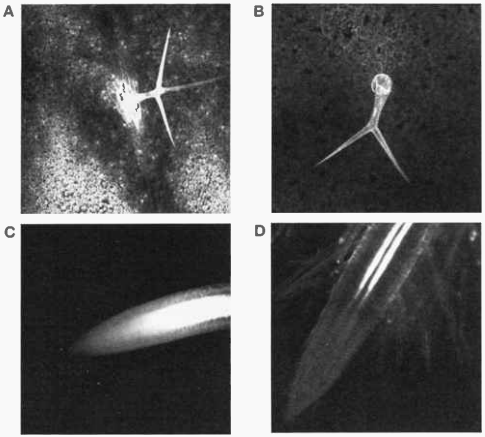

FIGURE 1 Cytoplasmic GFP can freely diffuse between plant cells in *Arabidopsis* epidermis when driven by the *Glabra2* promoter (*A*), and unload out of the phloem into the root tip when driven by the *Sucrose H⁺ symporter* promoter (*C*) because GFP is smaller than the size exclusion limit of the plasmodesmata on these tissue boundaries. An ER-localized version of GFP does not move when driven by the *Glabra2* (*B*) and *Sucrose H⁺ symporter* (*D*) promoters because the ER in the plasmodesmata is oppressed. We designed a genetic screen for mutants defective in regulating the size exclusion limit of the plasmodesmata using transgenic *Arabidopsis* lines expressing the cytoplasmic version of GFP under the control of the *Glabra2* and *Sucrose H⁺ symporter* promoters. We identified several putative mutants that do not permit the intercellular trafficking of cytoplasmic GFP. Preliminary analysis of the mutants indicates that regulation of the plasmodesmal size exclusion limit is essential for development.

A Pilot Screen to Localize Proteins of Unknown Function in *Arabidopsis*

C. Kopec, A. Mohanty

The recent completion of the *Arabidopsis* genome sequence delivered a catalog of approximately 25,000 genes that function together to form a higher plant. However, approximately one third of these genes have no known function in plants or other systems. As an initial approach to characterize these "genes of unknown function," we plan to localize their encoded protein products using fusions to the yellow fluorescent protein (YFP). We developed a high-throughput overlap polymerase chain reaction (PCR) procedure to fuse the YFP-coding sequence at our chosen site within any gene-coding sequence. The fusions will be transformed into *Arabidopsis* plants for analysis of tissue-specific and subcellular localization of the protein under its native regulatory sequences. This project should discover novel developmental and subcellular domains and may identify proteins that localize to known plant structures for which we currently do not have molecular components, such as plasmodesmata.

Microarray Analysis of Maize Inflorescence Development

N. Satoh [in collaboration with V. Brendel, University of Iowa, and S. Hake, Plant Gene Expression Center, Albany, California]

The maize inflorescence is a complex developmental system, involving the progression of meristems through several distinctive states with different characteristics and potentials. Although several genes involved in these progressions have been characterized already, the recent availability of maize expressed sequence tag (EST) and genomic sequence collections permits a broader survey of developmental genes. With assistance from Rob Lucito and the CSHL microarray facility, we constructed a pilot oligonucleotide array and probed it with cDNAs from developing inflorescences and leaves. The initial results indicate that we can detect many of the known inflorescence developmental transcripts on our pilot array, and so we plan to proceed to the discovery phase by making arrays of all known maize genes. These arrays will be probed with cDNAs prepared from different inflorescence stages as well as from mutants that

arrest development at defined points. We will test novel candidate developmental genes using the maize targeted mutagenesis system here at CSHL.

High-resolution QTL Analysis of Seed Row Number in Maize

D. Jackson, U. Au, L.A. Haller, N. Satoh [in collaboration with T. Rocheford, University of Illinois, and E. Buckler, North Carolina State University]

A major factor in the human selection of maize as a crop was a huge increase in the number of seeds produced per plant. This was achieved by a combination of factors, but one major factor was a selection for an increase in the number of seed rows on the ear. For example, teosinte, the wild ancestor of maize, has only a single row of seeds, whereas modern maize cultivars have 12 to 18 rows. We have measured inflorescence meristem size in a range of maize cultivars and found a strong positive correlation with seed row number. This indicates that regulation of meristem size is an important factor in seed row number. Evidence that the *fasciated ear2* (*fea2*) gene may be important in this process has come from two independent lines of research. *fea2* mutants have unregulated meristem growth, and we found an association between *fea2* sequence haplotypes and seed row number in a diverse collection of maize germ plasm.

Our interest was also sparked by the observation that *fea2* maps close to a quantitative trait locus (QTL) for row number. In the past, QTL analysis in maize has been a relatively imprecise method to identify genomic regions controlling a particular trait, with relatively low resolution. A new mapping population, the integrated B73-Mo17 (IBM) population, has now been developed and is ideal for the fine mapping of quantitative traits. We have used this resource by measuring seed row number in the different IBM families. Our preliminary QTL analysis indicates that row number is under the control of a small number of loci, some of which map to previously identified row number QTL and some of which are novel. Of particular interest, one of the QTLs maps to the same region as *fea2*, and now with much higher resolution than shown in previous studies. Therefore, *fea2* is an excellent candidate for this QTL, and we will test this directly by a transgenic approach.

A Protein Complex Involved in Restriction of Stem Cell Proliferation

J. Wang, W. Deleu

The *fea2* gene has a role in restriction of stem cell proliferation; *fea2* mutants have massive inflorescence apical meristems due to a mis-specification of cell fate in the meristem tissues. *fea2* encodes a leucine-rich repeat receptor-like protein, and to ask what other proteins might be involved in this pathway, we generated a polyclonal antibody to a carboxy-terminal FEA2 peptide. We detected FEA2 protein in membrane-enriched fractions, and it runs slightly larger than its predicted size due to glycosylation. By gel filtration chromatography, we found that FEA2 is part of a complex of approximately 450 kD, and we are attempting to purify this complex. We will also ask if other fasciated mutants that we have characterized encode components of the FEA2 complex.

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PLANT DEVELOPMENTAL GENETICS

M. Timmermans T. Phelps-Durr J. Thomas
M. Juarez E. Wurtmann

Plants have the unique property to initiate new organs, such as leaves and flowers, throughout their lifetime, which frequently extends over many years. The growing tips of plants are called meristems and consist of a small group of indeterminate stem cells. These cells divide to maintain the stem cell population and indeterminate growth of the plant and to differentiate into determinate founder cells from which lateral organs arise. Coincident with their initiation, lateral organs are patterned along three axes: (1) the proximodistal axis, which leads to the differentiation of distinct tissues and cell types at the base and the tip of the developing organ; (2) the dorsoventral axis, leading to distinct cell fates in the upper and lower surfaces of the organ; and (3) the mediolateral axis, which distinguishes central and marginal domains of the organ.

Early surgical experiments suggested that signals from the meristem have an important role in the patterning of lateral organs. However, the genes involved in the initiation and patterning of lateral organs in plants are still largely unknown. We are using forward and reverse genetic approaches to generate mutants in maize and *Arabidopsis* that affect these processes. In particular, we are studying the repression of stem cell fate during organ initiation and growth, and we are analyzing the genetic pathways leading to dorsoventral polarity in leaves and leaf-like lateral organs. Variations in the expression patterns of such genes are likely to contribute to the diversity of leaf shapes among many plant species.

REPRESSION OF STEM CELL FATE DURING ORGAN DEVELOPMENT

The mechanism that distinguishes stem cells from organ founder cells in the shoot apical meristem (SAM) is largely unknown, but the regulation of *knotted1*-like homeobox (*Knox*) gene expression appears to be one of the key determinants. *Knox* genes are expressed in the meristem and are required for indeterminate growth, i.e., stem cell fate. Initiation of determinate lateral organs such as leaves is correlated with the down-regulation of *Knox* gene expression in a subset of cells within the meristem, the organ founder cells. We have previously shown that the *rough sheath2* (*Rs2*) gene from maize encodes a MYB-domain protein that acts as a negative regulator of *Knox* gene expression. Recessive mutations in *rs2* cause the misexpression of KNOX proteins in developing leaf primordia. Interestingly, KNOX protein accumulation in leaves of *rs2* null mutants occurs in just a subset of cells that express *Rs2* in wild type. KNOX proteins accumulate in patches with sharp lateral boundaries, suggesting that *rs2* leaves are clonal mosaics of $Knox^{+}$ and $Knox^{-}$ sectors. The numbers and sizes of such sectors vary among leaves and do not correlate with normal developmental domains. Furthermore, the down-regulation of *Knox* gene expression during leaf initiation precedes the onset of *Rs2* expression, suggesting that *Rs2* is required for maintenance of *Knox* gene repression. On the basis of these observations, we proposed that *Rs2* acts as an epigenetic regulator to keep *Knox* genes in an "off" state. *Rs2* thus prevents differentiated cells within lateral organs from reverting into indeterminate stem cells.

We are currently studying the mechanism by which *Rs2* represses *Knox* gene expression. Using a yeast 2-hybrid screen and glutathione *S*-transferase pull-down assays, we have identified seven RS2 interacting proteins (RS2-IPs). These have homology with the protein phosphatase 2A regulatory subunit A (PP2A-A), PP2A regulatory subunit B′ (PP2A-B′), a zinc finger transcription factor, ASYMMETRIC LEAVES2 (AS2) from *Arabidopsis*, a highly conserved hypothetical protein, histone 2B (H2B), and a known nucleosome assembly protein. Several of these proteins are known to function in the nucleus, consistent with a role in transcriptional regulation. We are testing the subcellular localization of the remaining RS2-IPs by transiently expressing chimeric proteins consisting of the RS2-IP fused to green fluorescent protein (GFP) in leaf tissue. Both the PP2A regulatory subunits were found to be localized in the nucleus. Interestingly, the fusion protein between GFP and the RS2-IP with unknown function colocalized with chromosomes in dividing cells, suggesting that it is associated with chromatin. This result together with the confirmed interaction

between RS2, H2B, and the nucleosome assembly factor suggested that the RS2 complex maintains *Knox* genes silenced by altering the organization of chromatin at these loci. Actively transcribed genes usually have a more open chromatin structure, whereas chromatin at silenced genes is densely packed. Nucleosome density can be determined using the enzyme micrococcal nuclease, which cuts DNA once between adjacent nucleosomes. We are analyzing the chromatin organization at the *Knox* loci in *Arabidopsis*. The *ASYMMETRIC LEAVES1* (*AS1*) gene is the *Rs2* ortholog and functions in the repression of several *Knox* genes during *Arabidopsis* leaf development. We have found that the nucleosome density at the promoter of one of the *Knox* genes, *KNAT1*, is indeed higher in wild-type leaves than in *as1* mutant leaves.

To determine the roles of the RS2-IPs in plant development, particularly in stem cell function, we are using reverse genetic resources available in *Arabidopsis*. Loss-of-function mutations in AS2 are known to affect *Knox* gene silencing during leaf development, but our results indicate that AS2 acts in the same complex as AS1/RS2. We have isolated mutations in *Arabidopsis* orthologs of the unknown protein and the nucleosome assembly protein. Loss-of-function mutants in the unknown protein are indistinguishable from wild type, but loss of the nucleosome assembly protein causes embryo lethality. Using an RNA interference (RNAi)-like approach, we have generated transgenic plants that have reduced levels of the nucleosome assembly protein. These transgenic plants have defects in leaf and floral development that resemble the defects observed in the *as1* and *as2* mutants. The sepals, petals, and stamens of the flower are reduced in size, such that the carpel is prematurely exposed. Leaves of these transgenic plants are asymmetrically lobed, and like *as1* and *as2*, they misexpress three of the four *Arabidopsis Knox* genes. AS2 expression is unaffected and AS1 transcript levels are increased in these mutants, indicating that the defects in these transgenic plants do not result from loss of AS1 or AS2. However, loss of the nucleosome assembly protein in these transgenic plants affects the chromatin organization at the *Knox* loci in that the density of nucleosomes is reduced.

Taken together, our observations suggest that AS1/RS2 and AS2 assemble into a protein complex which targets a nucleosome assembly factor to the *Knox* loci in organ founder cells. This nucleosome assembly factor establishes a condensed, silenced chromatin state at the *Knox* loci. As a result, stem cell fate remains repressed in determinate, differentiating cells of developing lateral organs.

DORSOVENTRAL PATTERNING OF LATERAL ORGANS IN MAIZE

Normal maize leaves develop as flattened dorsoventral organs with distinct cell types on the upper/dorsal and lower/ventral sides. We previously reported that *Leafbladeless1* (*Lbl1*) is required for the specification of dorsal identity in leaves and leaf-like lateral organs and that dorsoventral polarity in the developing organ is needed to grow out laterally into a flattened leaf. Loss of *Lbl1* gene function results in the formation of ventralized, radially symmetric leaves, or in ectopic laminar outgrowth surrounding ventralized sectors on the dorsal leaf surface. We have identified several additional loci required for dorsoventral patterning. Recessive mutations in two of these loci result in phenotypes resembling *lbl1*, indicating that these genes also function in the specification of dorsal cell fate. The *Rolled leaf1-Original* (*Rld1-O*) mutant is semidominant and is characterized by a curled leaf phenotype in which the leaf blades roll up toward the midvein. This phenotype results from a partial inversion in dorsoventral polarity. Cell types that are normally confined to the dorsal epidermis are ectopically expressed in the ventral epidermis of the mutant, and vice versa. To dissect the genetic pathway(s) leading to dorsoventral polarity, we are analyzing the double-mutant interactions between these dorsoventral patterning mutants. The vegetative phenotypes of *lbl1 Rld1-O* double mutants are much less severe than those observed in either single mutant. Depending on the environmental conditions, double-mutant leaves frequently appear normal. This mutually suppressive interaction indicates that RLD1 and LBL1 either have opposing functions or regulate each other.

We are also characterizing maize homologs of several *Arabidopsis* genes that are expressed specifically in either the dorsal or ventral domains of the leaf. The *Arabidopsis YABBY* genes act in the ventral domain of lateral organs and are required for the specification of ventral cell types in the leaf. As in *Arabidopsis*, the maize *Yabby* genes are expressed in the incipient primordium, but interestingly, expression is restricted to the dorsal side of the leaf. Moreover, *Yabby* expression is restricted to the margins of young leaf primordia. This expression pattern is altered in *lbl1* and *Rld1-O* mutants. The overall level of expression is reduced in

lbl1, and the narrow leaf primordia show no *Yabby* expression in the mutant margins. Weak *lbl1* mutants frequently develop ectopic blade outgrowths on the dorsal side of the leaf. The *Yabby* genes are expressed in these outgrowths. In *Rld1-O*, the level of expression of the *Yabby* genes is increased and expression is prolonged, but it remains dorsally localized. In contrast to *lbl1*, *Rld1-O* mutant leaves develop ectopic outgrowths on the ventral leaf surface. The *Yabby* genes are also expressed in these ventral ectopic outgrowths. These results suggest that the *Yabby* genes act downstream from LBL1 and RLD1. The *Yabby* genes may mediate dorsoventral polarity signals to direct lateral growth at the leaf margins. The establishment of *Yabby* expressing cells next to nonexpressing cells could be required for lateral growth. If so, there would be no evolutionary constraints to limit *Yabby* expression to, for instance, the ventral side of the leaf. Alternatively, the maize *Yabby* genes function in dorsoventral patterning. If so, their role and upstream regulators have diverged between maize and *Arabidopsis*, or the pathway for dorsoventral axis specification is conserved but inverted between *Arabidopsis* and maize.

We recently cloned the *Rld1* gene. It encodes a member of the class III homeodomain-leucine zipper (HD-ZIP III) family of proteins, which are known to function in dorsoventral patterning in *Arabidopsis*. In addition to the DNA-binding domains, HD-ZIP III proteins contain a START lipid-sterol binding-like domain, suggesting that they may function as nuclear receptors. Sequence homology suggests that expression of *Rld1* may be regulated at the posttranscriptional level by microRNAs (miRNAs). miRNAs are 20–24-nucleotide RNA molecules that negatively regulate expression of mRNAs which contain a complementary sequence. Both *Arabidopsis* and rice were shown to produce miRNAs that specifically regulate the HD-ZIP III family members by an RNAi-related mechanism. The miRNA complementary site is conserved in the maize *Rld1* gene, and all three dominant *Rld1* mutations result from a single-nucleotide change in this complementary site. This suggests that the dominant *Rld1-O* mutant phenotype results from misexpression of *Rld1*. Mosaic analysis has shown that

FIGURE 1 Model for the specification of dorsoventrality in maize leaves.

expression of the *Rld1-O* mutant allele in just the ventral epidermis is sufficient to invert the polarity of the leaf. This suggests that the miRNA normally represses *Rld1* expression on the ventral side of the leaf such that *Rld1* functions on the dorsal side of the leaf.

These results can be reconciled with the model for dorsoventral patterning shown in Figure 1. The pattern of expression, at the margins and in ectopic outgrowths, is consistent with a role for the *Yabby* genes in lateral outgrowth. In addition, *lbl1* leaves frequently fail to grow out laterally, which could result from the loss of *Yabby* expression. *Lbl1* is also required for dorsal cell specification, and this function could in part be mediated via the *Yabby* genes, which are expressed dorsally and act downstream from *Lbl1*. *Rld1* expression is limited to the dorsal domain due to the posttranscriptional negative regulation by an miRNA in the ventral domain. The *Rld1-O* mutation may lead to increased RLD1 activity in both the dorsal and ventral domains. This would result in increased LBL1 activity and *Yabby* expression. This model can thus explain the double-mutant data that *Rld1-O* suppresses *lbl1*, and the observed increased *Yabby* expression in *Rld1-O*. RLD1 activity in the ventral domain of *Rld1-O* mutant leaves could cause ectopic *Lbl1* function and dorsalization of the leaf. Consistent with this, the *Rld1-O lbl1* double-mutant analysis showed that *Rld1-O* requires *Lbl1* in order to affect dorsoventral polarity.

PUBLICATIONS

Kidner C., Timmermans M., Byrne M., and Martienssen R. 2002. Developmental genetics of the angiosperm leaf. *Adv. Bot. Res.* **38:** 192–234.

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 up to 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 interaction 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 (1987) and Scott Lowe (1995) are currently members of the faculty at the Laboratory. After 9 years at the Laboratory, Carol Greider (1988) left to join the Department of Molecular Biology and Genetics at Johns Hopkins University School of Medicine. Eric Richards (1989) is currently in the Department of Biology at Washington University. After finishing his fellowship, David Barford (1991) returned to the Laboratory of Molecular Biophysics at Oxford University. Ueli Grossniklaus (1994) was a member of our faculty before leaving to join the Friedrich Miescher Institut in Basel, Switzerland in 1998. Marja Timmermans, who joined us from Yale in 1998, ended her fellowship in June of this year, when she became an assistant professor at the Laboratory.

The two current CSHL Fellows, Gilbert (Lee) Henry and Terence Strick, joined the Laboratory in 2000. 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 taste bud development and the role of innervation in this process. Terence joined us after earning his Ph.D. in molecular and cellular biology at École Normale Supérieure in Paris with David Bensimon and Vincent Croquette. Terence is using single-molecule biophysics to study the mechanical response of DNA to stretching and twisting by enzymes that alter DNA topology, thus elucidating the properties of these enzymes.

L. Henry
T. Strick

Structural and Functional Studies of the Vertebrate Taste Bud

L. Henry, Y. Zhu

The goal of our research is to understand the molecular mechanisms that are required for the formation of taste buds during embryogenesis and the maintenance of their structure and function in the adult. The sensory cells of gustation are housed within ovoid structures called taste buds, which are embedded in the epithelium of the tongue, and to a lesser extent the epithelium of the palate and upper pharynx. Consisting of 70–100 cells, the vertebrate taste bud is a highly dynamic structure that possesses both epithelial and neuronal qualities. Like all other sensory cell types, the taste receptor cell membrane depolarizes in the presence of a suitable stimulus. Similar to the epithelial cells that line the intestine and other areas of the gut, cells within the bud turn over at a rapid rate (~8–10 days in rodents). Unlike the olfactory system, where the axons of newly formed receptor cells project for some distance back toward and synapse on to neurons in the glomeruli of the olfactory bulb, newly formed taste receptor cells lack classical projections and synapse on to sensory afferents that are associated with the taste bud.

Mammalian taste buds are embedded in epithelial specializations called papillae. On the surface of the tongue, three classes of papillae are easily distinguished. Toward the back of the tongue, there is a centrally located circumvallate papilla, two groups of foliate papillae are found to the lateral extremes of the

posterior tongue, and scattered over the anterior tongue are reddish colored fungiform papillae. The papillae form late during embryogenesis in the mouse (E13–E16) as the tongue begins to bud away from the pharynx. Soon after their formation, sensory afferents enter the papillae, and approximately 8–10 days after birth (P8–P10), taste buds form. In rodents, there is a single bud per fungiform papilla, tens of buds within the two foliate papillae, and hundreds of buds are found in the single circumvallate.

An intimate relationship between both the developing papillae and the mature taste bud with innervating sensory afferents has been established through a number of denervation studies. Papilla formation does not require innervation; however, the maintenance of these structures does in some way require contact with sensory afferents. Similarly, in adult animals, denervation of the tongue leads to the loss of taste buds. We are currently trying to understand the molecular basis for this neuroepithelial interaction. However, before regulatory mechanisms can be understood, it is first necessary to understand exactly what is being regulated. Toward this end, a primary aim of our current work is to understand, in molecular detail, how many different types of cells reside in the developing papilla and adult taste bud. What, if any, lineage relationships exist among these cell types? Is the distribution of the different cell types that reside in these structures spatially patterned?

DEVELOPMENT OF A QUANTITATIVE METHOD FOR THE ANALYSIS OF mRNA IN SINGLE CELLS?

A prerequisite for much of our work is the development of a quantitative method for the analysis of mRNA expression in single cells or small numbers of cells. A typical eukaryotic cell contains picogram amounts of mRNA, whereas the vast majority of the analytical procedures that we use to analyze gene expression require microgram amounts of material. Thus, the mRNA expressed in a single cell must be amplified before it can be studied. We have developed a novel scheme for performing such amplifications. In this technique, mRNA is bound to a magnetic bead, covalently coupled cDNA is produced, and after a series of molecular manipulations, the cDNA is released and amplified by either polymerase chain reaction (PCR) or in vitro transcription. The advantage of performing such manipulations in the solid phase is that precipitation and chromatographic steps are not required between manipulations, which allows the user a great deal of flexibility in modifying the cDNA so that it can be amplified. We have successfully amplified mRNA from single adult taste receptor cells and taste buds. Additionally, we are using microarray technology to assess the quantitative abilities of our procedure. These experiments simply involve comparing the expression profiles of diluted and amplified material versus undiluted material.

HOW DIVERSE ARE BOTH THE MATURE RECEPTOR CELL POPULATION AND THE PROGENITOR POOL FROM WHICH IT IS DERIVED?

Our goal is to develop a molecular fingerprint for each of the different cell types that reside in the adult taste bud. By fingerprint, we essentially mean a transcriptional profile. From the perspective of cell division, there are two types of cells within the adult taste bud: mitotically active progenitor cells at the base of the bud and differentiated quiescent cells at the apex. Mature taste receptor cells fall into the later category. In addition, the differentiated cells of the taste bud are highly elongated as compared to the progenitors and can release the neurotransmitter onto the sensory afferent network with which they form multiple synapses. These properties allow basal cells to be distinguished from their mature, apically located, daughter cells by the presence of mRNA transcripts that encode proteins which support the aforementioned processes. For example, there are a small number of genes known that are only expressed in dividing cells, and the production of certain neurotransmitters requires biosynthetic enzymes that are only expressed by the cells that make and release the transmitter. Altogether, we have isolated just more than 100 cDNA clones that can be used to pre-sort cells. Each of these clones has been spotted onto a low-density microarray which is hybridized to amplified and labeled cDNA derived from single cells. This pre-sorting hybridization allows progenitor and mature cells to be distinguished, as well as bitter-, sweet-, sour-, and salt-responsive cells.

HOW MANY TYPES OF CELLS ARE CONTAINED WITHIN THE DEVELOPING PAPILLAE?

To understand how papillae are formed and innervated, it is imperative that we first ask what are papillae, at the molecular level? The papillae of the tongue are very similar in structure to the various ectodermal placodes that cover the epidermis and give rise to hair, feathers,

and teeth. The secreted signaling factor sonic hedgehog (*shh*) is expressed in cells that will form these structures, and that expression is maintained after the papillae or placode has formed. We plan to exploit this finding by using the *shh* locus to ectopically express green fluorescent protein (GFP) in developing papillae. Bacterial artificial chromosome (BAC) clones carrying the *shh* gene have been isolated, and using a recently described recombination technique, a GFP cassette has been inserted into this locus. Transgenic mice will be generated using the modified BACs. Single GFP-positive cells from the lingual epithelium of E12–E17 embryos will be isolated and transcriptionally profiled using a single-cell cDNA synthesis method. Profiles will be obtained by screening microarrays and direct sequencing of cDNAs. From this work, we hope to determine the molecular diversity of the papilla during its formation and innervation. Once we can "fingerprint" the cells of the papillae in this manner, it should be possible to analyze the mechanisms that regulate this diversity, in particular, the contribution of innervation to the development of papillae.

Single-molecule DNA Analysis

T.R. Strick

We have been using single-molecule techniques to manipulate and study the structure and topology of DNA in processes ranging from transcription to packaging. We are particularly interested in the role played by DNA supercoiling. In our experiments, a single, linear 4-kb DNA molecule is tethered to a glass surface at one end and to a micron-sized magnetic bead at the other. Using a magnetic field to manipulate the bead, one can mechanically stretch and twist the molecule and thus reproduce the mechanical constraints it encounters in vivo (such as negative supercoiling). Determining the bead position gives the DNA end-to-end extension, which can be used to detect structural fluctuations along the double helix, as well as protein-DNA interactions in real time. These methods yield important new mechanistic insights into the molecular motors (such as polymerases and topoisomerases) which access the genome and maintain its integrity. At the same time, they also shed light on the nature of DNA structural transitions and their role in regulating gene activity. In this report, we briefly describe our use of DNA micromanipulation to observe spontaneous denaturation fluctuations in negatively supercoiled DNA. We also discuss the real-time measurement of the interaction between a single RNA polymerase and its promoter site. Finally, we consider the use of this technique to study the SMC (structural maintenance of chromosome) proteins responsible for compacting DNA into mitotic chromosomes.

The real-time observation of protein-DNA interactions involved in gene regulation and compaction is made possible by the detection and manipulation of single molecules. Continuous monitoring of a single pair of interacting molecules, such as bacterial RNA polymerase and DNA, gives a time course of their reaction. From this, the time scales of interactions can be measured between the protein and its DNA substrate and a quantitative analysis of association and dissociation rates can be performed. In addition, micromanipulation of the DNA substrate provides a way to study structural and mechanical features of the interaction, such as twist and bend deformations in the DNA that result from protein binding. Finally, the mechanical control of DNA topology enables us to change and measure DNA supercoiling in real-time, and to better understand the fundamental role of DNA topology in interactions with proteins. Here, we describe recent advances made in the study of structural fluctuations in DNA, transcription initiation by RNA polymerase, and the compaction of DNA by proteins belonging to the condensin family.

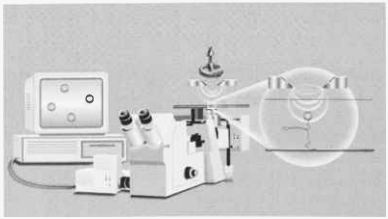

FIGURE 1 Depiction of the experimental setup. (*Inset*) DNA is tethered at one end to the wall of a glass capillary tube and at the other end to a magnetic bead. (*Overview*) The capillary tube is mounted on an inverted microscope whose focus is controlled by a computer. The computer also controls the displacements (translation and rotation) of the magnets used to manipulate the bead. A CCD camera connected to the microscope relays video images of the magnetic bead to the computer. The computer extracts from these images the mean position and the Brownian fluctuations of the bead, which can be used to determine the DNA's end-to-end extension *l* which results from an applied stretching force *F* and a supercoiling of *n* turns.

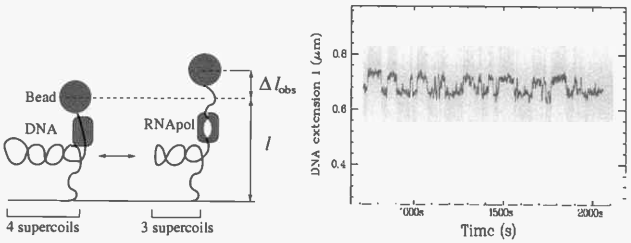

FIGURE 2 Observation of promoter melting by a single RNA polymerase. (*Left*) Sketch of the experiment. DNA containing a single bacterial promoter site is negatively supercoiled, causing it to form supercoiled loops and reducing the end-to-end extension. When RNA polymerase melts the promoter site, topological coupling in the DNA causes a supercoil to disappear and the extension increases. When the promoter site reanneals, the supercoil reappears and the extension decreases. (*Right*) In the presence of RNA polymerase, the DNA extension does indeed fluctuate between a low-extension and a high-extension position.

The single-molecule experiment we have implemented is depicted Figure 1. A 4-kb linear DNA molecule is shown anchored at one end to a glass surface and at the other end to a 1-μm-diameter magnetic bead. The field generated by magnets located above the sample is used to pull on and rotate the magnetic bead, thus stretching and twisting the tethered DNA. The stretching force applied to the DNA via the bead is calibrated as a function of magnet position (the closer the magnets, the higher the force). The torsion imparted to the DNA is exactly equal to the number of clockwise or counterclockwise rotations performed by the magnets and imposed on the bead. The double helix is thus quantitatively and reversibly supercoiled while held under tension. By determining the position of the magnetic bead, one measures and calibrates the end-to-end extension of the DNA molecule, and thus its mechanical response to stretching and twisting.

Observation of Denaturation Fluctuations in Unwound DNA

R. Sachidanandam, T. Strick

We have been studying spontaneous denaturation fluctuations in negatively supercoiled DNA. Such fluctuations could have a role in the initiation of DNA transcription and replication, which occurs at AT-rich sequences that are prone to unwinding. When we unwind a single DNA molecule, real-time monitoring of its end-to-end extension reveals large-scale fluctuations in DNA extension. This occurs for degrees of supercoiling for which DNA is expected to denature ($\sigma \sim -0.025$). These fluctuations do not appear on overwound DNA, suggesting that they are due to loss of regular B-DNA structure and the formation of alternative secondary structures (such as denaturation bubbles or cruciforms). As with the folding/unfolding transitions in RNA hairpins and protein domains observed by other groups in recent years, these fluctuations obey Poisson statistics and are well-described by a simple two-state model where thermal activation provides the energy for the system to flip between a "native" and an "unwound" state. The quantitative study of how DNA torsion affects its unwinding allows us to better understand the role of DNA topology in protein-DNA interactions.

Real-time Study of Transcription Initiation

A. Revyakin, R.H. Ebright, T. Strick

In the past year, we have made substantial progress in our efforts to observe promoter melting by RNA polymerase at the single-molecule level. One goal of these experiments is to determine how the topological state of the DNA molecule affects transcription initiation, a

fundamental process in the regulation of gene expression. A sketch of single-molecule detection of promoter melting is provided Figure 2.

For these experiments, we prepared a 4-kb DNA molecule containing a unique bacterial promoter site. Upon addition of RNA polymerase holoenzyme, we are able to observe fluctuations in the DNA end-to-end extension (see Fig. 2). Since these fluctuations are not observed if the DNA does not contain the promoter site, they must be due to interactions between an RNA polymerase and the promoter site. By measuring the amplitude of these fluctuations, we can estimate the extent of DNA untwisting that takes place at promoter melting, as well as observe a subtle compaction effect presumably due to association of the melted DNA with the surface of the RNA polymerase.

Since these observations are performed in real-time, we are also able to study the dynamics of formation and disappearance of this promoter-open complex known as RPo. Indeed, the high-extension state of the DNA is consistent with the protein-induced formation of an unwound region of DNA at the promoter site. Promoter melting and reannealing display single-exponential dynamics. As expected for a process where the rate-dependent step is affected by mechanical constraints on the DNA substrate, negative DNA supercoiling causes both the rate of promoter melting and the lifetime of melted complexes to increase. The propensity of different promoter sequences to melt can be studied in this manner, as can the role of activators such as CAP protein. By adding all four nucleotides, we confirm that these promoter-open complexes are transcriptionally competent. In this case, we observe the accumulation of several unwound bubbles on the DNA, as the same promoter is successively used by RNA polymerases to initiate transcription. This work shows that DNA micromanipulation is a robust methodology for the study of a broad range of protein-DNA interactions, and in particular those involved in transcription initiation. In principle, processes such as the origin of replication could also be studied using this technique. In the future, we will combine this micromanipulation technique with single-molecule fluorescence detection in order to measure protein conformational changes in parallel with our measurements on DNA topology.

DNA Compaction by SMC Proteins

T. Kawaguchi, M. Hirano, T. Hirano, T. Strick

A third project we have been pursuing involves the study of DNA compaction by the SMC proteins such as *Bacillus subtilis* SMC and *Xenopus laevis* condensin complex. Using DNA micromanipulation, we are able to observe progressive compaction of the molecule upon addition of purified *X. laevis* condensin and ATP. Discrete and transient changes in DNA extension are observed at low protein concentration and correspond to the association and dissociation of single condensin complexes from DNA. From these experiments, we can estimate the step-size of DNA compaction as well as the dynamics of the process. We are currently working to understand the role of DNA supercoiling in this compaction.

In Press

Revyakin A., Ebright R.H., Allemand J.-F., Croquette V., and Strick T.R. 2003. Single-molecule DNA nanomanipulation: Detection of promoter melting events by RNA polymerase. *Methods Enzymol.* (in press).

AUTHOR INDEX

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WATSON SCHOOL OF BIOLOGICAL SCIENCES DEAN'S REPORT

Just four years ago, in September 1998, Cold Spring Harbor Laboratory received accreditation from New York State to grant the Ph.D. degree and the Watson School of Biological Sciences was founded. In August of this year, the fourth class of Watson School students matriculated, bringing a full cohort of students for the Watson School's four-year Ph.D. program. The School now has 26 students from across the country and around the world. Indeed, except for Antarctica, all of the continents are represented in our student body! The international flavor of the student body reflects the long-standing tradition of Cold Spring Harbor Laboratory as an international center of excellence in the biological sciences.

These first years of the Watson School have been exciting indeed. Its innovative doctoral curriculum has taken hold and is rapidly achieving success owing in large part to the high quality of the students and to the dedication of students and faculty alike to the success of the program. Other established programs under the auspices of the Watson School—the Nature Study Program for young children, the Partners for the Future high-school student program, and summer Undergraduate Research Program—continued to flourish. This year, however, the School could begin to cast its attention beyond the doctoral and established programs and focus as well on establishing a program for postdoctoral research and education.

A Postdoctoral Program

Across our nation, postdoctoral education in the biomedical sciences is receiving much well-deserved attention. Cold Spring Harbor Laboratory is no exception. This year saw important progress in this regard. A postdoctoral working group, chaired by Lilian Gann and consisting of faculty (Zachary Mainen, Nicholas Tonks, and Linda Van Aelst), postdoctoral fellows (Frances Hannon, Alyson Kass-Eisler, Michael Packer, and Karen Zito), and the Laboratory Research Administrator Denise Roberts, completed its mission by summarizing the results of a survey of postdoctoral fellows and, together with me, prepared a series of recommendations to the Laboratory Director, Bruce Stillman, to enhance the postdoctoral experience at the Laboratory. Bruce Stillman was enthusiastic about the set of recommendations and he invited the entire postdoctoral community for an open discussion on the postdoctoral experience at the Laboratory. This meeting, in which there was a free-flowing exchange of ideas and opinions, represented the first of what will become an annual event, sponsored by the Watson School, to promote communication between the postdoctoral community and the Director.

A Postdoctoral Program Office

A major result of the postdoctoral working group's efforts and recommendations will be the establishment in 2003 of a formal postdoctoral program guided by Nicholas Tonks and Associate Dean Lilian Gann. With Bruce Stillman's encouragement and financial support, a postdoctoral program office will be established with the appointment of Alyson Kass-Eisler as postdoctoral program officer. As one of the postdoctoral representatives in the postdoctoral working group, Alyson has a firsthand awareness of the complex issues pertaining to postdoctoral education and success. And of course it is pleasing that she is no stranger to the Laboratory, as she was a postdoctoral fellow here with Carol Greider. I believe that these continued efforts and new resources for postdoctoral education will serve to improve the Laboratory's postdoctoral experience, which will aid in the recruitment of the best postdoctoral fellows to the Laboratory, who continue to represent the largest element of the Laboratory's research.

Postdoctoral program office members Lilian Gann, Nicholas Tonks, and Alyson Kass-Eisler

The Curriculum

The Watson School's Ph.D. curriculum continues to grow and develop. Both the spring and fall curricula of the Watson School are described in detail in the course descriptions following this report. Below are summarized the important new developments in the curriculum.

A New Topics in Biology Course

During the spring term, all Watson School students attend a one-week-long course at the Banbury Center arranged by Lilian Gann and Jan Witkowski. At these course "retreats," students attend lectures and seminars, read papers, and participate in discussions from morning till evening. The courses are an important and highly acclaimed element of the Watson School curriculum as they serve to extend the students' knowledge of the biological sciences beyond the Laboratory's scope by inviting experts from outside the Laboratory to teach the courses. Additionally, by having students attend a Topics in Biology course each year, the School engenders an appreciation that learning is a lifelong endeavor. With each new arriving class, more students enter the Watson School and it is not possible to have the intimate level of instruction that the School promotes with just one Topics in Biology course each year. Thus, this year, for the first time, two different Topics in Biology courses were offered. Hidde Ploegh from Harvard Medical School, returned to teach the entering class of 2001 his highly acclaimed *Immunology* course and Stanley Maloy and Ronald Taylor offered a new course on *Microbial Pathogenesis* to the entering classes of 1999 and 2000.

Stan Maloy came from the University of Illinois, Urbana, and Ron Taylor from Dartmouth Medical School to teach the course. The course and its instructors were extremely well-received, and the instructors were likewise very impressed by the quality of the students. The instructors implemented a number of novel didactic elements including daily "one-minute" quizzes in which students were posed a question, permitted five minutes to discuss the issue with their fellow students, and then given one minute to provide a written answer. The answers were then submitted for evaluation by the instructors and discussed together in class. These quizzes provided an easy and effective mechanism to evaluate the students performance in the course without requiring additional work by the students after the end of the one-week course. Although some students at the start of the week were not eager to take time away from their research to learn about microbial pathogenesis, by the end of the course, the students were unanimous in their enthusiasm for the time they spent in the course and how much they learned—a true compliment to the instructors. The instructors returned the compliment by noting that "this is the

Stan Maloy conversing with Rebecca Ewald of the Watson School and Anna Lena Chabes from Umeå University in Sweden.

best group of graduate students we have seen at any institution we have visited in the United States and abroad."

An Intensive Fall-Term Curriculum

The School continued to benefit from faculty commitment to didactic instruction of the students. This year, the same set of three Specialized Discipline courses—The Genome, Systems Neuroscience, and Cellular Structure and Function—were offered as in 2001. This conservation of courses permitted the instructors to improve upon each one by virtue of experience. The Scientific Reasoning and Logic course—the School's flagship core course—saw new leadership with Gregory Hannon taking over the reins from Scott Lowe as lead instructor and was joined by Hollis Cline and Senthil Muthuswamy as new instructors. We are indebted to founding course instructors Grigori Enikolopov and Scott Lowe who rotated off the course. I feel confident that we will be able to rely on their continued input in the future.

The Scientific Exposition and Ethics core course—an innovative course that melds student instruction in communication and ethics—continued to benefit from William Tansey's leadership. Together with the other course instructors—Adrian Krainer and Jan Witkowski—Bill is preparing a book based on the course to be published by the Cold Spring Harbor Laboratory Press. This development will go a long way toward one of the Watson School's goals of influencing graduate education beyond the confines of the Watson School itself.

By the end of the intensive Fall-Term Curriculum, the newly entering students had made great strides in acquiring the tools to become scholars in the biological sciences.

Watson School students Patrick Paddison (*left*) and Santanu Chakraborty.

DOCTORAL THESIS RESEARCH

Student	Academic Mentor	Research Mentor	Thesis Research
ENTERING CLASS OF 1999			
Amy Anne Caudy *George A. and Marjorie H. Anderson Fellow* *Howard Hughes Medical Institute Predoctoral Fellow*	Hollis Cline	Gregory Hannon	The biological function of RNA interference
Michelle Lynn Cilia *William R. Miller Fellow*	Nouria Hernandez	David Jackson	Mechanisms of intercellular trafficking via plasmodesmata
Ahmet M. Denli *David Koch Fellow*	Adrian R. Kralner	Gregory Hannon	Biochemical analysis of RNA-induced gene silencing
Emiliano M. Rial Verde *David and Fanny Luke Fellow* *Howard Hughes Medical Institute Predoctoral Fellow*	Jan A. Witkowski	Hollis Cline	Arc role in synaptic function
Elizabeth Ellen Thomas *Farish-Gerry Fellow* *Howard Hughes Medical Institute Predoctoral Fellow*	William Tansey	Michael Wigler	A de novo approach to identifying repetitive elements in genomic sequences
Niraj H. Tolia *Leslie C. Quick, Jr. Fellow*	David Helfman	Leemor Joshua-Tor	Structural analysis of a malarial surface antigen
ENTERING CLASS OF 2000			
Santanu Chakraborty *George A. and Marjorie H. Anderson Fellow*	Michael Wigler	Carlos D. Brody	Mechanisms of robust short-term memory in biological networks
Elena S. Ejkova *Engelhorn Scholar*	Jan A. Witkowski	William Tansey	Role of the proteasome in transcription
Rebecca C. Ewald *Engelhorn Scholar*	Bruce Stillman	Hollis Cline	NMDA receptor trafficking and its impact on neuronal functionality and morphology
Ira Hall *Beckman Graduate Student*	Alexander A.F. Gann	Shiv Grewal	Initiation, assembly, transfer, and genome-wide distribution of heterochromatin in *Schizosaccharomyces pombe*
Zachary Bela Lippman *Beckman Graduate Student*	William Tansey	Robert Martienssen	Comprehensive analysis of chromatin status on *Arabidopsis* chromosome 4
Marco Mangone *Charles A. Dana Foundation Fellow*	Linda Van Aelst	Winship Herr	The role of HCF-1 in cell proliferation
Masafumi Muratani *George A. and Marjorie H. Anderson Fellow*	Nouria Hernandez	William Tansey	Gene regulation by ubiquitin-mediated proteolysis
Patrick J. Paddison *Beckman Graduate Student*	Adrian R. Kralner	Gregory Hannon	An RNAi-based screen in mouse embryo fibroblasts for transformation-lethal gene targets
ENTERING CLASS OF 2001			
Catherine Y. Cormier *Beckman Graduate Student*	David J. Stewart	Yuri Lazebnik	Caspase activation in unidentified apoptotic pathways
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
Tomáš Hromádka *Engelhorn Scholar*	William Tansey	Anthony Zador	Stimulus optimization in the auditory cortex
Charles D. Kopec *Lindsay-Goldberg Fellow*	Anthony Zador	Roberto Malinow	Mapping the trafficking of AMPA receptors in dendritic compartments
Ji-Joon Song *Bristol-Myers Squibb Fellow*	Scott Lowe	Leemor Joshua-Tor	Structural studies of RNAi
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

Students Progress toward a Four-Year Ph.D. Degree with Research Successes

The students who matriculated in the first three classes—1999, 2000, and 2001—all progressed well throughout the course of the year. The students in the entering class of 2001 all passed the qualifying exam, selected research mentors, and as the year ended had prepared thorough descriptions of their proposed doctoral research (see box). These students were joined by 13 other students from Stony Brook University, who began their doctoral research at Cold Spring Harbor Laboratory this year.

Graduate students from the Hannon lab, Ahmet Denli (Watson School) and Yvette Seger (Stony Brook University).

A founding principle of the Watson School is that a Ph.D. degree need not take longer than on average four years. A question that has been posed by many is whether such an accelerated program can permit students the time to participate in serious research contributions. I am pleased to report that this year served as proof that this is indeed possible as demonstrated by the third- and fourth-year students. Fully one-half of the 14 third- and fourth-year students had already authored a published study by year's end and many had multiple publications. Additionally, these publications were in the very best of journals, including *Science* (five publications), *Proceedings of the National Academy of Sciences* (three), *Genes and Development* (two), and *Nature* (one).

Additionally, third-year student Ira Hall participated in seminal contributions to the new field of RNA interference, which together with other studies from Cold Spring Harbor Laboratory and elsewhere, were deemed the "Breakthrough of the Year" by *Science* Magazine. The School could not have imagined such stellar successes such as these when it first opened its doors. The success is made possible by the joined commitment of students, research and academic mentors, thesis committees, and the School administration. This early success augurs well for stellar dissertations emanating from the Watson School.

A Fourth Entering Class

This year saw the recruitment of the Watson School's fourth entering class, thus completing a full set of four classes in the School's four-year Ph.D. degree program. Of this year's six matriculating students, three come from the United States and the rest are from Australia, England, and Taiwan (see box and photo). They are from among 200 applicants reviewed by the Admissions Committee chaired by Nouria Hernandez and ably assisted by Janet Duffy. Two students, including Angélique Girard (see below), to whom we offered admission deferred the offer to join the entering class of Fall 2003. Others who turned down our offer of admission chose to attend the very best schools here and abroad, providing strong evidence of the high quality of the School's applicants.

Darren Burgess meets the Duke of York

ENTERING CLASS OF 2002

Allison L. Blum, American University, Washington, D.C.
Barbara McClintock Fellow

Darren J. Burgess, Christ Church, Oxford University, United Kingdom
Engelhorn Scholar

Beth L. Chen, Massachusetts Institute of Technology and University of California, Berkeley
George A. and Marjorie H. Anderson Fellow

Shu-Ling Chiu, National Tsing Hua University, Taiwan
Elisabeth Sloan Livingston Fellow

Jonathan S. Kui, Cornell University, Ithaca, New York
Alfred Hershey Fellow

Elizabeth Murchison, University of Melbourne, Australia
Elisabeth Sloan Livingston Fellow

Standing (*left to right*): Jonathan Kui, Allison Blum, Darren Burgess
Seated (*left to right*) Elizabeth Murchison, Beth Chen, Shu-Ling Chiu

A Successful Capital Campaign is Completed

One of the special features of the Watson School has been the establishment of an endowment committed to the School. This year saw the completion of the first phase of the Watson School Campaign, in which, under the direction of the former chairman of the Laboratory Board of Trustees, David Luke III, $32 million were raised to support the students, faculty efforts, administration, and building renovations for both the School and first-year student housing. As has been commented upon extensively elsewhere, the School will be forever indebted to David Luke and his colleagues for their generous effort and support. We are also thankful that Laboratory Trustee Robert Lindsay has agreed to lead the second phase of the Watson School Campaign. The goal of the second phase is to raise $15 million in the form of student fellowships so that the School may continue to grow.

An Endowment Opens Doors

The establishment of a committed endowment for the Watson School has had influences on the School that extend well beyond providing financial support. First, it has required the students and School administration to maintain regular contact with the donors to let them know how the students and School curriculum are developing. This exercise provides the students the opportunity to reflect on their development in the School and to appreciate that as scientists we are often the beneficiaries of others' generosity. For the School administration, it has taken much effort to monitor and maintain these contacts. In this regard, Janet Duffy, the Admissions and Academic Records Administrator, has played an invaluable role. Second, in some instances, the endowment has been provided to initiate specific programs, as exemplified by the Engelhorn Scholars program, which has become an illustrious program to support European students in the Watson School.

One of the School's endowed fellowships is the Florence Gould Fellowship, which is dedicated to the sponsorship of French students in the School. This year saw the appointment of the first Florence Gould Fellow with the recruitment of Angélique Girard from the École Polytechnique outside Paris. Ms. Girard is combining her doctoral studies at the Watson School with a degree from the French Corps des Mines, a prestigious three-year government program in policy and administration, and she will begin her doctoral studies in the Watson School in the fall 2003. During the process of recruiting a Florence Gould Fellow, the School had to learn much about the French educational system. This process has involved considerable effort, including my visiting the École Normale Supérieure and École Polytechnique during the summer, but, combined with the novelty of a four-year Ph.D. program in the United States, these efforts are likely to result in a source of outstanding students for the Watson School. These developments would not have come to pass were it not for a School endowment that arises from diverse sources with likewise diverse wishes.

National Institutes of Health Training Program Established

In the spring 2001, with the encouragement of the School's External Advisory Committee, the Watson School submitted a proposal for a doctoral training grant to the National Institutes of Health (NIH). As a result of the review of the proposal and a site visit in the fall 2001, the application was highly recommended for funding and accordingly the School has been awarded support for the training program from the NIH. The two inaugural appointments to the NIH-sponsored training program are Allison Blum, who is the Barbara McClintock Fellow, and Jonathan Kui, who is the Alfred Hershey Fellow, from the entering class of 2002.

A Diverse Doctoral Program

One of the joys of the Watson School is the diversity of its students and faculty who come from different backgrounds and different nationalities. An important challenge, however, for the School is to attain increased diversity by the inclusion of individuals from underrepresented minorities. Success in this endeavor will enrich the lives and education of our students and address a national agenda. With this

in mind, it is imperative that the Watson School receive effective advice from as many sources possible. Thus, at Lilian Gann's instigation, the Watson School has established a working group composed of representatives of the different Laboratory educational programs, all of which have an interest in the inclusion of underrepresented minorities—the Undergraduate Research Program (Leemor Joshua-Tor), the doctoral (Lilian Gann and Winship Herr) and postdoctoral (David Helfman) programs, Meetings and Courses (David Stewart), and the Dolan DNA Learning Center (David Micklos). The working group also includes current or former underrepresented minority students studying at the Laboratory to provide advice: two Stony Brook University students (Michelle Juárez and Hazeem Okunola) and Gilbert (Lee) Henry, a former student of the Undergraduate Research Program. Lee performed his doctoral studies with Doug Melton at Harvard University (before we had a doctoral program to offer!) and is now a Cold Spring Harbor Laboratory Fellow—an independent postdoctoral fellow position.

We are also obtaining advice from outside our institution. and in this regard the School's External Advisory Committee has been an important resource. Indeed, as described further below, this year the School recruited Victor Corces, who comes recommended by the NIH for having been successful in recruiting underrepresented minorities to his doctoral training program at Johns Hopkins University. At our August External Advisory Committee site visit, Victor, along with the other committee members, provided invaluable advice and encouragement.

The Watson School is additionally taking active measures to become recognized by students from underrepresented minorities. This fall, Lilian Gann and I attended the Annual Biomedical Research Conference for Minority Students in New Orleans. The meeting largely consisted of poster presentations by undergraduates and booths representing different American educational institutions, as well as programs dedicated to the sponsorship of underrepresented minority students. Lilian and I had the pleasure of being "official" judges of student posters. We were both extremely impressed with the quality of the research and the poise of the students in their research presentations. We also manned a booth on the last day of the meeting and were very excited to find considerable interest in the Watson School.

This year, we were also very fortunate to obtain private support for the Watson School endowment directed to the inclusion of underserved and underrepresented student populations in the School. The William Randolph Hearst Foundation provided the funds to establish a William Randolph Hearst Scholarship expressly to support students from underrepresented minorities—another example of how directed support can help the School achieve its diverse goals.

Mentoring: The Thesis Committee

One of the founding principles of the Watson School is that mentoring is a key component to an efficient and rewarding education. The School's administration and faculty provide one important mentoring process in the form of effective thesis advisory committees. The School's administration ensures that the students have regular thesis committee meetings, and the committee members have shown great dedication to making certain that the meetings are rewarding and promote student progress. Each student meets with his or her committee every six to nine months, and given that each committee is composed of five faculty members, some coming from outside the institution, arranging the meetings involves considerable administrative effort, largely shouldered by Lilian Gann. Additionally, the faculty show immense dedication, often serving on multiple thesis committees. The effort is very much appreciated by the students, research mentors, and School alike as good sound advice on which ways to proceed with the research is provided—an important ingredient to a four-year doctoral program. The results of the thesis committee meetings as well as student progress generally are monitored by the Watson School's Executive Committee, a dedicated group of School faculty, students, and administration.

In speaking to non-Watson School students studying at the Laboratory, from both Stony Brook University and other institutions, the heavy Watson School emphasis on thesis committee meetings

and the effort taken by the School administration in arranging the meetings for the students is considered one of the most attractive features of the School's curriculum. I am indebted to all those involved for their dedication to this process.

Two-tier Mentoring

As I have described amply in previous reports, I believe that the Watson School academic mentoring program, led by William Tansey, has had an indelible imprint on the success of the School. In this program, entering students select by mutual agreement a member of the research or nonresearch faculty to serve as an academic mentor—a guardian angel 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. This program has been a vital ingredient in the Watson School's excellent record in retaining the students who matriculate—none of the Watson School students has yet chosen not to complete a Ph.D. This year's new academic mentors for the entering class of 2002 are:

Allison L. Blum	Leemor Joshua-Tor
Darren J. Burgess	Nicholas Tonks
Beth L. Chen	Senthil K. Muthuswamy
Shu-Ling Chiu	Alea A. Mills
Jonathan J. Kui	David Jackson
Elizabeth Murchison	John R. Inglis

A School also Needs Mentoring

A Watson School Executive Committee Dedicated to the Success of the Doctoral Program

To develop a successful curriculum and for it to evolve and improve requires the effort of many people dedicated to the success of the School. In this regard, the School is most fortunate to have an executive committee dedicated to excellence. The Watson School Executive Committee meets monthly to oversee the graduate program and the progress of the students, and to address the wide range of related issues. The objectives of the committee are to oversee the graduate program including:

- the roles of faculty in the program,
- the committee for admissions,
- the qualifying examinations committee,
- graduate student progress in the program,
- curriculum development and monitoring,
- the welfare of the graduate student community,
- advice on ways of enhancing relationships with other graduate programs, and
- training grants awarded to the Watson School.

The Executive Committee meetings are characterized by thoughtful and lively discussions of issues important to the School. Its highly committed members have devoted considerable time and intellectual resources to the program's development, for which I am most grateful. This year, we had the benefit of input from Zachary Lippman of the Watson School and Sabrina Nuñez of the Stony Brook University Genetics Program.

The External Advisory Committee Gains a New Member

In addition to generous financial support, the School has benefited greatly from a distinguished external advisory committee for advice on how to achieve the goals of the Watson School. Under Keith Yamamoto of the University of California at San Francisco, the committee continues to provide sage

Zachary Lippman educates local school children about plants.

and erudite as well as down-to-earth advice on how to succeed in graduate education in the biological sciences. An important element of this program is to include individuals from underrepresented minority groups in the School's student body. The School has made recruitment of these individuals a top priority. The School's associate dean, Lilian Gann, is coordinating our efforts in this regard. Additionally, to replace Shirley Tilghman, who stepped down from the School's external advisory committee upon becoming president of Princeton University last year, the School recruited Victor Corces from Johns Hopkins University, who has considerable experience in involving underrepresented minority students in graduate education.

Dean's Teas

Another important element of monitoring how things are going and providing mentoring to the students has been regular Dean's Teas that Lilian Gann and I attend. These teas are biweekly in the fall term and monthly in the spring term for the first-year students and bimonthly for the more senior students. These teas provide an opportunity for the students to raise common concerns as well as provide Lilian and me an opportunity to ensure that the students attend to important curricular requirements and their academic progress.

Quality of Life

In addition to the continuing success of the School's curriculum, much effort was placed this year on improving the quality of student life at the Laboratory. This effort saw increases in student stipends, a new housing development at Uplands Farm for students and postdocs, and a shuttle service to the train station and the Cancer Genome Research Center in Woodbury. Additionally, a program was developed to help introduce first-year students from Stony Brook University to research at the Laboratory. These developments were the result of efforts by the Facilities and Human Resources Departments led by Art Brings and Katie Raftery, respectively, as well as cooperation and encouragement from Lawrence Martin, dean of the graduate school at Stony Brook University, and the leaders of Stony Brook University graduate programs in the biomedical sciences.

NEW STUDENTS FROM SHARED STONY BROOK UNIVERSITY GRADUATE PROGRAMS

Student	CSHL Research Mentor	Program
Alyssa Carlberg	Hollis Cline	Genetics
Sihem Cheloufi	Alea A. Mills	Genetics
Yi-Chun Chen	David L. Spector	Molecular and Cell Biology
Carmine Chiariello	Z. Josh Huang	Genetics
Ikuko Hotta	Greg Hannon	Molecular and Cell Biology
Helen Hsieh	Roberto Malinow	Neurobiology and Behavior
Michael Kwofie	Jacek Skowronski	Genetics
Alexandra Lucs	Senthil K. Muthuswamy	Molecular Genetics and Microbiology
Marissa Moore	Senthil K. Muthuswamy	Genetics
Hazeem Okunola	Adrian R. Krainer	Physiology and Biophysics
Togay Oztaskin	Nouria Hernandez	Genetics
Andriy Tovkach	Leemor Joshua-Tor	Genetics
Hatim Zariwala	Zachary Mainen	Molecular and Cell Biology

One of the main concerns from students last year was the level of the Watson School stipend, which was, in and of itself, arguably lower than those of the other institutions with which we compete for students (although, unlike Cold Spring Harbor Laboratory, many other institutions do not provide free health insurance and subsidized food and housing to their students). In response to this challenge, the School has worked with Stony Brook University to raise graduate student stipends on Long Island. As a result, student stipends at both institutions continued to increase, with Watson School students receiving a $21,500 stipend for the 2002–2003 academic year. Additionally, coordinated with Stony Brook University, students now receive a 10% stipend bonus if they obtain their own independent predoctoral fellowship.

Departures

With each year come new departures. This year saw the following graduate students and postdoctoral fellows depart from the Laboratory:

Graduate Students

Sanae Irimura
Flavio Kamenetz
So Young Kim
John McNeil
Troy Messick
Patrick Reilly
Andrew Samuelson
Jason Stajich
Hongjae Sunwoo
Jiayuan Tong
Xiaorong Wang
Zhengwei Zhu

Postdocs

Isabel Cantallops
Marc Davenne
Jose Esteban
Robert Filipkowski
Jordan Fridman
Anna Giulini
Hirac Gurden
Scott Hammond
Edward Hofmann
Junjiro Horiuchi
Peter Krasnov
Miguel Maravall
Naoki Nakaya
Michael Packer
Emmanuelle Querido
Jyotishankar Raychaudhuri
David Roberts
Jose Rodriguez
Aravinthan Samuel
Gurumurthy Shankaranarayana
Maria Soengas
Yuri Stasiv

Staff Changes

Over the summer, Janet Silver, the Watson School's cheerful first point of contact, was taken away from us by an offer she could not refuse. Fortunately, we were able to replace her with Mark Beavers, our new Administrative Assistant, otherwise the person at the nexus of the operation. Prior to joining the Watson School staff, Mark was the Assistant to the Chair at the Department of Music and Performing Arts Professions at New York University's Steinhardt School of Education, where he received a Bachelor of Science in Music Education and Piano Performance in 1989. We have been very fortunate to recruit such a warm person to help make the Watson School a comfortable home for its students.

Mark Beavers

Dean Steps Down as Assistant Director

As the School has developed and grown, it has required more effort—not less—to ensure its success. Early on, the School was simply an idea—or a series of ideas—developed by the Cold Spring Harbor Laboratory faculty and administration, with advice from outside the Laboratory. Although developing those ideas took much effort, to implement them and to create the reality of a new school requires considerably more effort from all concerned. Indeed, I have learned that it takes more effort to ensure continued success than to generate initial success. The School is now at a critical stage with the first cohort of students expected to graduate next year with the Ph.D. degree. It then will begin to be known whether scientists of the highest quality can be trained and compete effectively in the scientific community after just four years of graduate training. I therefore decided to devote every effort possible to ensuring the success of the Watson School while maintaining a vigorous research program. I am therefore very grateful to Bruce Stillman for accepting and supporting my decision to resign as assistant director of the Laboratory. I look forward to continuing to work closely with Bruce in the upcoming years for both the Watson School and the Laboratory.

As the New Year begins, it seems most fitting that in this year of the 50th anniversary celebrations of the discovery of the structure of DNA by James Watson and Francis Crick, the School will also celebrate by conferring its first Ph.D. degrees.

April, 2003

Winship Herr

SPRING CURRICULUM

TOPICS IN BIOLOGY

ARRANGED BY **Lilian Gann**
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 seven-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 the Spring 2002, for the first time, there were two such courses: *Immunology* and *Microbial Pathogenesis*.

Immunology

Attended by the entering class of 2001

INSTRUCTOR **Hidde Ploegh,** Harvard Medical School

GUEST LECTURERS **Diane Mathis,** Joslin Diabetes Center
Uli von Andrian, Harvard Medical School

TEACHING FELLOWS **Margot Furman,** Harvard Medical School
Marianne Boes, Harvard Medical School

This course introduced the elements of the immune system, illustrating its unique characteristics as well as those it shares with other biological systems. The course ran from Sunday to Saturday, February 17–23, and was organized and largely taught by Hidde Ploegh. Two guests—Diane Mathis and Uli von Andrian—also lectured in the course, and two teaching fellows participated in all aspects of the course. As in previous years, the course was highly rated by all of the students, including course auditors.

Standing *(left to right)*: Sabrina Nuñez, Claudia Feierstein, Tomáš Hromádka, Elizabeth Murchison, Hidde Ploegh, Charles Kopec, Ji-Joon Song, Gowan Tervo. Seated *(left to right)*: Marianne Boes, Margot Furman, and Catherine Cormier, accompanied by Nellie the dog!

Microbial Pathogenesis

Attended by the entering classes of 1999 and 2000

INSTRUCTORS **Stanley Maloy,** University of Illinois
Ronald K. Taylor, Dartmouth Medical School

GUEST LECTURERS **James Bliska,** Stony Brook University
Darren Higgins, Harvard Medical School
Theresa Koehler, University of Texas–Houston Medical School
Karen Skorupski, Dartmouth Medical School

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 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 ran from Sunday to Saturday, May 5–11, and was organized and largely taught by Stanley Maloy and Ronald Taylor. It integrated lectures by the instructors, daily quizzes, readings of research papers, and seminars by the instructors plus the four invited lecturers who specialize in various aspects of bacterial pathogenesis. The students rated the course very highly, many of whom had little prior awareness of the subject.

(*Left to right*) Stan Maloy, Marco Mangone, Emiliano Rial Verde, Elizabeth Thomas, Ira Hall, Rebecca Ewald, Michelle Cilia, Anna Lena Chabes, Elena Ejkova, Niraj Tolia, Amy Caudy, Zachary Lippman, Ahmet Denli, Santanu Chakraborty, Masafumi Muratani, Patrick Paddison, and Ron Taylor.

Teaching Experience at the Dolan DNA Learning Center

DIRECTOR **David A. Micklos**

INSTRUCTORS **Scott Bronson**
Jenny Eisenman
Trisha Maskiell
Amanda McBrien

As science plays an increasing role in society, there is also an increasing need for biologists to educate nonscientists of all ages about biology. The Watson School of Biological Sciences doctoral program offers its students unique teaching experiences through the Laboratory's Dolan DNA Learning Center, where students teach laboratory courses to high school and middle school students. From these teaching experiences, they learn how to communicate with nonbiologists and to inspire and educate creative young minds. The teaching module entailed pairs of students teaching one morning or afternoon a week for 12 weeks. In the initial weeks, the Dolan DNA Learning Center instructors taught the Watson School students the didactic process—it was not until the fifth week that the graduate students taught on their own. At the end of the 12 weeks, the students were very excited about their teaching experience.

Laboratory Rotations

The most important element of a doctoral education is learning to perform independent research that leads to a unique contribution to human knowledge. After the fall course term, students participate in laboratory rotations. These rotations provide students and faculty the opportunity to get to know each other and to explore possibilities for doctoral thesis research. At the end of each rotation, students make short presentations of their studies to the other students and their rotation advisors. These talks give students an opportunity to share their laboratory experiences and to learn how to give a scientific presentation. With this latter goal in mind, in addition to the research mentors, the instructors of the Scientific Exposition and Ethics core course attend the talks and give individual feedback to students on their presentations. This year, 15 faculty members served as rotation mentors, some mentoring more than one student.

ROTATION MENTORS

Carlos D. Brody
David Helfman
Winship Herr
David Jackson
Leemor Joshua-Tor
Yuri Lazebnik
Scott Lowe
Zachary Mainen
Roberto Malinow
Andrew Neuwald
Jacek Skowronski
Lincoln Stein
Karel Svoboda
Tim Tully
Anthony Zador

FALL COURSE CURRICULUM

CORE COURSES

The Leslie C. Quick, Jr. Core Course on Scientific Reasoning and Logic

FUNDED IN PART BY **The Beckman Foundation**

INSTRUCTORS **Gregory Hannon (Lead)**
Hollis Cline
Winship Herr
Leemor Joshua-Tor
Senthil K. Muthuswamy
Arne Stenlund

GUEST LECTURERS **Alexander A.F. Gann**, **Shiv Grewal**, **Nouria Hernandez**, **David Jackson**, **Adrian R. Krainer**, **Scott Lowe**, **Zachary Mainen**, **Robert Martienssen**, **Alea A. Mills**, **Michael P. Myers**, **Jacek Skowronski**, **Bruce Stillman**, **William Tansey**, **Marja Timmermans**, **Tim Tully**, **Linda Van Aelst**, **Rui-Ming Xu**

VISITING LECTURER **Michael Hengartner**, University of Zurich

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 Fall Course Curriculum, students (1) acquired a broad base of knowledge in the biological sciences, (2) learned the scientific method, and (3) learned how to think critically about biological concepts. This course consisted of 12 weekly segments, each of which had a different

End of fall term celebratory breakfast. Standing (*left to right*): Lilian Gann, Elizabeth Murchison, Beth Chen, Jonathan Kui, Darren Burgess, Allison Blum, and Winship Herr. Front: Shu-Ling Chiu.

theme. Each week, students read an assigned set of research articles (generally four articles) and provided written answers to a problem set that guided them through two (or, on occasion, one) of the articles. Twice weekly, students attended lectures related to the week's topic, which included concepts and experimental methods. During the week, the students met to discuss the assigned papers not covered by the problem set among themselves. At the end of each weekly segment, the students submitted their problem sets and spent the evening discussing with faculty the articles not covered by the problem set. The course culminated in the thirteenth week with a final exam. Studying for the final exam gave the students the opportunity to synthesize and integrate what they had learned over the course of the fall term. The weekly topics were:

Week 1	Macromolecular Structure
Week 2	Genetics
Week 3	Transcriptional Regulation
Week 4	Posttranscriptional Regulation
Week 5	Mobile Genetic Elements
Week 6	Chromosome Structure and Function
Week 7	Signal Transduction
Week 8	Cell Division Cycle
Week 9	Cancer Genes
Week 10	Cell-Cell Communication
Week 11	Development
Week 12	Neurobiology
Week 13	Final Exam

The Darrell Core Course on Scientific Exposition and Ethics

FUNDED IN PART BY	**The 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	**William Tansey (Lead)** **Adrian R. Krainer** **Jan A. Witkowski**
GUEST LECTURERS	**Terri Grodzicker** **Winship Herr**
VISITING LECTURERS	**Robert P. Charrow, Esq.,** Crowell & Moring LLP **Carol Ezzell,** Editor and Staff Writer, *Scientific American* **Jane Maienschein,** Ph.D., Department of Philosophy, Arizona State University **Mary Morry,** Partner, Morgan & Finnegan, LLP **Philip Reilly,** JD, MD, CEO of Interleukin Genetics

This core course offered instruction about 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. This year, the course continued a novel format established in 2000 in which the course was organized around the scientific process starting with how the ideas for an experiment develop and covering execution of the experiment, presentation of the results at seminars and in publication, funding, and the implications of the experimental results on scientists and society. As a part of learning how to make oral presentations, together with the instructors, the students also critiqued formal seminar presentations at the Laboratory. A primary objective of the course was for students to consider exposition and ethics as an integral part of scientific research.

Research Topics

ARRANGED BY **Lilian Gann**
David Helfman

This core course provided students with an in-depth introduction to the fields of research that the Laboratory scientists investigate. Students and faculty attended a weekly Research Topics seminar, at which faculty members presented their current research topics and methods of investigation each Wednesday evening over dinner. The students learned how to approach important problems in biology. These seminars, together with the annual fall Laboratory in-house symposium, provided students with a basis for selecting laboratories in which to do rotations. Due to the increasing number of Faculty at the Laboratory, this year for the first time the Research Topics seminars were extended into January 2003. The weekly speakers were:

Week 1	Leemor Joshua-Tor, Scott Lowe, Rui-Ming Xu
Week 2	Gregory Hannon, Eli Hatchwell, W. Richard McCombie
Week 3	Nouria Hernandez, Winship Herr, William Tansey
Week 4	Roberto Malinow, Michael P. Myers, Marja Timmermans
Week 5	Hollis Cline, Robert Martienssen, Andrew Neuwald
Week 6	Shiv Grewal, Zachary Mainen, Anthony Zador
Week 7	Carlos D. Brody, Dmitri Chklovskii, Tatsuya Hirano
Week 8	Karel Svoboda, Yi Zhong
Week 9	Grigori Enikolopov, Nicholas Tonks, Linda Van Aelst
Week 10	Jacek Skowronski, Arne Stenlund, Bruce Stillman
Week 11	Yuri Lazebnik, Alea A. Mills, Michael Wigler
Week 12	David Helfman, Senthil K. Muthuswamy, David L. Spector
Week 13	David Jackson, Lincoln Stein, Jerry Yin
Week 14	Z. Josh Huang, Adrian R. Krainer, Tim Tully
Week 15	Masaaki Hamaguchi, Robert Lucito
Week 16	Josh Dubnau, Vivek Mittal

SPECIALIZED DISCIPLINES COURSES

The Genome

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 **Robert Martienssen**
W. Richard McCombie
Lincoln Stein

GUEST LECTURERS **Eli Hatchwell**
Andrew Neuwald
Tim Tully
Jan A. Witkowski

VISITING LECTURER **Marc Vidal,** Harvard Medical School

The first draft of the human genome sequence has been completed, and the new science of genomics promises to revolutionize biological concepts and approaches, making the computer a tool for research

as essential as the microcentrifuge and electrophoresis unit. With computer software, scientists can digest the enormous amount of genetic information produced by the genome project and answer questions about evolution, model complex processes such as signal transduction and gene regulation, and manage and organize experiments.

This course provided essential background as to how sequence and mapping information is generated and interpreted, the principles of microarray and other functional strategies, and the history of the genome project. It also provided a practical introduction to bioinformatics, including computational biology, biological data modeling, and laboratory workflow management. Topics covered included genetic and physical mapping, DNA sequence acquisition and interpretation, Web-based resources for genome data, gene modeling and protein- and DNA-sequence analysis, phylogenetic analysis, and functional genomics.

Systems Neuroscience

FUNDED IN PART BY **The George W. Cutting Lectureship; The Klingenstein Lectureship; The William Stamps Farrish Lectureship**

INSTRUCTORS **Carlos D. Brody**
Zachary Mainen
Anthony Zador

GUEST LECTURERS **Hollis Cline**
Roberto Malinow

Cognition and behavior arise from complex interactions between billions of neurons. This systems neuroscience course examined fundamental properties of neurons, including synaptic transmission and plasticity, and how in higher-order organization they give rise to brain functions such as visual perception.

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 Helfman**
David L. Spector

GUEST LECTURERS **Tatsuya Hirano**
Yuri Lazebnik

VISITING LECTURERS **Fred Chang,** Columbia University
Graham Warren, Yale University

With the complete set of instructions available for many organisms—i.e., their genome sequence—there is now an increasing emphasis on understanding the function of the gene products. This understanding will require an increasing appreciation of the structure and function of the cell. This course provided a basic overview of the structural and functional organization of cells with particular emphasis on cellular compartmentalization and communication. In addition, it provided insight into the basic toolbox of the cell biologist of the twenty-first century.

UNDERGRADUATE RESEARCH PROGRAM

Program Director: Leemor Joshua-Tor
Associate Program Director: Gregory Hannon
Program Administrator: Jane Reader

An important aspect of the summer program at the Laboratory is the participation of college undergraduate students in active research projects under the supervision of full-time faculty members. Since the program was initiated in 1959, 604 students have participated, and many have gone on to productive careers in the biological sciences.

The objectives of the program are to provide (1) a greater understanding of the fundamental principles of biology; (2) an increased awareness of experimental approaches to science; (3) a deeper understanding of the major issues in the fields of biochemistry, genetics, molecular, cellular, and structural biology, neuroscience and genomics; and (4) a personal acquaintance with research, research workers, and centers for study.

Left to Right: (*Front row*) Hiroki Asari, Gautam Agarwal.
(*Second row*) Sarah Whitcomb, Rachel Kalmar, Erin Kurten, Honor Hsin, Boo Shan Tseng, Grace Teng, Daniel Herman, Cindy Lee, Marisa Rodriguez, Jonathan Hertz, Jacqueline Ou.
(*Third row*) Anna Belkina, Lieven van der Veken, Vishal Patel.
(*Back row*) Thomas Denkenberger, Jamie Newman, Michalis Agathocleous, Cory Lindsay, Winfred Frazier, Sherry Aw, Renatta Knox.

During the program, the students are housed together on the Laboratory grounds. Nearly all of the students arrive at the same time and share the entire experience. For programmatic reasons, we limit the number of students to 25. In this manner, we ensure a cohesive program with substantial scientific and social interactions among the students. The students are required, at the beginning of the program, to present to their peers a concise oral description of the background and the design of their experiments. At the end of the ten-week program, the undergraduates present a 20–30-minute seminar describing the background, design, and results of the experiments they have been involved in during the course of a two-day undergraduate symposium. During their stay, the participants attend a series of Faculty Talks given by both young and established scientists at the Laboratory. These seminars are attended by only the students and Program Directors to assure that the seminars remain at a level appropriate for the undergraduates. It also encourages questions in an informal and comfortable setting. In addition to scientific discussion, these presentations cover important issues such as personal experiences and choices that led the scientists to their current area of research and position. The following students, selected from 443 applicants, took part in the 2002 program:

Gautam Agarwal, University of Texas
Advisor: **Zachary Mainen**
Sponsor: Burroughs Wellcome
Modeling odor recognition by neural synchrony.

Michalis Agathocleous, Trinity College
Advisor: **Hollis Cline**
Sponsor: Burroughs Wellcome
CPG expression changes tyrosine phosphorylation in vivo.

Hiroki Asari, University of Tokyo
Advisor: **Masaaki Hamaguchi**
Sponsor: The Emanuel Ax Fund
Suppression of DBC2 by RNA interference.

Sherry Aw, University of Wisconsin
Advisor: **David Jackson**
Sponsor: Burroughs Wellcome
Protein trafficking via plasmodesmata in *Arabidopsis thaliana*.

Anna Belkina, Russian State Medical University
Advisor: **David Helfman**
Sponsor: The Read Fund
Characterization of S100A4 function.

Thomas Denkenberger, Penn State University
Advisor: **Arne Stenlund**
Sponsor: Burroughs Wellcome
Studies of the bovine papillomavirus E1 helicase.

Winfred Frazier, University of Houston
Advisor: **Senthil K. Muthuswamy**
Sponsor: National Science Foundation
Phenotypic consequences of activating ErbB2 receptor mutants in epithelial cells.

Daniel Herman, Massachusetts Institute of Technology
Advisor: **Grisha Enikolopov**
Sponsor: Burroughs Wellcome
Characterization of noxin1 function using hairpin RNA interference.

Jonathan Hertz, Massachusetts Institute of Technology
Advisor: **Z. Josh Huang**
Sponsor: Glass Fund
Subcellular localization of protocadherins in GABAergic interneurons and their role in synaptic plasticity.

Honor Hsin, Harvard University
Advisor: **Roberto Malinow**
Sponsor: Jephson Educational Trust
Neurons ReAsHed: Imaging a molecular model of memory.

Rachel Kalmar, University of California, San Diego
Advisor: **Anthony Zador**
Sponsor: National Science Foundation
How does the auditory cortex encode complex sound?

Renatta Knox, Harvard University
Advisor: **David L. Spector**
Sponsor: National Science Foundation
Visualizing UAP56 in living cells.

Erin Kurten, University of Wisconsin
Advisor: **William Tansey**
Sponsor: National Science Foundation
Developing tools to study interactions between the proteasome and Med 8.

Cindy Lee, Stony Brook University
Advisor: **Jerry Yin**
Sponsor: Jephson Educational Trust
Molecular mechanism of atypical PKM regulation.

Cory Lindsay, Wayne State College
Advisor: **Eli Hatchwell**
Sponsor: Libby Fund
A common microdeletion at 8q24.3: Population frequency analysis.

Jamie Newman, Amherst College
Advisor: **Yuri Lazebnik**
Sponsor: Olney Fund
Can Primus regulate apoptosis?

Jacqueline Ou, Duke University
Advisor: **Michael Q. Zhang**
Sponsor: National Science Foundation
Toward genome-wide first exon annotation: Computational prediction and experimental protocol.

Fatih Ozsolak, Washington University
Advisor: **Rob Lucito**
Sponsor: Burroughs Wellcome
Gene copy number changes in breast and prostate cancers.

Vishal Patel, University of Illinois
Advisor: **Leemor Joshua-Tor**
Sponsor: Garfield Fund
Expression, purification, and crystallization trials of candidate plasticity gene 15.

Marisa Rodriguez, University of Houston
Advisor: **Alea A. Mills**
Sponsor: National Science Foundation
Using chromosome engineering to study functional genomics.

Grace Teng, Yale University
Advisor: **Robert Martienssen**
Sponsor: Bliss Fund
Analysis of *Schizosaccharomyces pombe* centromeric transcripts.

Boo Shan Tseng, Massachusetts Institute of Technology
Advisor: **Winship Herr**
Sponsor: National Science Foundation
Life without HCF-1: A way to create Siamese cells.

Lieven van der Veken, Leuven University
Host: **Linda van Aelst**
Sponsor: Frederica van Stade Fund
Molecular and cellular characterization of oligophrenin and potential partners.

Sarah Whitcomb, Columbia University
Advisor: **Gregory Hannon**
Sponsor: Shakespear Fund
Attempting to assay RNA-dependent RNA-polymerase activity of a putative RdRp from *Schizosaccharomyces pombe*.

Elisabeth Wurtmann, Carleton College
Advisor: **Marja Timmermans**
Sponsor: National Science Foundation
Regulation of knox genes by rough sheath2 in maize leaf initiation.

PARTNERS FOR THE FUTURE

Program Director: Yuri Lazebnik

Program Administrator: Lynn Hardin

The CSHL Partners for the Future program, established in 1990, gives young students who are interested in science an opportunity to do real biomedical research. Each year, six to eight Long Island high school students entering their senior year are chosen on the basis of a written application and an interview. Selected students are assigned to scientists at CSHL who become their mentors and supervise their research, which usually continues from October through March. During the six months of the program, students give several scientific presentations, including one to describe a plan of research and another to report how research is progressing. At the conclusion of the program, students present the results of their research projects to an audience of scientific mentors, high school teachers, friends, and relatives. Participants in the program frequently credit it for the first spark of their scientific imagination. To date, 68 students have completed the program, all of whom have enrolled in top-ranked colleges or universities. The 2002–2003 Partners for the Future are:

Partner	High School	CSHL Mentor	Laboratory
Kristen Aliano	Cold Spring Harbor High School	Michelle Cilia	David Jackson
Brandon Buckley	Cold Spring Harbor High School	Eric Drier	Jerry Yin
Andrew Chang	Jericho High School	Ravi Sachidanandam	Ravi Sachidanandam
Adam Graver	Friends Academy	Michael Regulski	Tim Tully
Marie Mizuno	Cold Spring Harbor High School	Dom Duelli	Yuri Lazebnik
Jonathan Lee Mogen	Half Hollow Hills High School West	Frances Hannan	Jerry Yin
Theresa Reimels	St. Dominic High School	Anindya Bagchi	Alea A. Mills
Jonathan Widawsky	Huntington High School	Eli Hatchwell	Eli Hatchwell

(*From left to right*) Jonathan Widawsky, Adam Graver, Marie Mizuno, Brandon Buckley, Theresa Reimels, Kristen Aliano, Jonathan Mogen, and Andrew Chang.

NATURE STUDY PROGRAM

The Nature Study Program gives elementary and secondary school students the opportunity to acquire a greater knowledge and understanding of their environment. Through a series of specialized field courses, younger students can engage in introductory programs such as *Nature Bugs, Nature Detectives*, and *Nature Discovery*, and older students can enroll in more advanced programs such as *Marine Biology*.

During the summer of 2002, students participated in 24 courses within the program. The major change to the program this year was the location. Instead of holding classes at the West Side School in Laurel Hollow, the classes were held outdoors, weather permitting, at Southdown School in Huntington.

This facility is used as a base for the students' exploration of the local environment. Continuation of the program in its present location would be beneficial but this is yet to be determined. Field classes are held on Laboratory grounds, St. John's Preserve, Fire Island National Seashore, the Nature Conservancy, the Cold Spring Harbor Fish Hatchery and Aquarium, as well as other local preserves and sanctuaries.

In addition to the three, two-week sessions, the *Adventure Education* course met on July 26, 2002, for a six-mile canoe trip on the Nissequogue River in Smithtown to navigate and explore the waters of Long Island. The course emphasizes the plant and animal life indigenous to the area as well as historic points of interest.

PROGRAM DIRECTOR: William M. Payoski, M.A., Adjunct Professor, Nassau Community College

REGISTRAR: Sharon Bense, Cold Spring Harbor Laboratory

INSTRUCTORS: **Alison Smith,** B.S., Marine Science, University of Rhode Island
Michael Zarzicki, B.A. in English, Adelphi University
Amy Friedank, B.S. in Marine Science, Long Island University, Southampton
Jessica Badalucco, B.S. in Biology, Adelphi University
Ann Marie LaRuffa, B.A in Natural Science, Adelphi University

COURSES

Nature Bugs (Kindergarten): Exploration, games, stories, and dramatics are used to introduce the young child to a variety of natural habitats.

Nature Detectives (Grades 1–2): An introductory course in nature study, stressing interrelationships between plants and animals. A variety of habitats are explored.

Nature Discovery (Grades 1–2): Students continue their discovery of nature through activities and concepts.

Ecology Explorers (Grades 3–4): Natural communities, food webs, and a succession of communities are studied in this course. Students study the diversity of plant and animal forms native to the Cold Spring Harbor Laboratory area.

Pebble Pups (Grades 3–4): Elementary geology for students interested in a basic study of rocks and minerals available on Long Island. Each student completes a rock and mineral collection. Dinosaurs and fossils are featured themes. Some of the highlights of this course include field trips to local museums.

Frogs, Flippers, and Fins (Grades 3–4): Program designed for younger students as an introduction to aquatic ecosystems. Fresh water and marine habitats are explored.

Seashore Life (Grades 5–7): Children examine plant and animal life found below the tidemark. Fish, marine worms, algae, shellfish, beach plants, and shore birds are studied.

Freshwater Life (Grades 5–7): Students consider the vertebrate, invertebrate, and plant life found in area bogs, ponds, lakes, and streams.

Adventure Education (Grades 6–10): This course is a 6-mile canoe trip up and down the Nissequogue River exploring the flora and fauna of the waterway.

Marine Biology (Grades 8–10): This course offers a more sophisticated study of plants and animals native to the inner and outer harbors and provides field trips, dissection, using the microscope, and laboratory experiments.

COLD SPRING HARBOR LABORATORY
MEETINGS AND COURSES

ACADEMIC AFFAIRS

The academic program of meetings and courses has evolved from a group of summer laboratory courses and the annual symposium to a diversified year-round effort. It now includes advanced laboratory courses, summer lecture and workshop courses held at the Laboratory's Banbury Center, large meetings and winter biotechnology conferences held in Grace Auditorium, short bioinformatics courses at Woodbury, and even off-campus meetings. Scientists ranging from graduate students to senior faculty participate in the program.

The 27 laboratory and lecture courses held in 2002 covered a diverse array of topics in molecular biology, neurobiology, structural studies, and bioinformatics. The primary aim of these courses remains to teach students the latest advances in techniques and concepts that can be immediately applied to the students' own research. New courses are always being started to reflect current needs and existing courses are updated by the instructors. This year, a laboratory course on *Proteomics* was begun, as well as a new lecture course on *Stem Cells*. Both proved to be immensely popular, were heavily oversubscribed, and will be taught again in future years. The *Advanced Bacterial Genetics* course, now more than 50 years old, has taken advantage of the knowledge of the genome sequence of many organisms to teach genetic analysis of pathogenic and "undomesticated" bacteria. Similarly, the *Eukaryotic Gene Expression* course now teaches students sophisticated in vivo methods, such as chromatin immunoprecipitation analysis, and has devised a plan for the use of RNA interference to make mutations in mammalian cells. Additionally, a new short course covering introductory bioinformatics, the *Genome Access* course, was initiated at the Genome Research Center.

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 extraordinary enterprise and dedication make the course program possible. The full program of 2002 courses and instructors are listed below.

Courses are supported by grants from a variety of sources, including multiple grants from the National Institutes of Health and National Science Foundation. We also depend on a large education grant from the Howard Hughes Medical Institute for the support of new courses and neurobiology courses and are grateful for the support of a neurobiology course grant from the Esther and Joseph A. Klingenstein Fund. Funds from the John Merck Fund and the Eppley Foundation have also supported neurobiology lecture courses at the Banbury Center. The courses also depend on equipment and reagents loaned or donated by a large number of vendors, without which the faculty and students would not have access to the latest techniques and technology.

Twenty meetings were held at the laboratory this year and brought almost 6000 scientists from more than 50 countries to discuss their latest research findings. Almost all of the meetings have no—or very few—"pre-invited" speakers. Talks are selected from submitted abstracts by the meeting organizers. This format ensures that the latest findings will be presented and that young scientists will have a chance to describe and discuss their work. The regular meeting season started with a conference on the *Evolution of Developmental Diversity* and finished with one on *Human Origins and Disease*. Several of the meetings were oversubscribed including those on *Genome Sequencing and Biology*, *Retroviruses, Translational Control*, and *Axon Guidance and Neural Plasticity*. Many of the meetings have become exciting and essential for their respective fields and are held on a biannual basis. This year, three winter biotechnology conferences, including a new conference on *Tissue Engineering*, were held outside of the regular meetings schedule. In a new initiative, archived streaming videos of the oral sessions of each meeting were made available to meeting attendees on a short-term basis via a private web portal called *The Leading Strand*. The limited access period of the Web Site is designed to prevent the material from being considered as competitive with publications. Partial support for individual meetings is provided by federal agencies, foundations, and companies, while core support for the program is provided by our Corporate Benefactors, Sponsors, Affiliates, and Contributors.

The Symposium continues to be the centerpiece of the meetings program. This year, the topic was *The Cardiovascular System*. The Symposium addressed many aspects of cardiovascular science

including normal heart and cardiovascular development and function, the failing heart, hypertension, atherosclerosis, vascular biology, angiogenesis, and tumor angiogenesis. The Symposium attracted 256 participants and featured 68 talks and 108 posters. Opening night speakers included Richard Harvey, Mark Fishman, Jonathan Seidman, and Rakesh Jain, and the summary was presented by Christine Seidman. Richard Lifton delivered the Dorcas Cummings lecture on "Salt and Blood Pressure: New Insights from Human Genetic Studies" to a mixed audience of scientists and lay friends of the Laboratory.

The success of the very large number of courses and meetings 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. They include Drew Mendelsohn, Associate Director for Marketing and Planning; Andrea Stephenson, Course Registrar and Operations Manager; Ed Campodonico, Manager of Audiovisual Services; Barbara Zane, the Course Coordinator; and Mary Smith, the Abstracts Coordinator. The staff of the Meetings and Courses office also include Joe Carrieri, Andrea Newell, Maureen Morrow, Val Pakaluk, Lauren Postyn, Marge Stellabotte, Jenna Williams, and various part-time staff. Audiovisual services under Ed Campodonico's management are ably provided by William Dickerson, Gerald McCloskey, John Parsons, and the part-time audiovisual staff. In the latter part of 2001, Michael Cannavarro transferred from the Information Technology Department to the Meetings and Courses programs because computer support for all of the courses and meetings has become a full-time occupation. Staff from several other departments also do crucial work for the courses and meetings. They include: Wendy Crowley, the Educational Grants Manager; Cliff Sutkevitch and his staff, who set up and maintain course equipment; and Leigh Johnson in the Library, who handles the book and journal needs of the courses. 2002 saw the departure of two valuable staff members, Michael Glaessgen and Nancy Weeks, to new pastures. We are grateful to them all.

Terri Grodzicker
Assistant Director for Academic Affairs
David Stewart
Executive Director, Meetings and Courses

67TH COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

The Cardiovascular System

May 29–June 3 257 participants

ARRANGED BY **Bruce Stillman,** Cold Spring Harbor Laboratory
David Stewart, Cold Spring Harbor Laboratory

It has been unusual to hold the Cold Spring Harbor Symposium on Quantitiative Biology on a topic devoted to a single organ. One notable exception was the successful Symposium in 1990 on the brain, in part to mark President Bush's proclamation of the 1990s being the decade of the brain and also to mark the beginning of the current Cold Spring Harbor initiative in neuroscience. There has been no such presidential pronouncement about the heart, and Cold Spring Harbor has not focused its own research on the cardiovascular system, but there have been remarkable developments in molecular understanding of the cardiovascular system over the last decade. It seemed only fitting, therefore, that this year's Symposium focus on such an important biological system.

Development of the cardiovascular system during embryogenesis has appropriately been a major area of research, particularly the patterning that forms the branches of the peripheral blood and lymphatic systems and development of the heart itself. Developmental biologists now have a good general understanding of how the system comes about. Equally important has been the focus on signaling pathways that function in the cardiovascular system to maintain its function. Perhaps most important is the relevance of this topic to human disease. The cardiovascular system remains a major focus of research and efforts to tame the western world's most common cause of death. Thus, in planning this Symposium, appropriate attention was paid to the various aspects of disease in the cardiovascular system.

Help in organizing this Symposium came form many sources, particularly my co-organizer David Stewart, Executive Director of our Meetings and Courses program, who played a key role in identifying speakers and topics for this Symposium. I wish to thank Shaun Coughlin, Richard Lifton, Eric Olson, Christine Seidman, Celeste Simon, and Sandy Williams for their generous advice about a field whose details were rather foreign to me.

The meeting ran for five days and included 257 participants with 108 oral presentations and 68 poster presentations. I wish to thank Dr. Richard Lifton for his superb presentation at the Dorcas Cummings Memorial public lecture of the genetics of hypertension, an example of how the human genome project is having a large impact on understanding human disease. I also wish to thank the first-night speakers, Drs. Richard Harvey, Mark Fishman, Jonathan Seidman, and Rakesh Jain, for their superb overview presentations, and I particularly thank Dr. Christine Seidman for agreeing to summarize the Meeting.

R. Lifton, Dorcas Cummings Lecture

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 for these Symposia to remain a success and we are most grateful for their continued support. *Corporate Benefactors:* Aventis Pharma AG; Bristol-Myers Squibb Company; Eli Lilly and Company; GlaxoSmithKline; Novartis Pharma AG; Pfizer Inc. *Corporate Sponsors:* Abbott Laboratories; Amgen Inc.; Amersham Biosciences, Inc.; Applied Biosystems; AstraZeneca; BioVentures, Inc.; Chugai Pharmaceutical Co., Ltd.; Cogene BioTech Ventures, Ltd.; Diagnostic Products Corporation; Forest Laboratories, Inc.; Genentech, Inc.; Hoffmann-La Roche Inc.; Johnson & Johnson Pharmaceutical Research & Development, LLC; Kyowa Hakko Kogyo Co., Ltd.; Merck Research Laboratories; New England Biolabs, Inc.; OSI Pharmaceuticals, Inc.; Pall Corporation; Pharmacia Corporation; ResGen, Inc.; Schering-Plough Research Institute; Wyeth Genetics Institute. *Plant Corporate Associates:* MeadWestvaco Corporation; Monsanto Company; Pioneer Hi-Bred International, Inc. *Corporate Affiliates:* Affymetrix, Inc.; Ceptyr, Inc. *Corporate Contributors:* Biogen, Inc.; EpiCentre Technology.; Immuno-Rx, Inc.; Integrated DNA Technologies, Inc.; Invitrogen; KeyGene; Lexicon Genetics, Inc.; Prolinx, Inc.; Qbiogene; ZymoGenetics, Inc. *Foundations:* Albert B. Sabin Vaccine Institute, Inc.

B. Stillman, R. Hynes, C. Seidman, R. Benezra

R. Hynes, D. Stewart

R. Harvey, P. Krieg, M. Nemer

PROGRAM

Introduction
Chairperson: C. Simon, *University of Pennsylvania School of Medicine, Philadelphia*

Angiogenesis and Lymphangiogenesis
Chairperson: E. Olson, *University of Texas Southwest Medical Center, Dallas*

Early Cardiogenesis
Chairperson: M. Rupnick, *Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts*

Congenital Heart Disease and the Failing Heart
Chairperson: P. Krieg, *University of Arizona College of Medicine, Tucson*

Reginald G. Harris Lecture: Class II Histone Deacetylases Act as Signal-responsive Repressors of Cardiac Hypertrophy and Heart Failure
Speaker: Eric Olson, *University of Texas Southwestern Medical School*

Genetics and Genomics of the Heart
Chairperson: E. McNally, *University of Chicago, Illinois*

Blood Pressure/Atherosclerosis/Lipid Homeostasis
Chairperson: R.S. Williams, *Duke University Medical Center, Durham, North Carolina*

Cardiovascular Development
Chairperson: R. Pasqualini, *University of Texas/M.D. Anderson Cancer Center, Houston*

Vascular Biology
Chairperson: D. Stainier, *University of California, San Francisco*

Regulation of the Heart
Chairperson: T. Mohun, *National Institute for Medical Research, London, United Kingdom*

Dorcas Cummings Lecture: Salt and Blood Pressure: New Insights from Human Genetic Studies
Speaker: Richard Lifton, *Yale University School of Medicine*

Angiogenesis and Disease
Chairperson: K. Moulton, *Children's Hospital, Boston, Massachusetts*

Cardiovascular Repair and Therapy
Chairperson: R. Hynes, *Massachusetts Institute of Technology, Cambridge*

Summary
Christine Seidman, *Harvard Medical School*

L. Goyal, E. Olson

G. Yancopoulos, R. Kalluri

U. Savla, N. Rosenthal

MEETINGS

Evolution of Developmental Diversity

April 17–21 149 participants

ARRANGED BY **Richard Behringer,** The University of Texas/M.D. Anderson Cancer Center
Nipam Patel, HHMI/University of Chicago

This first Evolution of Developmental Diversity meeting brought together a diverse group of scientists from around the world studying various molecular genetic and developmental aspects of animal and plant biology, with a strong emphasis on morphological and functional comparisons of organismal diversity and evolution.

The meeting opened with a keynote lecture given by Walter Gehring (Basel) on eye evolution. The meeting was organized into eight sessions whose topics reflected the most advanced and interesting areas of study in the evolution and development field, including genetics, the evolution of gene regulation, genomics, axial pattern, segmentation, and regionalization, speciation, and organogenesis. Each session had three invited talks from senior researchers. Between these more in-depth talks, six speakers per session were selected from the abstracts for more concise presentations. The meeting also included the presentation of many posters in two afternoon sessions.

Research utilizing the primary animal and plant model systems to understand developmental mechanisms has made great strides over the last few decades, providing a strong molecular and cellular understanding of conserved developmental pathways. However, it is clear that the very few primary model organisms are not sufficient to understand the tremendous diversity of animal and plant life on earth. Understanding the generation of this diversity, honed over eons of evolution, most likely will provide important insights into the mechanisms that cause organ variation and the evolution of species. These insights should provide novel information for understanding human health and disease mechanisms. Thus, it is now clear that other animal and plant models must be used to complement studies utilizing the primary model organisms. This use of diverse organisms to study biological questions was most evident during the session on Axial Pattern chaired by Nori Satoh (Kyoto) who introduced the session as nine talks on nine different organisms!

This meeting was funded in part by the National Science Foundation. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

R. Behringer, A. Godwin, R Thummel

PROGRAM

Genetics I
Chairperson: M. Felix, *Institut Jacques Monod, Paris, France*

Evolution of Gene Regulation
Chairperson: S. Carroll, *University of Wisconsin, Madison*

Genomics
Chairperson: J. Postlethwait, *University of Oregon, Eugene*

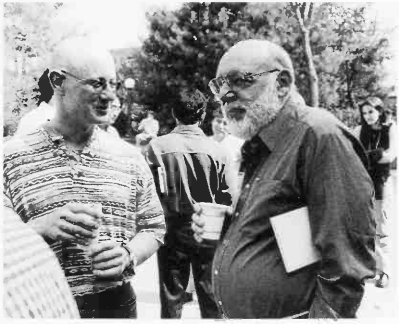
Axial Pattern
Chairperson: N. Satoh, *Kyoto University, Japan*

Segmentation and Regionalization
Chairperson: T. Kaufmann, *Indiana University, Bloomington*

Genetics II
Chairperson: D. Weigel, *Salk Institute for Biological Studies, La Jolla, California*

Speciation
Chairperson: C.-I. Wu, *University of Chicago, Illinois*

Organogenesis: Morphological Innovation
Chairperson: A. Burke, *Wesleyan University, Middletown, Connecticut*



J. Wittbrodt, W. Gehring

L. Pick, U. Schmidt-Ott

N. Patel, C. Sackerson, N. King, S. Carroll

Gene Expression and Signaling in the Immune System

April 24–28 385 participants

ARRANGED BY **Richard Flavell,** Yale University, New Haven, Connecticut
Rudolf Grosschedl, University of Munich, Institute for Biochemistry and Gene Center, Munich, Germany
Stephen Smale, HHMI/University of California School of Medicine, Los Angeles

This meeting included eight platform and two poster sessions that covered 275 abstracts. The first session on stem cells and early developmental decisions highlighted presentations on signaling in hematopoietic stem cells and on the role of Pax5 in B-cell commitment. The second session on the regulation of immune cell differentiation included presentations on mouse knock-out models for several key transcriptional regulators of differentiation. The session on chromatin structure and epigenetic regulation was highlighted by presentations on the role of Runx proteins in CD4 silencer function and on the relationship between histone acetylation, chromatin accessibility, and V(D)J gene rearrangement at T-cell receptor loci. In the session on control of antigen receptor gene assembly, the molecular dissection of the V(D)J recombination pathway was described. In addition, groundbreaking studies of a recently discovered regulator of both somatic hypermutation and Ig class switching, the activation-induced cytidine deaminase, were discussed. The session on antigen receptor signaling included presentations on the role of a G-protein-coupled receptor in the regulation of lymphocyte pools and on the effect of the T-cell maturation state on the formation of the T-cell synapse. The session on cytokine signaling and cell activation included presentations on the activation of cytokine genes by STAT proteins and on development of autoimmunity in IL-6-deficient mice. Highlighting the session on cell death were presentations on pro-apoptotic and anti-apoptotic Bcl-2 family members and on the production of autoantibodies in Fas-deficient mice. In the last session on T helper cell differentiation, the function of the transcription factor T-bet in the activation of the Th1 differentiation program and in B-cell isotype switching was discussed. This session was also highlighted by presentations on the role of the transcription factor Gata-3 in Th2 differentation and on functional antagonism between T-bet and Gata-3.

This meeting was funded in part by Amgen, Inc.; Boehringer Ingelheim Pharmaceuticals; Immunex; Pfizer; Wyeth-Ayerst Pharmaceuticals; Abbott Laboratories; Biogen; and Merck & Co., Inc. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

R. Grosschedl, S. Smale, R. Flavell

PROGRAM

Stem Cells and Early Developmental Decisions
Chairperson: I. Weissman, *Stanford University School of Medicine, California*

Regulation of Immune Cell Differentiation
Chairperson: K. Calame, *Columbia University College of Physicians & Surgeons, New York*

Chromatin Structure and Epigenetic Regulation
Chairperson: A. Rao, *Harvard Medical School, Boston, Massachusetts*

Control of Antigen Receptor Gene Assembly
Chairperson: D. Schatz, *Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, Connecticut*

Antigen Receptor Signaling
Chairperson: O. Witte, *Howard Hughes Medical Institute, University of California, Los Angeles*

Cytokine Signaling and Cell Activation
Chairperson: J. Darnell, *The Rockefeller University, New York*

Cell Death
Chairperson: C. Thompson, *University of Pennsylvania, Philadelphia*

T-helper Cell Differentiation
Chairperson: L. Glimcher, *Harvard School of Public Health, Boston, Massachusetts*

A. Feeney, P. Goebel, M. Nussenzweig

F. Radtke, H. Salminen, O. Lassila, P. Nieminen

X. Zhang, A. Khlaed

Molecular Chaperones and the Heat Shock Response

May 1–5 299 participants

ARRANGED BY **Elizabeth Craig,** University of Wisconsin, Madison
Carol Gross, University of California, San Francisco
Arthur Horwich, HHMI/Yale University School of Medicine

Twenty years after the inaugural molecular chaperones and the heat shock response meeting, this meeting featured advances in the areas of structure and mechanism of action of molecular chaperones; mechanisms of induction of the stress response; and nature and mechanism of protein misfolding in disease. In the first area, further advances were described for the classic heat shock proteins, the Hsp20, 60, 70, 90, and 100 classes, as well as more recently identified chaperones such as Hsp33. In a session of particular interest, the relationship between chaperone function and proteolysis was featured, with talks emphasizing the proteosome regulatory particle, targeting of substrates to Clp proteases, and the interaction between classic molecular chaperones and cellular proteolytic machinery. In the area of regulation of stress responses, a highlight of the meeting was a discussion of a systems approach to the complex problem of a stress response. In addition, progress toward an understanding of the function of heat shock transcription factors in complex organisms was discussed. In the area of protein misfolding and disease, a number of talks dealt with progress in the use of model systems in yeast, flies, and worms to attack this complex problem, particularly with regard to polyglutamine diseases. In addition, a number of talks discussed the biogenesis and maintenance of prions in yeast, and the roles that different chaperones have in this process.

This meeting was funded in part by the National Institute of Neurological Disorders and Stroke, a branch of the National Institutes of Health; Invitrogen Life Technologies; Merck & Co., Inc.; and the Alzheimer Association. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

A. Howrich, C. Gross, E. Craig

J. Carver, A. Aquilina, T. Treweek

PROGRAM

Diseases of Protein Misfolding
Chairperson: C. Dobson, *University of Cambridge, United Kingdom*

Quality Control and Protein Trafficking
Chairperson: P. Walter, *Howard Hughes Medical Institute, University of California, San Francisco*

Cellular Response to Stress
Chairperson: B. Bukau, *Albert-Ludwigs-University, Freiburg, Germany*

Chaperone Function in Disease and Development
Chairperson: A. Horwich, *Yale University School of Medicine, New Haven, Connecticut*

Regulation of the Stress Response
Chairperson: R. Morimoto, *Northwestern University, Evanston, Illinois*

Chaperones and Proteolysis
Chairperson: D. Finley, *Harvard Medical School, Boston, Massachusetts*

Chaperone Biochemistry and Protein Folding
Chairperson: K. Wüttrich, *Swiss Federal Institute of Technology, Zürich*

A. Chadli, N. Benaroudj

D. Toft, M. Welsh

Genome Sequencing and Biology

May 7–11 521 participants

ARRANGED BY **Pui-Yan Kwok,** Washington University
Jane Rogers, The Sanger Center
Edward Rubin, Lawrence Berkeley National Laboratory

This meeting marked the 15th 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 a "working draft" of the mouse and human genome sequences and several "finished" human chromosomes. Just over 500 people from around the world attended the meeting, with 331 abstracts presented describing a broad array of topics relating to the analysis of genomes from a number of different organisms.

The session topics included areas such as biological insights from comparative genomics, functional genomics, organismal biology, computational genomics, and the use of sequence variations to study populations and mechanisms of disease. Once again, projection-style, interactive computer demonstrations in Grace Auditorium effectively highlighted the critical new bioinformatics tools being developed for storing, organizing, and analyzing various genomic data sets, including expression, mapping, and sequence data. An ELSI (Ethical, Legal, and Social Implications) panel discussion, moderated by Francis Collins, focused on issues surrounding genetic enhancement and in-depth functional analysis and annotation of human chromosome 21.

The major themes of the meeting included the analysis and comparisons of the human and mouse draft genome sequences and in-depth functional analysis and annotation of human chromosome 21. Specific presentations continued to report major achievements in the sequencing of model organisms, including *Fugu rubripes* and *Ciona intestinalis*, the development of new technologies for genetic analysis, and various high-throughput approaches aimed at comparative sequence-based strategies "cracking" the gene regulatory code. The Friday afternoon keynote talks were delivered by Richard Gibbs and Svante Pääbo.

P.-Y. Kwok, M. Boguski, J. Rogers

This meeting was funded in part by the National Human Genome Research Institute, a branch of the National Institutes of Health. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

PROGRAM

Human Mouse Symposia
Chairpersons: J. McPherson, *Washington University School of Medicine, St. Louis, Missouri;* T. Taylor, *RIKEN Genomic Sciences Center, Tokyo, Japan*

Variations I
Chairpersons: A. Gnirke, *Exelixis, Inc., South San Francisco, California;* L. Cardon, *University of Oxford, United Kingdom*

Bioinformatics
Chairpersons: J. Mullikin, *Wellcome Trust Sanger Institute, Cambridge, United Kingdom;* D. Haussler, *Howard Hughes Medical Institute/University of California, Santa Cruz*

Comparative Genomics
Chairpersons: L. Pachter, University of California, Berkeley; *A. Bradley,* Sanger Centre, Hinxton, United Kingdom

ELSI Panel Discussion: Pharmacogenomics, Identified Populations, and Group Stigma

Moderator
Francis Collins, M.D., Ph.D., *National Human Genome Research Institute*

Panelists
Mark A. Rothstein, J.D., *University of Louisville School of Medicine*
Pamela L. Sankar, Ph.D., University of Pennsylvania Center for Bioethics

Organismal Genomics
Chairpersons: D. Kingsley, *Stanford University, California;* M. Fishman, *Massachusetts General Hospital/Harvard Medical School, Boston*

High-throughput Biology
Chairpersons: J. Ahringer, *University of Cambridge, United Kingdom;* U. Landegren, *University of Uppsala, Sweden*

Keynote Speakers
Richard Gibbs, *Baylor College of Medicine*
Svante Pääbo, *Max-Planck Institute for Evolutionary Anthropology*

Variations II
Chairpersons: H. Hobbs, *University of Texas Southwestern Medical Center, Dallas;* L. Almasy, *Southwest Foundation for Biomedical Research, San Antonio, Texas*

R. Wilson, J. McPherson

J. Kere, M. Daly

Laptops, Laptops, Laptops!!

The Cell Cycle

May 15–19 346 participants

ARRANGED BY **Stephen Elledge,** Baylor College of Medicine
J. Wade Harper, Baylor College of Medicine
David O. Morgan, University of California, San Francisco

This seventh biannual 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. We were particularly fortunate this year, due to the fact that the Nobel Prize in Physiology or Medicine was awarded in the fall of 2001 to three pioneers in cell cycle research: Paul Nurse, Tim Hunt, and Lee Hartwell. The meeting featured two exciting plenary lectures given by both Tim Hunt and Paul Nurse. Paul Nurse presented a wide-ranging and exciting talk describing the discovery of the central genes that control the cell division cycle in *Schizosaccharomyces pombe*, and their role in the discovery of parallel systems in human cells. This was followed by a mid-meeting lecture from Tim Hunt on the biochemical discovery of cyclins and their role in embryonic cell cycles. Three broad themes were dominant throughout the meeting: the interaction of cell cycle control with developmental and cancer biology; the mechanisms of action of cell cycle checkpoints and their integration with signal pathways that control mitotic transitions; and the emerging use of high-throughput and whole-genome approaches to uncover new aspects of cell biology.

Research in proteolysis for both G_1/S and G_2/M regulation in particular continues to receive intense focus, with much progress reported. As in previous years, there was also emphasis on the long-standing problem of regulation of DNA replication in the cell cycle, its onset, and its restriction to once per cell cycle. New to the discussion was a more detailed understanding of how DNA-damage checkpoint pathways interface with replication pathways. There was also an emphasis on the use of emerging technologies, both in tissue culture cells and in live animals such as *Caenorhabditis elegans*, to study the dynamics of signaling pathways and rapid changes in the localization of components of the mitotic apparatus in response to checkpoint activation. As in other years, scientists studying cell cycle regulation in yeast, *Xenopus, Drosophila*, nematodes, and mammals were well represented. Once again, the striking phylogenetic conservation of cell cycle regulatory mechanisms was readily evident. This meeting also served to demonstrate the impact of whole-genome analysis on basic cell biology, and

F. Luca, D. Morgan, W. Harper

these achievements will no doubt continue to drive rapid progress in this field. The cell cycle conference brought together 353 scientists for the presentation of 240 abstracts. It was another landmark meeting for the cell cycle field, and the participants all continued to look forward to equally exciting meetings in future years.

This meeting was funded in part by the National Cancer Institute, a branch of the National Institutes of Health, and the National Science Foundation. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

PROGRAM

Keynote Speaker
Paul Nurse, *Cancer Research, United Kingdom*

CDK Regulation
Chairperson: R. Deshaies, *California Institute of Technology, Pasadena*

G_1 Control
Chairperson: B. Andrews, *University of Toronto, Canada*

Replication
Chairperson: J. Diffley, *Imperial Cancer Research Fund, South Mimms, United Kingdom*

Mitosis I
Chairperson: A. Amon, *Massachusetts Institute of Technology Cancer Center/Whitehead Institute, Cambridge*

Mitosis II
Chairperson: E. Salmon, *University of North Carolina, Chapel Hill*

Mitotic Checkpoints
Chairperson: S. Reed, *Scripps Research Institute, La Jolla, California*

Controls
Chairperson: A. Carr, *University of Sussex, Falmer, United Kingdom*

Development and Cancer
Chairperson: J. Lees, *Massachusetts Institute of Technology Center for Cancer Research, Cambridge*

K. Dej, J. Claycomb, I. Ivanovska

J.D. Watson, L. Gann, T. Hunt, P. Nurse

J. Singer, S. Elledge

Retroviruses

May 21–26 450 participants

ARRANGED BY **Karen Beemon,** Johns Hopkins University
Julie Overbaugh, Fred Hutchinson Cancer Research Center

This annual meeting originated in 1975 as a meeting on RNA Tumor Viruses and evolved to its current focus on Retroviruses in 1993. This 27th meeting was enriched by two keynote speakers, who discussed challenges of HIV-1 prevention and treatment, both in Africa and in the United States. Julie Overbaugh gave a moving talk on "The HIV-1 epidemic in Africa—What are the opportunities and challenges for molecular retrovirologists?" This was followed by a talk by Bob Siliciano who discussed "Mechanisms of viral persistence in HIV infection—Basic science and clinical implications."

This meeting included 369 platform and poster presentations, distributed into 13 scientific sessions. The field seems to be shifting from studies of a single viral gene product and its role in the viral life cycle toward more complex events such as viral assembly or pathogenesis, involving host-virus interactions. Due to the great interest in virus assembly this year, two sessions were devoted to this topic. In addition, this meeting introduced a new session on evolution, which included discussions of both viral and host evolution and presented genomic data. Another new session was devoted to RNA processing, export, and translation. Three poster sessions, including one evening session, were arranged alphabetically rather than topically this year. The meeting was particularly enjoyable due to perfect weather and wonderful cuisine.

Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

K. Beemon, J. Overbaugh

R. Swanstrom, G. Kalpana

PROGRAM

Entry
Chairpersons: L. Albritton, *University of Tennessee Health Sciences Center, Memphis;* J. Kimata, *Southwest Foundation for Biomedical Research, San Antonio, Texas*

Assembly I
Chairpersons: C. Carter, *State University of New York, Stony Brook;* S. Goff, *Howard Hughes Medical Institute/Columbia University, New York*

Assembly II
Chairpersons: L. Parent, *Pennsylvania State University College of Medicine, Hershey;* A. Rein, *National Cancer Institute, Frederick, Maryland*

Transcription and Packaging
Chairpersons: F. Yoshimura, *Wayne State University, Detroit, Michigan;* M. Linial, *Fred Hutchinson Cancer Research Center, Seattle, Washington*

RNA Processing, Export, and Translation
Chairpersons: D. Purcell, *University of Melbourne, Parkville, Australia;* K. Boris-Lawrie, *Ohio State University, Columbus*

Keynote Address: The HIV-1 epidemic in Africa: What are the opportunities and challenges for molecular retrovirologists?
Speaker: Julie Overbaugh, *Fred Hutchinson Cancer Research Center*

Pathogenesis
Chairpersons: S. Ross, *University of Pennsylvania, Philadelphia;* C.-Z. Giam, *Uniformed Services University, Bethesda, Maryland*

Keynote Address: Mechanisms of viral persistence in HIV infection: Basic science and clinical implications.
Speaker: Bob Siliciano, *Johns Hopkins University*

Evolution
Chairpersons: H. Levin, *National Institutes of Health, Bethesda, Maryland;* J. Coffin, *Tufts University School of Medicine, Boston, Massachusetts*

RT and Recombination
Chairpersons: R. Bambara, *University of Rochester Medical Center, New York;* V. Pathak, *National Cancer Institute, Frederick, Maryland*

Integration and FV1
Chairpersons: M. Katzman, *Pennsylvania State University Medical Center, Hershey;* P. Bieniasz, *Aaron Diamond AIDS Research Center, New York*

Therapy and Vectors
Chairpersons: V. KewalRamani, *National Cancer Institute, Frederick, Maryland;* P. Pitha-Rowe, *Johns Hopkins University School of Medicine, Baltimore, Maryland*

J. Coffin, L. Hammarskjöld, C. Carter

M. Laughrea, M. Mougel, P. Boulanger

New York Structural Biology Group

June 26 230 participants

ARRANGED BY **Larry Shapiro,** Mount Sinai School of Medicine
Leemor Joshua-Tor, Cold Spring Harbor Laboratory

The summer meeting of the New York Structural Biology Discussion Group was the fifth in day-long meetings allowing structural biologists from all over the region to meet and discuss their latest results. The meeting was open to structural biologists from different disciplines, including crystallographers, spectroscopists, computational biologists, and biochemists, with more than 225 participants from academia and industry from the tri-state area. The program featured nine talks and a poster session and concluded with a beach barbecue, 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.

This meeting was co-sponsored by the New York Academy of Sciences. Financial support was provided by Amersham Pharmacia Biotech Inc., Bruker Nonius Crystallographic Systems, Hampton Research, New York Structural Biology Center, and Rigaku/MSC.

PROGRAM

SESSION I
Chairperson: Marilyn Gunner, *City College, New York*

Eddy Arnold, *Rutgers University:* Structural basis of HIV reverse transcriptase resistance to AZT and other nucleoside analog drugs.
David Eliezer, *Weill Medical College of Cornell University:* NMR studies of α-synuclein.
Stewart Shuman, *Memorial Sloan-Kettering Cancer Center:* RNA repair.

SESSION II
Chairperson: Hermann Schindelin, *SUNY, Stony Brook*

Andrej Sali, *The Rockefeller University:* 3D modeling of protein assemblies.
Terrence Strick, *Cold Spring Harbor Laboratory:* Single-molecule detection of mechanically and enzymatically driven DNA denaturation.

SESSION III
Chairperson: Larry Shapiro, *Mount Sinai School of Medicine*

Qun Shen, *Cornell University:* Direct phasing using a reference beam.
David Stokes, *New York University:* Electron tomography of desmosomes.
Wayne Hendrickson, *Columbia University College of Physicians & Surgeons:* Signal transduction by histidine kinase sensors.

H. Schindelin, L. Joshua-Tor

W. Hendrickson, H. Wu, L. Shapiro, D. Eliezer

Cancer Genetics and Tumor Suppressor Genes

August 14–18 429 participants

ARRANGED BY **Doug Hanahan,** University of California, San Francisco
Jacqueline Lees, MIT Center for Cancer Research
Scott Lowe, Cold Spring Harbor Laboratory
Charles Sherr, HHMI/St. Jude Children's Research Hospital

This fourth cancer meeting focused on topics related to cancer genetics and cancer biology. More than 425 scientists, most of whom presented unpublished research through oral or poster presentations, attended the conference. The talks were highlighted by two keynote lectures: Anton Berns discussed recent advances in the use of mouse models to model human cancer, highlighting their potential utility for identifying new cancer genes and use for studying cancer therapeutics. Steven Elledge discussed recent advances in DNA, including the molecular framework of cellular DNA damage responses and their roles in tumor damage responses.

An international team of established investigators, whose expertise covered many of the topics highlighted during the meeting, chaired the talks. The first oral session discussed new mouse models, which continue to be an important topic at the meeting. Indeed, many other talks and posters highlighted the use of these mice to study gene function and to model human cancer. Other sessions highlighted recent advances in our understanding of DNA-damage responses, apoptosis, and cellular senescence, the Rb and p53 tumor suppressor pathways, and signal transduction pathways that are altered in human tumors. One session highlighted technological advances for identifying and characterizing cancer genes, which included a presentation by Greg Hannon on RNA interference. The meeting again featured a special session called "NCI Listens," chaired by William Kaelin, and also had a special presentation by Cheryl Marks (NCI) on the Mouse Models of Human Cancer Consortium. This meeting continued to be enthusiastically supported, and all sessions found the lecture and poster halls packed with extensive discussions and exchanges of information and ideas.

This meeting was funded in part by the National Cancer Institute, a branch of the National Institutes of Health. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

S. Lowe, D. Hanahan, C. Sherr, J. Lees

PROGRAM

Mouse Models
Chairpersons: S. Cory, *Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia;* L. Chin, *Harvard Medical School, Boston, Massachusetts*

Keynote Address: Mouse Models for Cancer Have Come a Long Way
Speaker: Anton Berns, *The Netherlands Cancer Institute*

Checkpoints and DNA-damage Responses
Chairpersons: M. Jasin, *Memorial Sloan-Kettering Cancer Center, New York;* J. Petrini, *University of Wisconsin, Madison*

Keynote Address: The DNA Damage Response
Speaker: Stephen Elledge, *Baylor College of Medicine*

Signaling and Transcription in Oncogenesis
Chairperson: J. Downing, *St. Jude Children's Research Hospital, Memphis, Tennessee;* R. Hynes, *Massachusetts Institute of Technology, Cambridge*

RB/E2F
Chairpersons: D. Livingston, *Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts;* J. Lees, *MIT Center for Cancer Research, Cambridge, Massachusetts*

"NCI Listens"
Chairperson: William G. Kaelin, Jr., M.D., *Associate Investigator for Howard Hughes Medical Institute; Associate Professor, Department of Adult Oncology, Dana Farber Cancer Institute; and Harvard Medical School*

Speaker: Dinah S. Singer, *National Cancer Institute, National Institutes of Health: An Overview of NCI Programs and Initiatives.*
Moderator: Paulette S. Gray, *National Cancer Institute, National Institutes of Health*

C. Richardson, C. Mueller, J. Adams

The p53 Family
Chairpersons: C. Prives, *Columbia University, New York;* G. Evan, *University of California Comprehensive Cancer Center, San Francisco*

Apoptosis and Tumor Progression
Chairpersons: J. Adams, *Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia;* L. Matrisian, *Vanderbilt University School of Medicine, Nashville, Tennessee*

Senescence and Immortalization
Chairpersons: D. Galloway, *Fred Hutchinson Cancer Research Center, Seattle, Washington;* T. de Lange, *The Rockefeller University, New York*

New Cancer Genes, Targets, and Therapies
Chairpersons: L. Iruela-Arispe, *University of California, Los Angeles;* G. Hannon, *Cold Spring Harbor Laboratory*

M. Fried

S. Cory, V. Ivanov

Molecular Genetics of Bacteria and Phages

August 20–25 205 participants

ARRANGED BY **Robert Landick,** University of Wisconsin, Madison
Thomas Silhavy, Princeton University
Catherine Squires, Tufts University School of Medicine

The meeting featured 90 talks arranged in 10 sessions, two poster sessions, and a special workshop. Young scientists gave most of the talks, and a senior scientist who presented an introductory overview moderated each session. Topics included The Bacterial Genome: Structure, Recombination, and Transposition; RNA Polymerase Structure and Function, Regulation of Transcription; Posttranscriptional Control, Ribosomes, and Translation; Signaling and Global Circuits; Cell Surfaces, Secretion and Import; Chaperones, Heat Shock, and Protein Degradation; Cell Division and Development; and Host/Pathogen Interactions. One of the themes that emerged from this meeting was the remarkable conservation in biology. van den Ent (representing the Gerdes, University of Southern Denmark, and Lowe, MRC, Cambridge England, groups) reported that the shape-determining protein MreB from *Bacillus* has a three-dimensional structure remarkably similar to actin, and this finding complements the previously identified bacterial homolog of tubulin, FtsZ. The structures of the multisubunit bacterial and eukaryotic RNA polymerases are strikingly similar, and this conservation of structural motifs across great evolutionary distances was highlighted in the afternoon workshop on RNA polymerase. Two new structures of active transcription elongation complexes formed by the single-chain phage T7 RNA polymerase were described in detail by Vassylyev (Riken Cell Signalling Group, Japan) and Yin (Steitz group, Yale). Although T7 RNA polymerase exhibits little if any sequence similarity to the multi-

C. Squires, R. Landick

T. Silhavy, L. Rothman-Denes, O. Amster-Choder

subunit polymerases, the structures bore remarkable resemblance to the previously determined structure of the yeast RNAP polymerase II transcription elongation complex.

This meeting was funded in part by the National Science Foundation. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

PROGRAM

Cell Surfaces, Secretion, and Import
Chairperson: R. Misra, *Arizona State University, Tempe*

RNA Polymerase: Structure, Function, and Catalysis
Chairperson: W. McAllister, *State University of New York Downstate Medical Center, Brooklyn*

Posttranscriptional Control, Ribosomes, and Translation
Chairperson: A.E. Dahlberg, *Brown University, Providence, Rhode Island*

Signaling and Global Circuits
Chairperson: L. Kenney, *Oregon Health Science University, Portland*

Workshop: New Insights into RNA Polymerase Structure
R. Landick, *University of Wisconsin, Madison*

Regulation of Transcription I
Chairperson: S. Wickner, *National Institutes of Health, Bethesda, Maryland*

Chaperones, Heat Shock, and Protein Degradation
Chairperson: B. Bukau, *Albert-Ludwigs-University Freiburg, Germany*

Cell Division and Development
Chairperson: P. Levin, *Washington University, St. Louis, Missouri*

The Bacterial Genome: Structure, Recombination, and Transposition
Chairperson: S. Rosenberg, *Baylor College of Medicine, Houston, Texas*

Host/Pathogen Interactions
Chairperson: A. Camilli, *Tufts University, Boston, Massachusetts*

Regulation of Transcription Initiation and Elongation
Chairperson: M. Gottesman, *Columbia University, New York*

W. Williams, K. Ramamurthi

T. Inada, K. Kvint

Mouse Molecular Genetics

August 28–September 1 334 participants

ARRANGED BY **Richard Behringer,** The University of Texas/M.D. Anderson Cancer Center
Carmen Birchmeier, Max Delbruck Center, Germany
Hiroshi Hamada, Osaka University
Terry Magnuson, University of North Carolina, Chapel Hill

This meeting, held every other year, attracted more than 325 researchers from across the globe. The meeting brings together a vast cadre of scientists with very diverse backgrounds who are linked by the common thread of working with mouse mutants. Consequently, the scope of the biology presented at the meeting is enormous, covering topics from early developmental decisions in gastrulating embryos to the behavior of mice.

The meeting opened with the 2nd Annual Rosa Beddington Lecture given by Kathryn Anderson. Following a historically successful format, the meeting was organized into eight sessions each arranged so that a session begins and ends with an invited senior researcher. Between these more in-depth talks (which are selected to be of general interest), eight to nine speakers per session were selected from the abstracts for more concise presentations. The meeting also included the presentation of several hundred posters in two afternoon sessions. To highlight current interests in the mouse molecular genetics community, two workshops on Sperm Cryopreservation and Recovery of Mouse Strains by Assisted Reproduction (Larry Mobraaten, discussion leader) and BAC Modification and Transgenesis (Francis Stewart, discussion leader) were arranged where several speakers presented technical information and their approaches. Although the workshops partially overlapped with the poster sessions, these workshops were very well attended.

The mouse meeting has evolved over the years to have a stronger genetic and organogenesis/disease emphasis, and this year, invited presentations included detailed presentations on the newly assembled and annotated public mouse genome, complex genetic traits, and models of neural tube, breast, prostate, skeleton, germ cell, and endoderm development and disease. Richard Palmiter, one of the organizers of the first meeting and a pioneer in transgenic research, returned to give an invited lecture in the Neurobiology session. The meeting exemplified some of the special characteristics of the mouse genetics community—sharing of unpublished data and reagents (including mice). Inevitably, this meeting is also a mouse dating service! Mice described in the meeting have found their way to new homes to mate with other mutants to prove (or disprove) hypotheses developed during interactions at Cold Spring Harbor.

R. Behringer, C. Birchmeier

This meeting was funded in part by the National Cancer Institute, The National Institute of Diabetes and Digestive and Kidney Diseases, the National Institute of Child Health and Human Development, and the National Institute of Neurological Disorders and Stroke, all branches of the National Institutes of Health. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

PROGRAM

Rosa Beddington Lecture: Genetic Analysis of Early Embryonic Patterning
Speaker: K. Anderson, *Memorial Sloan-Kettering Cancer Center, New York*

Functional Genomics
Chairperson: L. Stubbs, *Lawrence Livermore National Laboratory, California*

Signals, Receptors, and Transcription
Chairperson: R. Grosschedl, *University of Munich, Germany*

Workshop I: Sperm Cryopreservation and Recovery of Mouse Strains by Assisted Reproduction
L. Mobraaten, *Jackson Laboratory, Bar Harbor, Maine*

Patterning
Chairperson: Y. Saga, *National Institute of Genetics, Mishima, Japan*

New Technologies
Chairperson: J. Schimenti, *Jackson Laboratory, Bar Harbor, Maine*

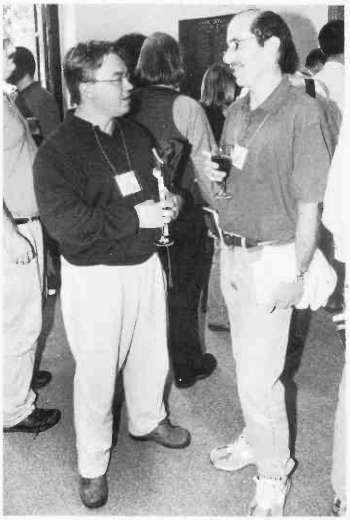
Workshop II: BAC Modification and Transgenesis
F. Stewart, *Technische Universität Dresden, Germany*

Organogenesis
Chairperson: L. Hennighausen, *National Institutes of Health, Bethesda, Maryland*

Neurobiology
Chairperson: R. Palmiter, *Howard Hughes Medical Institute/ University of Washington, Seattle*

Models of Human Disease
Chairperson: C. Abate-Shen, *UMDNJ-Robert Wood Johnson Medical School, Piscataway, New Jersey*

Genetics
Chairperson: E. Li, *Massachusetts General Hospital, Charlestown*



S. Duncan, K. Zaret

J. Mager, K. Tremblay

J. Hadchovel, F. Relaix, T. Natoli

SECOND CSHL/WELLCOME TRUST CONFERENCE
Genome Informatics

September 4–8 232 participants

ARRANGED BY **Ewan Birney,** European Bioinformatics Institute
Suzanna Lewis, University of California, Berkeley
Lincoln Stein, Cold Spring Harbor Laboratory

This second conference on Genome Informatics again focused on large-scale genome annotation and utilization. We chose to repeat the conference in this joint venue following the overwhelmingly positive reception of the first conference given in August 2001. The conference was again held September 4–8 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 Wellcome Trust Sanger Institute, the European Bioinformatics Institute, and the HGMP-MRC Resource Centre. The conference followed a format similar to that of traditional Cold Spring Harbor meetings, in that the majority of oral presentations were drawn from openly submitted abstracts.

The explosion of biological data requires a concomitant increase in the scale and sophistication of information technology. This ranges from the storage of data and their associated data models, to the design of effective algorithms to uncover nonobvious aspects of these data sets, to ontologies to concisely describe biological information, and finally to software systems to support curation, visualization, and exploration. The conference brought together some of the leading scientists in this growing field, and researchers from other large-scale information-handling disciplines were also invited to attend. In addition to the organizers, discussion leaders included Arek Kasprzyk, Fritz Roth, Lior Pachter, Sean Eddy, James Gilbert, Mark Yandell, Wyeth Wasserman, and Michael Eisen. The keynote speaker was Nobel Laureate Sydney Brenner. More than 50% of delegates came from North America, a highly unusual statistic for a European meeting, and the meeting hosted 111 scientific presentations in talks and posters.

PROGRAM

In Silico Data Discovery I
Chairpersons: A. Kasprzyk, *EMBL-EBI, Hinxton, United Kingdom;* F. Roth, *Harvard Medical School, Boston, Massachusetts*

Comparative Genomics
Chairpersons: L. Pachter, *University of California, Berkeley;* S. Eddy, *Washington University School of Medicine, St. Louis, Missouri*

Annotation Pipelines
Chairpersons: J. Gilbert, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom;* M. Yandell, *University of California, Berkeley*

Functional Genomics
Chairpersons: W. Wasserman, *Karolinska Institute, Stockholm, Sweden;* M. Eisen, *Lawrence Berkeley National Laboratory, California*

In Silico Data Discovery II
Chairpersons: A. Kasprzyk, *EMBL-EBI, Hinxton, United Kingdom;* F. Roth, *Harvard Medical School, Boston, Massachusetts*

Keynote Address
Speaker: Sydney Brenner, *Salk Institute for Biological Studies*

Integrative Biology
Chairpersons: R. Stevens, *University of Manchester, United Kingdom;* C. Mungall, *Berkeley Drosophila Genome Project, California*

The Hinxton Hall Conference Center

Translational Control

September 10–15 462 participants

ARRANGED BY **Matthias Hentze,** European Molecular Biology Laboratory
Alan Hinnebusch, National Institute of Child Health and Human Development, National Institutes of Health
Robert Schneider, New York University School of Medicine

The meeting included three keynote speakers, eight platform sessions, and three poster sessions that covered a record 387 oral and poster presentions. Highlights in the area of translation mechanisms included the three-dimensional structures of elongation factors eEFs 2 and 1Bγ and the binding domains in eIF4G (the scaffolding subunit of eIF4F) for cap-binding protein eIF4E, helicase eIF4A, and eIF4E kinase, Mnk1. It was shown that eIF4G interacts with eIF1 and participates in AUG selection, and multiple contacts between eIF3 and eIF2 in the multifactor complex containing eIFs 1 and 5 were shown to function downstream from initiator tRNA binding. The binding sites for eIF3, eIF1A, and eIF1 on the ribosome have been mapped, and it was proven that GTP hydrolysis by eIF5B is required for its release from the ribosome following subunit joining. Translation reinitiation can be stimulated by interaction of poly(A)-binding protein (PABP) with termination factor eRF3. The 60S protein L3 stimulates peptidyltransferase activity of the ribosome by enhancing peptidyl-tRNA binding to the P-site.

On the regulation of translation factors, it was reported that p53 down-regulates general translation initiation, tumor suppressor protein Pdcd4 inhibits eIF4A helicase activity, and p21-activated protein kinase γ-PAK inhibits eIF4G by phosphorylation. The GEF for eIF2, eIF2B, is inhibited during sepsis through phosphorylation by GSK-3, and eIF2α kinase GCN2 is regulated by TOR kinases and UV irradiation. The resumption of translation following ER stress requires the inhibition of eIF2α kinase PERK by p58-IPK and GADD34, a positive regulator of eIF2α phosphatase. PERK is critically involved in the secretion of extracellular matrix proteins during skeletal development in mice. The dsRNA-activated eIF2α kinase PKR is inhibited during HSV-1 infections by the viral protein Us11, which antagonizes PKR activator PACT. Adenovirus 100K protein inhibits host protein synthesis by competing with Mnk1 for eIF4G binding.

In the arena of gene-specific translational control, it was reported that translation of Sendai virus P/C mRNA involves a ribosomal shunt and methionine-independent translation initiation. A pseudoknot in interferon-γ mRNA inhibits translation in *cis* by localized activation of PKR, and c-Src activates 15-LOX mRNA translation by overcoming the repressing effects of hnRNPs K and E1, acting through the 3′UTR element DICE. Mitogen activation of TOP mRNA translation requires PI3 kinase but not mTOR

M. Hentze, A. Hinnebusch, R. Schneider

R. Jackson, T. Pestova

or S6 kinase-1. The inhibitory uORF in *arg-2* mRNA can mediate arginine-dependent translational repression when embedded in the coding sequence, and the uORF-encoded peptide in the CMV *UL4* gene inhibits its own termination and other initiation events downstream by interacting with release factor eRF1. The ELAV protein HuD negatively regulates the internal ribosome entry site (IRES) that controls p27Kip1 translation, whereas eIF2A inhibits the IRES in yeast URE2 mRNA in vivo. The binding site for cricket paralysis virus (CrPV) IRES was mapped on the 40S ribosome, and translation by this IRES was reconstituted with purified ribosomes and elongation factors, confirming its independence of eIFs. Programmed frameshifting in bacteria can be modulated by tRNA modification, or by a Shine-Delgarno sequence that destabilizes E-site tRNA.

Regarding translational control in development, a new cytoplasmic poly(A) polymerase, GLD-2, involved in regulating germ-line fate and early embryogenesis, was discovered in *C. elegans*. The worm GLD-1 regulates *tra-2* translation through interaction with PABP, and also represses *glp-1* mRNA via the 3′UTR. *Drosophila* sex-lethal (SXL) protein represses *msl2* mRNA in vitro by blocking mRNA binding to the 43S complex. The cytoplasmic polyadenylation element-binding protein (CPEB) and maskin positively and negatively regulate cyclin B mRNA translation, respectively, in *Xenopus* embryo extracts. Src negatively regulates RNA binding by QKI protein, regulating accumulation of myelin basic protein mRNA in the developing nervous system of the mouse. Finally, a cellular IRES in egg-laying hormone mRNA was shown to be regulated by physiological activity in *Aplysia* neurons.

This meeting was funded in part by the National Cancer Institute and the National Institute of Child Health and Human Development, branches of the National Institutes of Health. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

PROGRAM

Keynote Lectures

Speaker: Gideon Dreyfuss, *HHMI/University of Pennsylvania School of Medicine:* The SMN complex: An assembly machine for RNPs.

Speaker: Phillip Sharp, *Massachusetts Institute of Technology:* siRNAs.

Speaker: Eric Kandel, *HHMI/Columbia University:* Translational control in long-term memory and its persistence.

Initiation

Chairperson: O. Meyuhas, *Hebrew University–Hadassah Medical School, Jerusalem, Israel*

Translation Mechanisms

Chairperson: P. Farabaugh, *University of Maryland, Baltimore*

Regulation of Translation Factors

Chairperson: D. Ron, *New York University School of Medicine, New York*

Viral Strategies of Translational Control

Chairperson: V. Pain, *University of Sussex, Brighton, United Kingdom*

Regulatory Elements in mRNAs

Chairperson: M. Berry, *University of Hawaii, Manoa, Honolulu*

Internal Initiation

Chairperson: T. Pestova, *State University of New York Health Sciences Center, Brooklyn*

Developmental Regulation of Translation

Chairperson: J. Richter, *University of Massachusetts Medical School, Worcester*

Mechanisms of mRNA Turnover

Chairperson: G. Brewer, *University of Washington, Seattle*

J. Richter, A. Jacobson

J. Li, S.-H. Chiu, L. Maquat

Dynamic Organization of Nuclear Function

September 18–22 377 participants

ARRANGED BY **Wendy Bickmore,** MRC Human Genetics Unit
Gideon Dreyfuss, HHMI/University of Pennsylvania School of Medicine
David L. Spector, Cold Spring Harbor Laboratory
Katherine Wilson, Johns Hopkins University School of Medicine

The meeting brought together a diverse group of investigators to discuss the relationship between nuclear structure and function. In the opening session devoted to diseases, infection, and DNA damage, D.M. Livingston discussed recent observations indicating that the tumor suppressor protein BRCA1 may promote the localization of Xist RNA to the inactive X chromosome and thereby have a role in the maintenance of X chromosome inactivation. In BRAC1$^{-/-}$ cells, an absence of both the Xist RNA signal and histone H3 lysine 9 methylation was observed. Other talks on the role of BRAC1/BARD1 and RNA polymerase II in coordinating the response to DNA damage (F.E. Kleiman), laminopathies (T. Haraguchi, J.M. Holaska, K. Hoffmann), Emery-Dreyfus muscular dystrophy (B. Reichart), the assembly of DNA repair foci (R. Rothstein, C.M. Green), DNA replication checkpoints (C. Smythe), and dysmyelination (J. Pilotte) all highlighted the close association of nuclear structure to disease.

Numerous talks demonstrated the dynamics of chromatin and chromatin-associated proteins in living cells. S. Gasser discussed the dynamics of yeast chromatin in living cells using the *lac* operator/repressor system. Telomeres, centromeres, as well as silent mating-type loci exhibited constrained motion. Telomere anchoring to the nuclear periphery was shown to involve both the Sir and Ku proteins, suggesting that parallel pathways may exist. Interestingly, upon loss of silencing, increased chromosome motion was observed. In mammalian cells, J.R. Chubb demonstrated that chromatin associated with the nucleolus or nuclear periphery is more constrained in its movement than more nucleoplasmic loci, and D. Gerlich showed that chromosomes exhibit ordered positions that are inherited through mitosis in mammalian cells. S. Janicki discussed the development of a stable cell line in which an inducible DNA locus as well as its RNA and protein products could be directly visualized in living cells. Using this system, a loss of HP1 was observed as the locus transited from an inactive to an active state. In addition, RNA was detectable at the locus within 5 minutes of induction. Using FRAP analysis, T. Cheutin also demonstrated the dynamic nature of HP1 which exhibited complete recovery within 1 minute. G. Hager showed that whereas the glucocorticoid receptor and its coactivators exhibit short residence times at a promoter (5 sec), RNA polymerase II exhibited a longer residency time of 12–15 minutes. By combining advances in computational fluorescence microscopy with multiplex probe design, R.H. Singer described studies in which the expression of many genes can be visualized simultaneously inside single cells with high spatial and temporal resolution.

D. Spector, R. Nayak

Several studies reported on unexpected chromatin associations. P. de Lanerolle showed that nuclear myosin 1 coimmunoprecipitated with RNA polymerase II, and antiactin antibodies inhibited transcriptional initiation but not elongation. T. Kohwi-Shigematsu showed that SATB1 localized in a framework-like structure around heterochromatin in thymocyte nuclei and was resistant to DNase I

and salt extraction. SATB1 was shown to form protein complexes with ATP-dependent chromatin remodeling enzymes and histone deacetylases. M.S. Schmidt-Zachmann reported on NO145, a protein that localizes to a thin cortical layer forming a cage-like perinucleolar structure composed of a meshwork of filaments.

Proteomics was also widely discussed as R. Reed presented a comprehensive analysis of the human spliceosome, indicating that it contains 145 distinct proteins, and J.M. Cronshaw described a proteomic analysis of the mammalian nuclear pore complex that has identified 29 nucleoporins and 18 nuclear-pore-associated proteins. In addition, W. Bickmore described the development of a nuclear protein database that contains information on more than 1000 vertebrate nuclear proteins.

Numerous studies discussed the dynamic associations of the nuclear lamins (R.D. Goldman, T. Dechat, Y. Gruenbaum) and the dynamic organization of the nuclear pore complex (G. Rabut). In particular, R.D. Goldman reported that disruption of lamin organization resulted in an inhibition of RNA polymerase II, but not polymerases I and III transcription, and Y. Gruenbaum reported that reduction in *C. elegans* lamin protein causes embryonic lethality. Analysis of protein components of the nuclear pore complex by FRAP analysis indicated that individual nucleoporins exhibited very different dynamics, with average residency times ranging from a few seconds to several hours. The most stable nucleoporins appeared to be components of the spoke ring complex, whereas the most dynamic ones were found at the nuclear basket. A variety of other studies were presented on the dynamics of various nuclear bodies as well as nuclear import and export processes.

Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

PROGRAM

Nuclear Structure in Disease, Infection, and Damage
Chairperson: D.M. Livingston, *Dana-Farber Cancer Institute, Boston, Massachusetts*

Genome Organization and Transcription
Chairperson: S.M. Gasser, *ISREC, Epalinges, Switzerland*

RNA Processing
Chairperson: J.A. Steitz, *Yale University School of Medicine, New Haven, Connecticut*

Dynamic Properties of the Nuclear Envelope/Lamina
Chairperson: R.D. Goldman, *Northwestern University Medical School, Chicago, Illinois*

Nucleoli and Other Organelles
Chairperson: A.I. Lamond, *University of Dundee, United Kingdom*

New Approaches to Study Nuclear Structure/Function
Chairperson: R.H. Singer, *Albert Einstein College of Medicine, Bronx, New York*

Chromosomes and the Cell Cycle
Chairperson: W.A. Bickmore, *MRC Human Genetics Unit, Edinburgh, United Kingdom*

Nuclear Transport
Chairperson: I.W. Mattaj, *European Molecular Biology Laboratory, Heidelberg, Germany*

T. Pederson, R. Goldman, S. Krauss, G. Maul

L. Wieslander, J. Steitz

Axon Guidance and Neural Plasticity

September 25–29 450 participants

ARRANGED BY **Cori Bargmann,** HHMI/University of California, San Francisco
Tobias Bonhoeffer, Max-Planck Institute of Neurobiology, Germany
Larry Zipursky, HHMI/University of California, Los Angeles

The remarkable feats of information processing performed by the human brain are determined by the intricate network of connections between nerve cells (or neurons). The magnitude of the task involved in wiring the nervous system is staggering. In adult humans, each of over a trillion neurons makes connections with, on average, over a thousand target cells in an intricate circuit whose precise pattern is essential for the proper functioning of the nervous system. How can this pattern be generated during embryogenesis with the necessary precision and reliability? Neuronal connections form when each developing neuron sends out an axon, tipped by a growth cone, which migrates through the embryonic environment to its synaptic targets, guided by attractive and repulsive proteins that instruct it to migrate in particular directions. Once in their appropriate target regions, axons must seek out particular target cells with which to form synaptic connections. These connections are then further refined, through the making and breaking of synaptic contacts, under the control of specific patterns of electrical activity in the neurons and targets, until a highly tuned circuit is established.

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 CSHL conference series on "Axon Guidance and Developmental Plasticity of the Nervous System" was initiated in 1998. This year, the third of these meetings involved sessions devoted to particular stages 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.

As for the first two meetings, the response of the field to this conference was one of overwhelming enthusiasm. Of the 450 registrants, 300 submitted abstracts; 65 abstracts were selected for talks, in eight sessions. Senior researchers, starting Assistant Professors, postdoctoral fellows, and graduate students were all well represented. All of the major areas of research in the field were covered, as were all of the major approaches (cellular, physiological, anatomical, biochemical, and genetic). In addition, there were two special lectures: Dr. Tom Pollard focused on basic mechanisms controlling the cytoskeleton, and Dr. Eric Knudsen focused on mechanisms of development, function, and plasticity of the auditory system in the barn owl. The meeting provided an

C. Bargmann, J. Witkowski

important clearing house for ideas and approaches, and helped scientists in the field to get the most up-to-date information, as well as enabling them to meet, to network, and to establish collaborations. From 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 further, the meeting was deemed a great success.

This meeting was funded in part by the National Institute of Neurological Disorders and Stroke, a branch of the National Institutes of Health. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

PROGRAM

Guidance I: Semaphorins and Slits
Chairpersons: B. Dickson, *Institute of Molecular Pathology, Vienna, Austria;* M. Tessier-Lavigne, *Stanford University, California*

Synaptic Plasticity and Behavior
Chairpersons: M. Stryker, *University of California, San Francisco;* H. Cline, *Cold Spring Harbor Laboratory*

Special Lecture I
Speaker: Tom Pollard, *Yale University*

Regeneration
Chairpersons: C. Holt, *University of Cambridge, United Kingdom;* M.-M. Poo, *University of California, Berkeley*

Gene Expression and Translation
Chairpersons: R. Axel, *Columbia University, New York;* Y. Jin, *University of California, Santa Cruz*

Cytoskeleton and Signaling
Chairpersons: L. Luo, *Stanford University, California;* T. Pawson, *Samuel Lunenfeld Research Institute, Toronto, California*

Targeting and Synapse Formation
Chairpersons: S. Smith, *Stanford University, California;* E. Schuman, *California Institute of Technology, Pasadena*

Special Lecture II
Speaker: E. Knudsen, *Stanford University*

Guidance II
Chairpersons: C. Goodman, *Renovis, Inc., South San Francisco, California;* J. Sanes, *Washington University, St. Louis, Missouri*

Guidance III: Netrins and Ephrins
Chairpersons: R. Klein, *Max-Planck Institute of Neurobiology, Martinsried, Germany;* J. Flanagan, *Harvard Medical School, Boston, Massachusetts*

T. Godenschwege, K. Hofmeyer

C. Sabatier, J. Flanagan, M. Tessier-Lavigne

T. Bonhoeffer

Molecular Genetics of Aging

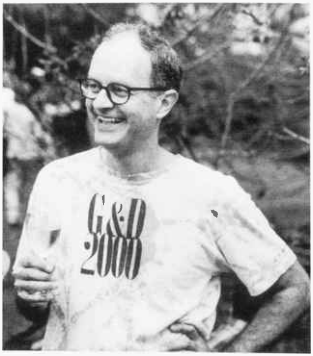
October 2–6 232 participants

ARRANGED BY **Ronald DePinho,** Dana Farber Cancer Institute
Terri Grodzicker, Cold Spring Harbor Laboratory
Leonard Guarente, Massachusetts Institute of Technology
Cynthia Kenyon, University of California, San Francisco

Aging is a process that leads to many degenerative changes and death. Recently, specific processes closely associated with aging as well as genes that may regulate aging have been identified. This meeting focused on these advances and provided a forum for discussion of many theories, old and new. Two regulators of aging were featured in several sessions, one involving signaling pathways by insulin-like molecules and the other, a novel class of NAD-dependent deacetylases, called SIR2 proteins. Altering these pathways was shown to extend life span in yeast, *Caenorhabditis elegans*, *Drosophila*, and mice. There was also a session dedicated to stem cell biology and transcription profiling of aging cells. Two other sessions related to telomere biology and cellular senescence. Premature aging syndromes and diseases of aging were also featured. Finally, recent findings relating aging to metabolism and oxidative stress closed the conference. It was clear that very rapid progress is being made to understand aging and its regulation at a molecular level.

This meeting was funded in part by the National Institute on Aging, a branch of the National Institutes of Health. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

C. Kenyon, A. de Grey



L. Guarente

V. Lundblad, J. Mitchell

PROGRAM

Model Systems I
Chairpersons: L. Guarente, *Massachusetts Institute of Technology, Cambridge;* J. Tower, *University of Southern California, Los Angeles*

Stem Cells and Genomics
Chairperson: H. Blau, *Stanford University School of Medicine, California*

Telomere Dynamics and DNA Damage Pathways
Chairpersons: S. Jackson, *University of Cambridge, United Kingdom;* T. de Lange, *The Rockefeller University, New York*

Cellular Senescence, Telomeres, and Cancer
Chairpersons: J. Campisi, *Lawrence Berkeley National Laboratory, California;* S. Lowe, *Cold Spring Harbor Laboratory*

Model Systems II
Chairpersons: G. Ruvkun, *Massachusetts General Hospital, Boston;* V. Lundblad, *Baylor College of Medicine, Houston, Texas*

Aging Syndromes and Diseases
Chairpersons: G. Martin, *University of Washington, Seattle;* D. Hanahan, *University of California, San Francisco*

Special Lecture from the Director
Speaker: Richard Hodes, *Director, National Institute on Aging*

Metabolism, Stress, and the Biology of Aging
Chairpersons: D. Wallace, *Emory University School of Medicine, Atlanta, Georgia;* A. Richardson, *Audie L. Murphy Memorial VA Hospital, San Antonio, Texas*

R. McKay, R. DePinho

L. Cao, T. Ide

Germ Cells

October 9–13 224 participants

ARRANGED BY **David Page,** Massachusettes Institute of Technology
Ruth Lehmann, New York University School of Medicine, Skirball Institute

Germ cells, by the virtue of the next generation, are the only cells in the body that have the potential of being immortal. Over the past few years, it has become apparent that many of the fundamental developmental mechanisms required for the establishment and function of germ cells have been conserved during evolution. This fourth Germ Cells meeting was organized with the goal of bringing together researchers who study germ-line development in both vertebrate and invertebrate systems and researchers studying human reproduction. The questions addressed in this meeting were: How are germ cells initially specified? Are germ cells a "totipotent" stem cell population? How do primordial germ cells acquire stem-cell character in the ovary or testis? Despite the apparent differences in the way germ cells are specified in the early embryo of different organisms, several genes, such as *nanos, DAZL, pumilio, piwi,* and *vasa*, show a remarkable degree of conservation, whereas other important players, such as the *Caenorhabditis elegans pie-1*, mouse *oct-4*, and *Drosophila oskar,* seem not have direct counterparts. An important and far-reaching issue dealt with the control of gene expression in the germ line. Whereas reprogramming of somatic nuclei is hampered by epigenetic factors such as gene silencing and imprinting, germ-line cells have evolved mechanisms to set and erase these marks, thereby allowing the perpetuation of the species. Given our growing understanding of the molecular mechanisms that control germ-line development and maturation in model organism, the implications of these findings for human reproduction were vividly discussed during the meeting. The next Germ Cells meeting will be held in the fall of 2004 with Judith Kimble and Blanche Capell serving as the organizers.

This meeting was funded in part by the National Institute of Child Health and Human Development, a branch of the National Institutes of Health; Lalor; and the March of Dimes. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

J. Maldonado, D. Page

M.-A. Handel, S. Mahadevaiah

PROGRAM

Germ Cell Origins
Chairpersons: G. Seydoux, *Johns Hopkins University School of Medicine, Baltimore, Maryland;* C. Wylie, *Children's Hospital Research Foundation, Cincinnati, Ohio*

The Embryonic Germ Line
Chairpersons: A. Spradling, *Carnegie Institution of Washington, Baltimore, Maryland;* B. Hogan, *Howard Hughes Medical Institute/Vanderbilt University Medical Center, Nashville, Tennessee*

Germ Line Stem Cells
Chairpersons: J. Kimble, *Howard Hughes Medical Institute/ University of Wisconsin, Madison;* M. Fuller, *Stanford University School of Medicine, California*

Spermatogenesis
Chairpersons: M.A. Handel, *University of Tennessee, Knoxville;* T. Schedl, *Washington University School of Medicine, St. Louis, Missouri*

Transcription
Chairpersons: R. Lehmann, *New York University School of Medicine, New York;* D. Page, *Massachusetts Institute of Technology, Cambridge*

Gamete Success and Evolution
Chairpersons: A. Clark, *Cornell University, Ithaca, New York;* J. Schimenti, *Jackson Laboratory, Bar Harbor, Maine*

Oogenesis
Chairpersons: J. Eppig, *Jackson Laboratory, Bar Harbor, Maine;* J. Richter, *University of Massachusetts Medical School, Worcester*

Germ Line–Soma Interactions/Sex Determination
Chairpersons: A. McLaren, *Wellcome/CRC Institute, Cambridge, United Kingdom;* S. DiNardo, *University of Pennsylvania Medical School, Philadelphia*

B. Hogan, J. Eppig

J. Kreidberg, R. Jaenisch

J. Kimble, J. Morris, T. Marty

Human Origins and Disease

October 30–November 3 150 participants

ARRANGED BY **Mary-Claire King,** University of Washington
Christopher Stringer, Natural History Museum
Douglas Wallace, University of California, Irvine

This fourth in a series of Cold Spring Harbor meetings on the evolution of modern humans brought substantial amounts of molecular genetic information together with fossil, archeological, and linguistic findings in an attempt to achieve a new synthesis in our understanding of human origins. Molecular anthropological studies of maternally inherited mitochondrial DNA and paternally inherited Y chromosomes continue to indicate that Africa harbors both the greatest genetic diversity and the oldest lineages. Most of the evidence points toward emigration of a small group of humans out of Africa between 40,000 and 50,000 years ago who populated first Asia and then Europe. But genetic evidence also hints at possible earlier emigrations along coastlines stretching to Papua New Guinea and Australia, a hypothesis itself challenged by the proposal that this variation may have become fixed through random genetic drift.

Besides being used to address global questions of human origins, molecular anthropology has permitted novel approaches to the study of more recent migrations and historical events. Meeting highlights included the reconstruction and analysis of a worldwide tree of the Y chromosome, which tracks the movements of humans across the globe. The tree suggests that while the cultural and linguistic contribution made by invaders may be large, the genetic contribution may be quite small if the conquered population is large in comparison with the invading force. In parallel, analyses of mitochondrial DNA lineages track the journeys of human females, which largely echo but sometimes differ from the Y-chromosome data, presumably because of different migratory behavior. The meeting also was host to intense speculation about genes that appear to be under strong and differential selective pressure, perhaps in response to particular diets, parasites, climates, or diseases. Most of the evidence for selective pressure comes from gene variants that are protective against malaria, but it is clear that we can expect other examples of rapid natural selection to emerge as research progresses. A number of intriguing examples were presented, without a clear picture of the underlying selective pressure yet being available. And finally, last year's discovery of the human-specific language gene present in all but a rare few human families is provoking debate about whether the acquisition of rapidly spoken language was the defining transition that

M.-C. King

A. Brooks, C. Stringer, F. Grine

pre-dated the appearance of many cultural artifacts and modern behaviors, a hypothesis hotly contested by the majority of archeologists who favor culture rather than genes to explain their findings.

The meeting was profiled in a November 12 *New York Times* piece by Nicholas Wade, entitled "Geneticists Track More of Earliest Humans' First Itineraries."

This meeting was funded in part by the Grace Professorship. Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

PROGRAM

Of Apes and Men
Chairpersons: D.C. Wallace, *University of California Irvine;* M.M. Lahr, *University of Cambridge, United Kingdom*

Africa and Early Human Evolution
Chairpersons: C.B. Stringer, *Natural History Museum, London, United Kingdom;* A.C. Stone, *University of New Mexico, Albuquerque*

Out of Africa Dispersals and Processes
Chairpersons: A.S. Brooks, *George Washington University, Washington, D.C.;* S. Pääbo, *Max-Planck Institute for Evolutionary Anthropology, Leipzig, Germany*

Global Human Genetic Variation
Chairpersons: A. Torroni, *Urbino University, Italy;* P. Underhill, *Stanford University, California*

African Radiation of Homo Sapiens
Chairpersons: S. McBrearty, *University of Connecticut, Storrs;* M. Hammer, University of Arizona, Tuscon

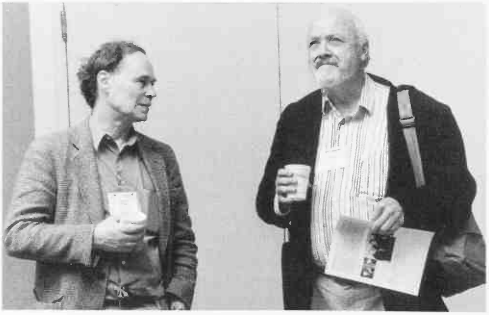
Southeast Asia, Australia, and Oceania
Chairpersons: M. Stoneking, *Max-Planck Institute for Evolutionary Anthropology, Leipzig, Germany;* J.C. Stephens, *Genaissance Pharmaceuticals, New Haven, Connecticut*

Asia and the Americas
Chairpersons: P. Forster, *University of Cambridge, United Kingdom;* R. Villems, *Estonian Biocentre, Tartu*

Western Eurasia and Europe
Chairpersons: K.K. Kidd, *Yale University School of Medicine, New Haven, Connecticut;* M. Seielstad, *Genome Institute of Singapore*

Human Radiation and Infectious Disease
Chairpersons: N.K. Yankovsky, *Institute of General Genetics, Moscow, Russia;* J. Hey, *Rutgers University, Piscataway, New Jersey*

Human Variation and Disease
Chairpersons: M.C. King, *University of Washington, Seattle;* J. Bertranpetit, *Universitat Pompeu Fabra, Barcelona, Spain*



N. Wade, W. Calvin

J. Swanson, S. Pääbo

A. Lovell, R. King

BIOTECHNOLOGY CONFERENCE
Tissue Engineering

November 21–24 101 participants

ARRANGED BY **Farshid Guilak,** Duke University Medical Center
Rocky Tuan, National Institute of Arthritis, Musculoskeletal, and Skin Diseases

The goal of this Tissue Engineering Biotechnology Conference, a first of its kind for Cold Spring Harbor Laboratory, was to bring together investigators in this emerging research and biotechnology discipline to exchange ideas, present new discoveries, and interact in order to develop novel concepts for the scientific enhancement of the field. The meeting consisted of both platform and poster presentations by researchers from a wide spectrum of research areas related to tissue engineering. Presenters included established investigators as well as graduate students and postdoctoral fellows. The topics for the platform sessions represented areas of key, active research development in tissue engineering, and included (1) biology of tissue progenitor cells; (2) application of progenitor and stem cells; (3) novel biomaterials, biomimetics, and bioinspired materials; (4) functional tissue engineering; (5) gene-based approaches in tissue engineering; and (6) applications and in vivo models of engineered tissues. The experimental approaches reported covered cellular and molecular biology, gene therapy, stem cells, biomaterials, biomechanics, biomechanical engineering, computer-aided design, bioinformatics, bioreactors, and in vivo models, reflecting the inherently multidisciplinary nature of the field of tissue engineering.

Some of the highlights included new insights on the biology of adult tissue-derived, multipotential, mesenchymal stem cells, novel biomaterials based on natural and synthetic polymers, novel approaches to the design of bioreactors for tissue engineering, integration of mechanically active environment in functional tissue engineering, and how to bring tissue-engineered products to the market. Because tissue engineering is a new, emerging discipline, the Conference was a wonderful opportunity to bring together investigators of diverse background and expertise to a congenial, interactive setting to fully explore the scientific concepts necessary to develop and enhance the sophistication of the field. The quality of the presentations was very high, and many exciting impromptu discussions, among both senior and junior investigators, took place during and after the presentations, and late into the night

R. Tuan, J. Huard, D. Stewart

throughout the course of the Conference. The Conference was viewed as a wonderful beginning to foster the translation of basic scientific principles of biology, in conjunction with the principles of engineering, into research areas that have tremendous potential in biomedicine, particularly in the new field of regenerative medicine. The environment here at CSHL further contributed to the highly interactive nature of the Conference. Many attendees expressed the hope that the CSHL will continue to host meetings on tissue engineering in the future.

Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

PROGRAM

Biology of Tissue Progenitor Cells
Chairperson: A. Caplan, *Case Western Reserve University, Cleveland, Ohio*

Application of Progenitor and Stem Cells in Tissue Engineering
Chairperson: F. Guilak, *Duke University Medical Center, Durham, North Carolina*

Novel Biomaterials, Biomimetics, and Bioinspired Materials I
Chairperson: J. Elisseeff, *Johns Hopkins University, Baltimore, Maryland*

Novel Biomaterials, Biomimetics, and Bioinspired Materials II
Chairperson: S. Hollister, *University of Michigan, Ann Arbor*

Functional Tissue Engineering
Chairperson: R. Tuan, *NIAMS, National Institutes of Health, Bethesda, Maryland*

Gene-based Approaches in Tissue Engineering
Chairperson: J. Huard, *University of Pittsburgh, Pennsylvania*

Applications and In Vivo Models of Engineered Tissues
Chairperson: J. Glowacki, *Brigham and Women's Hospital, Boston, Massachusetts*

C. Williams, R. Mauck

R. Oki, J. Gimble

A. Ratcliffe, J. Elisseeff

BIOTECHNOLOGY CONFERENCE
Therapeutic Opportunities in Neurodegenerative Diseases

December 5–8 108 participants

ARRANGED BY **Sam Gandy,** Farber Institute for Neuroscience at Thomas Jefferson University
Harry LeVine III, Pfizer Inc.
Marcy MacDonald, Massachusetts General Hospital/Harvard Medical School

As many as one half of those aged 65 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 hereditability or identifiable genetic mutations, it has been possible over the past 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 behavioral 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 the living animal models. Most importantly, the animal models have enabled discovery of entirely unanticipated therapeutic strategies (such as amyloid β immunotherapy). In December 1999, some of these rationally discovered compounds and unexpected immunotherapies entered Phase I clinical trials. The progress of compounds and rational strategies from the animal model to the human clinical trial; the design, results, and conclusions of 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.

H. LeVine, S. Gandy, M. MacDonald

For the 2002 meeting, six 3.5-hour platform sessions were organized around common technological themes. Chairpersons, invited speakers, and speakers selected from submitted abstracts were drawn from the academic and pharmaceutical sectors. Although the discussion of new unpublished data was emphasized, the group was tolerant of a wide range of intellectual property conventions. Poster presentations were also encouraged: Posters were displayed throughout the meeting, and poster viewing was especially encouraged during the cocktail hours before the evening meals.

Topics of the meeting included protein aggregation inhibitors, protein processing enzymology and pharmacology, gene transfer, stem cell transfer, immunotherapy, human genetics, design and interpretation of genomic analyses in mammalian and lower systems, bioinformatics, mouse and fly models of neurological disease, mechanisms of neurodegeneration, metabolic and hormonal influences on disease protein metabolism in cell culture and living animal models as well as in human clinical trials, and the legal and regulatory obstacles to drug discovery for neurodegenerative diseases. Diseases considered at the 2000 meeting included Alzheimer's, Huntington's, spinocerebellar atrophies, ALS, prion diseases, Parkinson's, tauopathies, and synucleinopathies.

Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

PROGRAM

Prion and Triplet Diseases: Heat Shock Proteins as Model Therapeutics
Chairpersons: S. Lindquist, *Howard Hughes Medical Institute/University of Chicago, Illinois;* M. MacDonald, *Massachusetts General Hospital, Charlestown*

Alzheimer's Aβ: Targeting Secretase Enzymes and Protein Aggregation
Chairpersons: H. LeVine, *Pfizer, Ann Arbor, Michigan;* D. Frail, *Pharmacia Corporation, Kalamazoo, Michigan*

Immunopathology and Immunotherapy of Neurodegeneration
Chairpersons: L. van Eldik, *Northwestern University Medical School, Chicago, Illinois;* G.E. Landreth, *Case Western Reserve University School of Medicine, Cleveland, Ohio*

Challenges in Bringing Neurodegeneration Drugs to the Clinic: Patient Selection, Quantitative Outcomes, Unprecedented Trial Expense, and Regulatory Disincentives
Chairpersons: D. Murphy, *NINDS, National Institutes of Health, Rockville, Maryland;* N. Ishaque, *Advanced Technology Leader, Niskayuna, New York*

Optimizing Animal and Cellular Models for Drug Evaluation
Chairpersons: K. Duff, *Nathan Kline Institute, New York University, Orangeburg;* J.Q. Trojanowski, *University of Pennsylvania School of Medicine, Philadelphia*

New Genes, New Strategies
Chairpersons: M. Gurney, *deCode Genetics, Reykjavic, Iceland;* R. Mayeux, *Columbia University College of Physicians & Surgeons, New York*

M. Gurney

BIOTECHNOLOGY CONFERENCE
Comparative Plant Genomics

December 12–15 98 participants

ARRANGED BY **Mike Bevan,** The John Innes Centre, United Kingdom
Susan McCouch, Cornell University

This meeting featured 37 talks in 8 sessions, a poster session, and a workshop of evolutionary genomics. The keynote lecture given by Rob Martienssen from Cold Spring Harbor Laboratory in honor of the Centenary of Barbara McClintock's birth fittingly described the deep insights recently gained into the epigenetic mechanisms involved in establishing and maintaining plant heterochromatin and regulating transposon activity. Talks, which were mostly selected from abstracts, covered progress in sequencing plant genomes, genome dynamics, functional genomics, and genome-related informatics and databases. Since the completion of the *Arabidopsis* genome sequence in 2000, several new plant genome projects have been initiated, including rice, representing the first cereal genome to be sequenced, Lotus and Medicago representing the legume family, poplar representing tree species, and Brassica and tomato. Furthermore, strategies for tackling larger and more complex genomes such as maize were discussed. *Arabidopsis* functional genomics activities, including progress toward the goal of obtaining disruptions of all 27,000+ genes by the end of the year, were reviewed at a meeting of the Multinational *Arabidopsis* Steering Committee. An impressive total of more than 200,000 independent T-DNA and transposon insertions in the *Arabidopsis* genome were cataloged. A workshop on Evolutionary Genomics, organized by Gloria Coruzzi and Rob Martienssen, focused on reviewing the tools needed

R. McCombie, M. Bevan, R. Martienssen

Z. Lippman, S. McCouch, E. Vollbrecht

to establish and interpret relationships between plant genomes, and on identifying the species providing the most insight into the evolution of higher plants. These so-called nodal species include the primitive seed plants called cycads that bear seeds on leaves, and Ginkgo and Gnetum, two early gymnosperms. Extensive expressed sequence tag sequencing of these plants will form important foundations for understanding the evolution of seed-bearing plants.

Contributions from our Corporate Benefactors, Corporate Sponsors, Plant Corporate Associates, Corporate Associates, Corporate Contributors, and Foundations also provided core support for this meeting.

PROGRAM

Sequence and Analysis of Plant Genomes I
Chairperson: M. Bevan, *John Innes Centre, Norwich, United Kingdom*

The McClintock Centenary Lecture: Heterochromatin, Transposable Elements, and Epigenetics of the Plant Genome
Speaker: R. Martienssen, *Cold Spring Harbor Laboratory*

Sequence and Analysis of Plant Genomes II
Chairperson: S. McCouch, *Cornell University, Ithaca, New York*

Evolutionary Genomics Workshop
R. DeSalle, *American Museum of Natural History, New York:* Tools for evolutionary phylogenomics.

V. Irish, *Yale University, New Haven, Connecticut:* Genomics and plant evolution.

G. Coruzzi, *New York University, New York:* Future directions for plant evolutionary genomics.

Genome Dynamics
Chairperson: J. Bennetzen, *Purdue University, W. Lafayette, Indiana*

Functional Genomics I
Chairperson: R. McCombie, *Cold Spring Harbor Laboratory*

Functional Genomics II
Chairperson: J. Ecker, *The Salk Institute for Biological Studies, La Jolla, California*

Informatics and Databases
Chairperson: C. Town, *The Institute for Genomic Research, Rockville, Maryland*

M. Rosso

I. Small, C. Town

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 they are not adequately treated by universities. 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.

Acquiring and Analyzing Genomic Sequence Data

March 12–28

INSTRUCTORS

E. Birney, EBI, Hintxon, United Kingdom
E. Mardis, Washington University School of Medicine, St. Louis, Missouri
W.R. McCombie, Cold Spring Harbor Laboratory
J. McPherson, Washington University School of Medicine, St. Louis, Missouri
T. Wood, Bryan College, Dayton, Tennessee

ASSISTANTS

V. Balija, Cold Spring Harbor Laboratory
M. Clamp, The Sanger Centre, Hinxton, United Kingdom
M. de la Bastide, Cold Spring Harbor Laboratory
J. Kramer, Millennium Pharmaceuticals, Watertown, Massachusetts
D. Main, Clemson University Genomics Institute, Clemson, South Carolina
L. Nascimento, Cold Spring Harbor Laboratory
A. O'Shaughnessy, Cold Spring Harbor Laboratory
L. Palmer, Cold Spring Harbor Laboratory
T. Rohlfing, Washington University School of Medicine, St. Louis, Missouri
L. Spiegel, Cold Spring Harbor Laboratory
T. Zutavern, Cold Spring Harbor Laboratory

This year's course consisted of four modules, which featured (1) production sequencing and sequence finishing, (2) sequence variation analysis, (3) cross-species sequence comparison, and (4) gene prediction and annotation. The first part of the course emphasized the technical and management aspects of large-scale physical mapping and sequencing projects, including polymorphism detection. The second part focused on computational analysis of sequence data, including sequence variation (such as single-nucleotide polymorphisms [SNPs]), comparative genomics, gene prediction, and sequence annotation. Where appropriate, the computational analysis was coupled with data acquisition. A sequencing project was carried out during the course, with an emphasis on the technical nuances of large-scale sequencing. In the past years, this phase has targeted a bacterial artificial chromosome (BAC) clone from various species, including *Arabidopsis thaliana*, mouse, chimpanzee, and human. Techniques for generating both "working draft" and completely finished genome sequences were covered. Whole-genome shotgun and clone-based sequencing methods utilizing capillary-based sequence acquisition was taught. The project this year was a low-coverage whole-genome shotgun of a bacterium. Sequence variation analysis featured SNP generation and detection methods. A laboratory project included sequencing of targeted regions from multiple individuals and additional SNP analysis. Computational analysis focused on using readily available tools for comparative genomics, gene prediction, and annotation methods. Data from the course production sequencing module was utilized, as were examples from the publicly accessible sequence archives. Sequence homology was used to analyze orthologous and parologous sequences. Ab initio gene prediction as well as supporting evidence using expressed sequence tags (EST), mRNA, and homologous sequences were profiled. These modules focused on the use of the tools themselves and on the underlying algorithms. For a more in-depth coverage of sequence analysis theory, the Computational Genomics Course was recommended.

PARTICIPANTS

Dore, C., DipL., Montreal Genome Center, Montreal, Canada
Elgin, S., B.A., Ph.D., Case Western Reserve University, Cleveland, Ohio
Jungerius, B., B.Sc., M.Sc., Wageningen University, The Netherlands
La Rota, C., B.S., M.S., Cornell University, Ithaca, New York
Liu, X., B.S., Ph.D., Harvard Medical School, Boston, Massachusetts
Moody, Adrian, B.Sc., Ph.D., AstraZeneca, Cheshire, United Kingdom
Orjeda, G., M.S., Ph.D., Genoscope, Evry, France
Ortega, M., Tech., Institute of Biochemical Research, Argentina, Buenos Aires
Peterlongo, P., B.S., Ph.D., Memorial Sloan-Kettering Cancer Center, New York
Song, Z., B.S., Ph.D., Massachusetts Institute of Techology, Cambridge
Turpeinen, H., M.Sc., Ph.D., University of Turku, Finland
Wan, K.-L., B.Sc., Ph.D., Universiti Kebangsaan Malaysia, Malaysia
Wang, Y.-C., B.A., Ph.D., National Taiwan Normal University, Taiwan, Republic of China
Young, K.S., B.A., Samsung Advanced Institute of Technology, Taejon, Korea
Zhu, C., M.D., Ph.D., Karolinska Hospital, Stockholm, Sweden
Zuccolo, A., M.Sc., University of Udine, Italy

SEMINARS

Altshuler, D., Massachusetts General Hospital, Boston: SNPs and association studies I.
Altshuler, D., Massachusetts General Hospital, Boston: SNPs and association studies II.
Green, E., National Institutes of Health, NHGRI, Bethesda, Maryland: Comparative sequence analysis.
Martienssen, R., Cold Spring Harbor Laboratory: DNA methylation.
Wilson, R., Washington University School of Medicine, St. Louis, Missouri: Sequence-finishing challenges.

Protein Purification and Characterization

April 3–16

INSTRUCTORS
R. Burgess, University of Wisconsin, Madison
A. Courey, University of California, Los Angeles
S.-H. Lin, M.D. Anderson Cancer Center/University of Texas, Houston
K. Severinov, Waksman Institute, Rutgers University, Piscataway, New Jersey

ASSISTANTS
L. Anthony, University of Wisconsin, Madison
V. Bergendahl, University of Wisconsin, Madison
K. Earley, M.D. Anderson Cancer Center/University of Texas, Houston
M. Galfione, M.D. Anderson Cancer Center/University of Texas, Houston
S. Jia, University of California, Los Angeles
D. Markov, Columbia University, New York
S. Nechaev, University of California, San Diego, La Jolla
G. Ratnaparkhi, University of California, Los Angeles
N. Thompson, University of Wisconsin, Madison
J. Yuzhenkova, Waksman Institute, Rutgers University, Piscataway, New Jersey

This course was intended for scientists who are 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 *E. coli*; and (4) a membrane-bound receptor. A variety of bulk fractionation, electrophoretic, and chro-

matographic 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, protein-protein interaction studies by far-western analysis, peptide mapping, amino acid analysis, protein sequencing, and mass spectrometry. Emphasis was placed on strategies of protein purification and characterization. Guest lecturers discussed protein structure, modification of proteins, methodologies for protein purification and characterization, and applications of protein biochemistry to cell and molecular biology.

PARTICIPANTS

K. Barton, B.S., Ph.D., Carnegie Institute of Washington, Stanford, California
Brandi, L., B.S., Ph.D., University of Camerino, Italy
Brodersen, P., B.S., M.S., University of Copenhagen, Denmark
Cegelski, L., B.S., Washington University, St. Louis, Missouri
Chamberlin, M.E., B.S., Ph.D., Ohio University, Athens, Ohio
Chipuk, J.E., B.S., Ph.D., Case Western Reserve University, Cleveland, Ohio
Dangond, F., B.A., M.D., Brigham & Women's Hospital, Boston, Massachusetts
Labib, K., B.A., M.D., Paterson Institute for Cancer Research, Manchester, United Kingdom
Malashkevich, V., B.S., Ph.D., Whitehead Institute, Cambridge, Massachusetts
Melnick, A., M.D., Mount Sinai School of Medicine, New York
Rogers, M., B.S., Ph.D., Stanford University School of Medicine, Stanford, California
Saccani, S., B.S., Ph.D., Institute of Research in Biomedicine, Bellinzona, Switzerland
Sato, T., B.S., Ph.D., The Scripps Research Institute, La Jolla, California
Shureiqi, I., B.S., M.D., University of Texas/M.D. Anderson Cancer Center, Houston
Smeets, M., M.Sc., Ph.D., University Medical Centre, Utrecht, The Netherlands
Stehr, F.C., DipL., University Institute of General Botany, Hamburg, Germany

SEMINARS

Burgess, R., University of Wisconsin, Madison: Overview of protein purification, immunoaffinity purification; biochemical studies of RNA polymerase/Sigma factor interactions.
Courey, A, University of California, Los Angeles: Mechanisms of activation and repression by the dorsal morphogen.
Guidotti, G., Harvard University, Cambridge, Massachusetts: Membrane proteins and extracellular ATP.
Hart, G., Johns Hopkins University School of Medicine, Baltimore, Maryland: Dynamic interplay between *O*-glycosylation and *O*-phosphorylation in cell signaling and metabolic regulation.
Joshua-Tor, L., Cold Spring Harbor Laboratory: Proteins in 3-D.
Lin, S.-H., M.D. Anderson Cancer Center, Houston, Texas: Strategies for identification of prostate cancer bone metastasis-related factors and 16-kD fragment of prolactin as an angiogenesis inhibitor.
Mische, S., Boehringer-Ingelheim Pharmaceuticals, Ridgefield, Connecticut: Proteomics and why pharmaceutical companies are interested in it.
Sauer, B., Massachusetts Institute of Technology, Cambridge: Energy-dependent protein degradation and unfolding.
Severinov, K., Waksman Institute, Rutgers, Piscataway, New Jersey: Regulation of host RNA polymerase during viral development.

Cell and Developmental Biology of *Xenopus*

April 6–16

INSTRUCTORS **J. Christian,** Oregon Health Sciences University, Portland
K. Cho, University of California, Irvine

ASSISTANTS **I. Blitz,** University of California, Irvine
C. Degnin, Oregon Health and Sciences University, Portland
R. Hackenmiller-Paradis, Oregon Health Sciences University, Portland
N. Hirsch, University of Virginia, Charlottesville

The frog *Xenopus* is an important vertebrate model for studies of maternal factors, regulation and molecular mechanisms of tissue inductions, and regulation of cell fate decisions. In addition, *Xenopus* oocytes and embryos provide a powerful system in which to conduct a number of cell biological and gene regulation assays. This course provided extensive laboratory exposure to the biology, manipulation, and use of oocytes and embryos of *Xenopus*. The course consisted of intensive laboratory sessions, supplemented by daily lectures and demonstrations from experts in cellular, experimental, and molecular development. Areas that were covered included (1) care of adults; (2) oocyte isolation and embryo production; (3) stages of embryonic development and anatomy; (4) whole-mount in situ hybridization and immunocytochemistry; (5) microinjection of eggs and oocytes with lineage tracers, DNA constructs, mRNA, and antisense oligonucleotides; (6) micromanipulation of embryos, including explant and transplantation assays; (7) in vivo time-lapse confocal imaging; (8) preparation of trans-

genic embryos; and (9) use of *Xenopus tropicalis* for genetic analyses. This course was suited both for investigators who have had no experience with *Xenopus* and for those who have worked with *Xenopus* and wished to learn new and cutting edge techniques. All applicants had current training in molecular biology and some knowledge of developmental biology.

PARTICIPANTS

Brownawell, A., M.S., Ph.D., University of Virginia, Charlottesville
Dave, V., M.S., Ph.D., University of Cincinnati, Ohio
Dorey, K., B.Sc., Ph.D., Imperial Cancer Research Fund, London, United Kingdom
Kee, Y., B.Sc., M.S., Ph.D., Caltech, Pasadena, California
LaFollette-Shumway, K., B.A., Ph.D., University of Texas Health Science Center, Houston
Leung Wai Lun, A., B.Sc., Ph.D., The University of Hong Kong, Hong Kong
Manson, F., B.Sc., Ph.D., Western General Hospital, Edinburgh, United Kingdom
Morris, A., B.S., Ph.D., Emory University School of Medicine, Atlanta, Georgia
Nasipak, B., B.A., Columbia University, New York
Palmer, M., B.S., Emory University, Atlanta, Georgia
Reddy, P., M.B.B.S., Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio
Stroubouli, J., Ph.D., Erasmus University, Rotterdam, The Netherlands
Sun, H., B.S., Ph.D., Johns Hopkins University School of Medicine, Baltimore, Maryland
Toreejon, M., B.S., Ph.D., National Research Council, Moffett Field, California
Vottari, T., Laurente, University of Pisa, Italy
Walsh, S., B.S., Ph.D., Duke University, Durham, North Carolina

SEMINARS

Amaya, E., Welcome, CRC Institute, United Kingdom: Transgenesis and insertional mutagenesis in *Xenopus*.
Cho, K., University of California, Irvine: Probing vertebrate development using DNA microarrays.
Christian, J., Oregon Health Sciences University, Portland: Regulation of BMP activity by proprotein processing.
Danilchik, M., Oregon Health Sciences University, Portland: Membrane dynamics during cleavage in *Xenopus*.
Harland, R., University of Californa, Berkeley: Signaling and morphogenesis.
Heasman, J., Children's Hospital Medical Center, Cincinnati, Ohio: Maternal control of embryonic cell fates.
Cline, H., Cold Spring Harbor Laboratory: Analysis of activity-regulated genes in brain development in *Xenopus*.
Moody, S., George Washington University Medical Center, Washington, D.C.: Introduction to early embryology; the role of FoxD5 in expanding the neural plate.
Ueno, N., National Institute for Basic Biology, Japan: A new player controlling *Xenopus* gastrulation.

Advanced Bacterial Genetics

June 5–25

INSTRUCTORS **K. Hughes,** University of Washington, Seattle,
W. Metcalf, University of Illinois, Urbana
P. Youderian, Texas A&M University, College Station

ASSISTANTS **P. Aldridge,** University of Washington, Seattle
L.T. Socias, Texas A&M University, College Station
M. Wilson, University of Illinois, Urbana

The course presented logic and methods used in the genetic dissection of complex biological processes in eubacteria. The following laboratory methods were used: 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 polymerase chain reaction (PCR) and cloning methods; epitope insertion mutagenesis; and site-directed mutagenesis. Key components of the course were 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.

PARTICIPANTS

Bachler, C., B.S., M.S., Universitat Bern, Switzerland
Bartoli, F., B.S., M.Sc., Instituto Venezolano Investigaciones, Cientificas, Caracas, Venezuela
Cowart, R., B.S., Ph.D., University of Dubuque, Iowa
Ferguson, G., B.Sc., Ph.D., Massachusetts Institute of Technology, Cambridge
Herskovits, A., B.S., M.S., Weizmann Institute of Science, Rehovot, Israel
Johansson, C., M.S., Uppsala University, Sweden
Laal, S., M.S., Ph.D., New York University School of Medicine, New York
Maeder, C., B.S., Johns Hopkins University, Baltimore, Maryland
Martins, L., B.S., Ph.D., Instituto de Tecnologia Quimica e Biologica, Oeiras, Portugal
Meng, J., B.S., Ph.D., University of Maryland, College Park
Nilsen, T., M.Sc., Ph.D., Massachusetts General Hospital, Cambridge
Putteet-Driver, A., B.S., University of California, Irvine
Rao, C., B.S., Ph.D., University of California, Berkeley
Sjolund, M., M.Sc., Ph.D., Uppsala University, Sweden
Srinivasan, B., B.S., M.S., Ph.D., Stanford University, Stanford, California
Tankson, J., B.S., M.S., Ph.D., USDA/Agricultural Research Services, Athens, Georgia

SEMINARS

Camilli, A., Tufts University, Boston, Massachusetts: Investigation of pathogen gene expression during infection using in vivo expression technology (IVET).
Galan, J., Yale University, New Haven, Connecticut: Close encounters of *Salmonella* with host cells: Type III secretion at work.
Groisman, E., Washington University School of Medicine, St. Louis, Missouri: Two-component signal transduction in *Salmonella* and *E. coli*.
Hughes, K., University of Washington, Seattle: Coupling flagellar gene expression to organelle assembly.
Jenal, U., University of Basel, Switzerland: Temporal and spatial control mechanisms during the *Caulobacter* cell cycle and development.
Kenney, L., Oregon Health Science University, Portland: OmpR regulation of porin gene expression.
Lory, S., Harvard Medical School, Boston, Massachusetts: Listening to conversations between pathogens and their hosts with DNA microarrays.
Maloy, S., University of Illinois, Urbana: Genetics of host specificity in *Salmonella*.
Metcalf, W., University of Illinois, Urbana: The global phosphate cycle.
Normark, S., Karolinska Institute, Stockholm, Sweden: Bacterial interactions with the innate immune system.
Roth, J., University of Utah, Salt Lake City: Adaptive mutation: A misinterpreted bacterial artifact reveals a genetic process that may be central to bacterial adaptation, evaluation of new genes, and origins of cancer.
Rothman-Denes, L., University of Chicago, Illinois: Expanding the family of single-subunit DNA-dependent RNA polymerases.
Silhavy, T., Princeton University, Princeton, New Jersey: Coping with envelope stress.
Youderian, P., Texas A&M University, College Station: Are bacterial outer-membrane channels ligand-gated?

Molecular Embryology of the Mouse

June 5–25

INSTRUCTORS	**R. Behringer,** University of Texas/M.D. Anderson Cancer Center, Houston **A. Gossler,** Institut fur Molekularbiologie, Hannover, Germany **A. Nagy,** Samuel Lunenfeld Research Institute, Toronto, Ontario, Canada
CO-INSTRUCTORS	**S. Colin,** National Cancer Institute/FCRDC, Frederick, Maryland **T. Lufkin,** Mount Sinai School of Medicine, New York
ASSISTANTS	**A. Kobayashi,** M.D. Anderson Cancer Center, Houston, Texas **M. Gerstenstein,** Samuel Lunenfeld Research Institute, Toronto, Canada **K. Serth,** Institut fur Molekularbiologie, Hannover, Germany

This intensive laboratory and lecture course was designed for biologists interested in applying their expertise to the study of mouse embryonic development. Laboratory components provided an introduction into the technical aspects of working with and analyzing mouse embryos, and lectures provided the conceptual basis for contemporary research in mouse development. Experimental techniques were covered in the practicals including in vitro culture and manipulation of pre- and postimplantation embryos, transgenesis by DNA microinjection, embryo transfer, establishment, culture and genetic manipulation of embryonic stem cells, production of chimeras by aggregation with and injection of embryonic stem cells, and the analysis of development by whole-mount in situ hybridization, skeletal preparation, and transgene expression.

On the last weekend of the course, a special reunion celebrating the 20th anniversary of the mouse course was arranged. The reunion included a series of talks by past and present instructors, and also featured a piano concert by Phillipe Soriano at Robertson House and an evening banquet around the Banbury pool. As well as the instructors, co-instructors, assistants, and students, reunion participants included Siew-Lan Ang, Kathryn Cheah, Frank Costantini, Susan Dymecki, Brigid Hogan, Randy Johnson, Alexandra Joyner, Peter Koopman, Elizabeth Lacy, Robin Lovell-Badge, Terry Magnuson, Gail Martin, Anne McLaren, Paul Overbeek, Virginia Papaioannou, Roger Pedersen, Nadia Rosenthal, Janet Rossant, Davor Solter, Philippe Soriano, and Patrick Tam.

PARTICIPANTS

Cambronero, F., M.D., Ph.D., Stowers Institute for Medical Research, Kansas City, Missouri
Christian, J., B.S., Ph.D., Oregon Health Sciences University, Portland
Filippova, G., M.S., Ph.D., Fred Hutchinson Cancer Research Center, Seattle, Washington
Jechlinger, M., Mag., Institute of Molecular Pathology, Vienna, Austria
Kobielak, A., M.Sc., Ph.D., The Rockefeller University, New York
Krummel, K., B.S., Ph.D., Salk Institute, La Jolla, California
Lossie, A., B.S., Ph.D., Baylor College of Medicine, Houston, Texas
Martin de Lara, F., B.S., M.S., CIB Spanish Research Council, Madrid, Spain
Naz, S., B.S., Ph.D., National Institute on Deafness and Other Communication Disorders, Rockville, Maryland
Nieto, M., M.S., National Center of Biotechnology, Madrid, Spain
Sachidanandan, C., M.Sc., Ph.D., Cancer Research UK, London, United Kingdom
Savage, J., B.A., M.S., Indiana University Purdue University, Indianapolis
Vokes, S., B.A., University of Arizona Health Sciences Center, Tucson
Vong, L., B.S., Ph.D., Albany Medical College, New York

SEMINARS

Aizawa, S., Kumamoto University School of Medicine, Kumamoto, Japan: Forebrain development.
Behringer, R., The University of Texas/M.D. Anderson Cancer Center, Houston: Transgenesis and gene targeting.
Church, D., National Institutes of Health, Bethesda, Maryland: Mouse genomics.
Cross, J., University of Calgary, Canada: Development of extra-embryonic tissue.
Gossler, A., Medizinische Hockschule, Hannover, Germany: Somitogenesis.
Gridley, T., The Jackson Laboratory, Bar Harbor, Maine: Notch signaling.
Hogan, B., Howard Hughes Medical Institute/Vanderbilt University Medical Center , Nashville, Tennessee: Genetic regulation of organogenesis.
Jessell, T., Columbia University/Hammer Health Sciences Center, New York: Regionalization and neuronal specification in the spinal cord.
Joyner, A., New York Medical Center, Skirball Institute of Biology, New York: Neural patterning.
Justice, M., Baylor College of Medicine, Houston, Texas: ENU mutagenesis.
Lovell-Badge, R., MRC National Institute for Medical Research, London, United Kingdom: Sex determination: Building genetic pathways.
Lufkin, T., Mount Sinai School of Medicine, New York: HOX genes and patterning.
Magnuson, T., University of North Carolina, Chapel Hill: Model system mouse: A synthesis and examples.
Martin, G., University of California, San Francisco: FGFs and limb development.
McLaren, A., Wellcome/CRC Institute, Cambridge, United Kingdom: Germ cells.
Nagy, A., Mount Sinai Hospital, Toronto, Canada: Embryonic stem cells and the biology of chimeras.
Rosenthal, N., EMBL-Monterotondo, Monterotondo-Scala, Italy: Heart development.
Schimenti, J., The Jackson Laboratory, Bar Harbor, Maine: Modern and classical forward approaches in mice for identifying novel developmental genes.
Scholer, H., University of Pennsylvania, Kenneth Square: Gene funciton and regulation in pluripotent stem cells.
Shen, M., UMNDJ-Robert Wood Johnson Medical School, Piscataway, New Jersey: Left-right patterning.
Solter, D., Max-Planck Institut für Immunbiologie, Freiburg, Germany: Fertilization and preimplantation development.
Stewart, A.F., Technische Universitaet Dresden, Dresden, Germany: Targeting strategies: Design, construction, and integration of conditional options.
Stewart, C., National Cancer Institute/FCRDC, Frederick, Maryland: Implantation.
Tam, P., Children's Medical Research Institute, Wentworthville, Australia: Postimplantation development and body plan.

Mouse Course Reunion

Physiological Approaches to Ion Channel Biology

June 5–25

INSTRUCTORS **M. Hausser,** University College London, United Kingdom
N. Spruston, Northwestern University, Evanston, Illinois
L. Trussell, Oregon Health Sciences University, Portland

ASSISTANTS **J. Dzubay,** University of California, Los Angeles
S. Malin, Washington University, St. Louis, Missouri
P. Monsivais, University College London, United Kingdom
N. Staff, Northwestern University, Evanston, Illinois

This intensive laboratory/lecture course introduced the 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 cultured cells or neurons in brain slice preparations. Different recording configurations (e.g., extracellular, whole-cell, cell-free patches, and single channel) were used. The advantages and disadvantages of each method and preparation were considered in order to match experimental approaches to scientific questions. Similarly, various methods for ligand/drug application were demonstrated. A primary goal was to identify properties of ion channels that allow neurons to

carry out their unique physiological or systems-level roles. These properties are related to information previously gained from molecular cloning and heterologous expression of ion-channel genes. Areas of particular interest included channels that (1) are activated by neurotransmitter at central and peripheral synapses, (2) interact with other channels to shape the response properties of neurons, (3) respond to neuromodulators with changes in functional properties, or (4) are developmentally required and regulated. The research interests of guest lecturers complemented and expanded this list. This course was intended for students with specific plans in applying these techniques to a defined problem.

PARTICIPANTS

Cassler, C., B.S., M.S., International School for Advanced Studies, Trieste, Italy
Chin, J., B.Sc., University of Alberta, Edmonton, Canada
Cho, W.K., B.A., B.S., M.D., Indiana University, Indianapolis
Craig, A.M., B.Sc., Ph.D., Washington University School of Medicine, St. Louis, Missouri
Feranchak, A., B.S., M.D., University of Colorado, Denver
Karpova, A., B.S., M.S., Ph.D., Cold Spring Harbor Laboratory
Luneborg, N., B.A., University College London, United Kingdom
McKay, B., B.S., M.S., University of Calgary, Alberta, Canada
Metz, A.E., B.A., Northwestern University, Evanston, Illinois
Ramos, R., B.A., M.A., University of Connecticut, Storrs
Roseberry, A., B.A., M.S., Ph.D., The Rockefeller University, New York
Wise, D., B.S., University of Texas, Austin

SEMINARS

Diamond, J., National Institutes of Health, Bethesda, Maryland: Glutamate transporters and synaptic transmission.
Farrant, M., University College London, United Kingdom: GABAergic synaptic transmission.
Hausser, M., University College London, United Kingdom: Recording voltage-gated and synaptic currents. Purkinje cells: Dendritic excitability and synaptic integration.
Huguenard, J., Stanford University Medical School, Stanford, California: Cortical synaptic networks.
Llinas, R., New York University School of Medicine, New York: A historical look at ion channel physiology.
McBain, C., National Institutes of Health, Bethesda, Maryland: Ion channels in interneurons.
Nerbonne, J., Washington University School of Medicine, St. Louis, Missouri: Molecular basis of functional voltage-gated K^+ channel diversity.
Malin, S., Washington University School of Medicine, St. Louis, Missouri: K channels in cardiac muscle. K channels in neurons.
Siegelbaum, S., Columbia University, New York: Cyclic-nucleotide-gated channels.
Spruston, N., Northwestern University, Evanston, Illinois: Cable theory and synaptic integration. Dendritic excitability and synaptic integraton in the hippocampus.
Stuart, G., John Curtin School of Medical Research, Canberra, Australia: Dendritic excitability and synaptic integration, neocortex layer V.
Sullivan, J., University of Washington, Seattle: Cannabinoid receptors and synaptic transmission.
Svoboda, K., Cold Spring Harbor Laboratory: Imaging dendritic spines.
Trussell, L., Oregon Health Sciences University, Portland: Synaptic transmission in the auditory system.

Computational Neuroscience: Vision

June 13–26

INSTRUCTORS **E.J. Chichilnisky,** The Salk Institute, La Jolla, California
P. Glimcher, New York University, New York
E. Simoncelli, New York University, New York

ASSISTANTS **A. Churchland,** University of California, San Francisco
J. Pillow, New York University, New York

Computational approaches to neuroscience produced important advances in our understanding of neural processing. Prominent success came 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.

PARTICIPANTS

Alitto, H., B.S., University of California, Davis
Allred, S., B.S., University Washington, Seattle
Borghuis, B., M.A., Utrecht University, Holland
Boucher, L., B.A., Ph.D., Dartmouth College, Hanover, New Hampshire
Chatterjee, S., A.B., Salk Institute, La Jolla, California
Corrado, G., B.A., Stanford University, Stanford, California
Curnow, T., M.Sc., University of Oxford, United Kingdom
Hillis, J., B.Sc., University of Berkeley, California
Knappmeyer, B., M.S., Max-Planck Institute for Biological Cybernetics, Tuebingen, Germany
Lau, B., B.A., New York University, New York
Mante, V., M.S., ETH Zurich, Switzerland
Neri, P., D.Phil., Stanford University, Stanford, California
Olman, C., B.S., University of Minnesota, Minneapolis
Osindero, S., B.A., M.Sc., Gatsby Computational Neurosciences Unit, London, United Kingdom
Peirce, J., Ph.D., New York University, New York
Rokers, B., B.A., M.A., University of California, Los Angeles
Ruppertsberg, A., M.Sc., Ph.D., Keele University, United Kingdom
Rust, N., B.S., New York University, New York
Schluppeck, D., M.A., D.Phil., University of California, Los Angeles
Smith, M., B.A., M.A., York University, Toronto, Canada
Swinehart, C., B.S., Brandeis University, Waltham, Massachusetts
Tcheang, L., M.Sc., University of Oxford, United Kingdom
Troncoso, X., B.S., University College London, United Kingdom
Wyder, M., B.A., Wake Forest University School of Medicine, Winston Salem, North Carolina

SEMINARS

Adelson, E., Massachusetts Institute of Technology, Cambridge: Elements of vision/lightness.
Brainard, D., University of California, Santa Barbara: Trichromacy.
Brainard, D., University of California, Santa Barbara: Color vision: Trichromacy and context effects.
Chichilnisky, E.J., The Salk Institute, La Jolla, California: Contextual effects.
Chichilnisky, E.J., The Salk Institute, La Jolla, California: Retinal processing. White noise analysis. Tetinal adaptation.
Cullen, K., McGill University, Montreal, Canada: Eye movements II.
Glimcher, P., New York University, New York: Review: Signal detection theory. Eye movements. Perceptual decisions II.
Grill Spector, K., Stanford University, Stanford, California: Object/shape human physiology (fMRI).
Heeger, D., Stanford University, Stanford, California: Human physiology (fMRI) of attention.
Movshon, J.A., New York University, New York: LGN/V1 physiology. MT physiology.
Rieke, F., University of Washington, Seattle: Photoreceptors and photon detection. Spikes and information.
Simoncelli, E., New York University, New York; Mathematical tools: Linear systems, Fourier, probability/statistics. Image statistics and visual processing. Motion perception and modeling.
Kersten, D., University of Minnesota, Minneapolis: Object/shape psychophysics.
Newsome, W., Stanford University, Stanford, California: Motion: Linking physiology and perception.
Shadlen, M., University of Washington, Seattle: Neural noise and perception. Perceptual decisions I.
Treue, S., University of Tuebingen, Germany: Attention physiology.
Verghese, P., Smith Kettlewell Eye Research Institute, San Francisco, California: Attention psychophysics.

Making and Using DNA Microarrays

June 18–25

INSTRUCTORS **J. DeRisi,** University of California, San Francisco
V. Iyer, University of Texas, Austin

ASSISTANTS

A. Carroll, University of California, San Francisco
M. Diehn, Stanford University School of Medicine, Stanford, California
P. Killion, University of Texas, Austin
J. Lieb, University of North Carolina, Chapel Hill
M. Llinas, University of California, San Francisco
C. Murphy, University of California, San Francisco
B. Pulliam, University of California, San Francisco
C. Seidel, University of California, Berkeley
A. Sil, University of California, San Francisco
D. Wang, University of California, San Francisco

PARTICIPANTS

Alberola, T., B.S., Ph.D., University of Valencia, Spain
Jarlebark, L., B.Sc., Ph.D., Karolinska Institute, Stockholm, Switzerland
Al-Murrani, S., B.Sc., Ph.D., Pennsylvania State College of Medicine, Hershey
Koukoulas, I., B.Sc., Ph.D., The University of Melbourne, Australia
Armstrong, L., B.S., M.S., University of Texas, San Antonio
Kovacs, S., B.A., Ph.D., California State University, Fresno
Chakraborty, S., B.Sc., Ph.D., Jawaharlal Nehru University, New Delhi, India
Laloraya, S., M.Sc., Ph.D., Indian Institute of Science, Bangalore, India
Collett, H., B.Sc., Ph.D., University of Cape Town, South Africa
Linney, E., B.S., Ph.D., Duke University Medical Center, Durham, North Carolina
Eudy, J., B.S., Ph.D., University of Nebraska Medical Center, Omaha

Minois, N., B.A., Ph.D., Max-Planck Institute for Demographic Research, Rostock, Germany
Feng, Q., B.S., Ph.D., Great Lakes Forestry Centre, Ontario, Canada
Shirsat, N., M.Sc., Ph.D., Tata Memorial Centre, Mumbai, India
Fowlks, E., B.S., Ph.D., Hampton University, Virginia
Simmons, S., B.A., Ph.D., Woods Hole Oceanographic Institute, Massachusetts
Gadea, J., B.S., Ph.D., Universidad Politecnica de Valencia, Spain
Vallar, L., M.A., Ph.D., CRP-Sante, Good Duchy of Luxembourg
Griffith, B., B.S., M.S., University of New Mexico Health Sciences Center, Albuquerque
Weaver, D., B.S., Ph.D., Medical College of Ohio, Toledo

SEMINARS

Carroll, A., University of California, San Francisco: Core.
DeRisi, J., University of California, San Francisco: Introduction. Field overview.
Diaz, E., University of California, Berkeley: Spatial and temporal maps of gene expression in the developing nervous system.
Diehn, M., Stanford University School of Medicine, Stanford, California: Genomic expression programs and the integration of the CD28 costimulatory signal on T-cell activation.
Imam, F., Stanford University, Stanford, California: A transcriptional analysis of development in *Drosophila melanogaster*.
Iyer, V., University of Texas, Austin: Exploring transcriptional programs and mechanisms on a genome-wide scale.
Jamieson, C., University of California, San Francisco: How I set up a microarray consortium: An evolutionary concept!
Li, J., University of California, San Francisco: Genome-wide analysis of DNA replication.
Lieb, J., University of North Carolina, Chapel Hill: Exploring specificity in protein-genome interactions.
Llinas, M., University of California, San Francisco: Microarray-based whole-genome approaches.
Murphy, C., University of California, San Francisco: Discovery of novel lifespan genes through microarrays analysis of the DAF-2 pathway.
Seidel, C., University of Berkeley, California: Robotics fundamentals.
Sil, A., University of California, San Francisco: Using genomics to probe pathogenesis in *Histoplasma capsulatum*.
Wang, D., University of California, San Francisco: Microarray-based detection and genotyping of viruses.

Biology of Developmental Disabilities

June 28–July 3

INSTRUCTORS **B.J. Casey,** Weill Medical College of Cornell University, New York
Y. Munakata, University of Denver/University of Colorado

This lecture course explored basic developmental principles of the brain and behavior and their application to understanding the biology of developmental disabilities. Leading developmental neurobiologists, neuroscientists, and psychologists presented models of typical and atypical brain development. Students were exposed to a wide spectrum of methods currently being used in developmental science including neuroimaging, computational modeling, and animal models.

PARTICIPANTS

Amso, D., B.S., Ph.D., Cornell University, Ithaca, New York
Ansari, D., B.A., M.Sc., Institute of Child Health, London, United Kingdom
Bonte, M., B.A., M.A., University of Maastricht, The Netherlands
Byrd, D., B.A., M.S., University of Florida, Gainesville
Creel, S., B.A., BS, University of Rochester, New York
Dyer, J., B.A., University of Michigan, Ann Arbor
Edgin, J., B.S., M.S., University of Denver, Colorado
Griffin, A., B.A., University of Texas, Austin
Hamilton, E., B.A., University of Michigan, Ann Arbor
Marcus, D., B.A., M.A., University of Minnesota, Minneapolis
Menzer, D., A.B., Wake Forest University, Winston-Salem, North Carolina
Raitano, N., B.S., M.A., University of Denver, Colorado

Rueda-Cuerva, M., B.S., Ph.D., Sackler Institute for Developmental Psychobiology, New York
Shohamy, D., B.A., Ph.D., Rutgers University, Newark, New Jersey
Snyder, K., B.S., University of Minneapolis, Minnesota
Stedron, J., B.A., M.A., University of Denver, Colorado
Townsend, E., B.A., MPT, University of Minnesota, Minneapolis
Webb, S., B.A., Ph.D., University of Washington, Seattle
Wilbrecht, L., B.A., The Rockefeller University, New York
Ziergler, D., B.S., Massachusetts General Hospital/Harvard University

SEMINARS

Amaral, D., University of California, Davis: Amygdala, social behavior, and neurodevelopmental disorders.
Bates, E., University of California, San Diego, La Jolla: Plasticity and reorganization of language.
Casey, B.J., Weill Medical College of Cornell University, New York: Development of frontostriatal circuitry and cognitive control.
Davidson, M., Weill Medical College of Cornell University, New York: Basic principles and acquisition of fM.
Fossella, J., Weill Medical College of Cornell University, New York: Genetics workshop.
Gould, E., Princeton University, Princeton, New Jersey: Neurogenesis in the adult brain.
Greenough, W., University of Illinois, Urbana: Environmental and genetic factors.
Kennedy, D., Massachusetts General Hospital Center for Morphometric Analysis, Charlestown: Basic principles and acquisition of MRI and DTI.
Lewis, D., University of Pittsburgh, Pennsylvania: Development and disruption of prefrontal cortex.
McCandliss, B., Weill Medical College of Cornell University, New York: Development and plasticity of reading and speech.
McEwen, B., The Rockefeller University, New York: Impact of stress on developmental and learning.
Munakata, Y., University of Denver/University of Colorado, Boulder: Computational modeling workshop.
Nottebohm, F., The Rockefeller University, New York: Vocal learning and neuronal replacement in the songbird.
Thomas, K., Weill Medical College of Cornell University, New York: Development of the amygdala response: Applications of fMRI.
Watts, R., Weill Medical College of Cornell University, New York: Hands-on workshop on DTI analysis.
Worden, M., Weill Medical College of Cornell University, New York: Basic principles and acquisition of EEG/ERP. Hands-on workshop on EEC/ERP analysis.

Mouse Behavioral Analysis

June 28–July 11

INSTRUCTORS **S. Anagnostaras,** Emory University, Atlanta, Georgia
M. Mayford, The Scripps Research Institute, La Jolla, California

ASSISTANTS **A. Matynia,** University of California, Los Angeles
S. Mitchell, Emory University, Atlanta, Georgia
L. Reijmers, The Scripps Research Institute, La Jolla, California
B. Wiltgen, University of California, Los Angeles

This course was intended to provide a theoretical and experimental introduction to behavioral analysis in the mouse, with a focus on learning and memory. It was specially designed for geneticists, molecular biologists, pharmacologists, and electrophysiologists with a need for a hands-on introduction to behavioral analysis of the mouse. Additionally, the course covered the principles of using mutant mice in behavioral studies, as well as the issues involved in integrating behavioral, neuroanatomical, neurophysiological, and molecular findings. Among the methods presented were the water maze, cued and contextual fear conditioning, natural/ethologically relevant learning, open field behavior, and the rotorrod and other activity tests.

PARTICIPANTS

Battaglia, M., B.A., M.D., S. Raffaele University, Milan, Italy
Furukawa, T., M.D., Ph.D., Osaka Bioscience Institute, Osaka, Japan
Murphy, B.A., B.S., M.S., Merck, Rahway, New Jersey
Pietras, M., B.Sc., M.Sc., Astrazeneca, Wilmington, Delaware
Pozueta, J., B.A., M.A., Universidad Autonoma de Madrid, Spain
Sadhu, A., M.S., Friedrich Meischer Institute, Basel, Switzerland
Seong, E., B.S., M.S., University of Michigan, Ann Arbor
Thomas, M., B.A., Ph.D., University of Michigan, Ann Arbor
Van Amerongen, R., M.Sc., Netherlands Cancer Institute, Amsterdam, The Netherlands
Watson, J., Vet.M.B., Johns Hopkins University, Baltimore, Maryland
Zhao, B., B.S., Ph.D., Karolinska Institute, Stockholm, Sweden

SEMINARS

Anagnostaras, S., Emory University, Atlanta, Georgia: Statistics.
Blanchard, R., University of Hawaii, Honolulu: Aggressive and defensive behavior in the rat and mouse. Crosspieces generality of aggression and defense.
Costa, R., University of California, Los Angeles: Mouse models of cognitive disorders.
Crawley, J., National Institutes of Mental Health, Bethesda, Maryland: Strategies for behavioral phenotyping of a new transgenic or knockout mouse.
Eichenbaum, H., Boston University, Massachusetts: The hippocampus.
Hen, R., Columbia University, New York: Genetic studies of 5-HT function.
Matynia, A., University of California, Los Angeles: Phenotypic screen for long-term memory mutants.
Mayford, M., The Scripps Research Institute, La Jolla, California: Ca^{++} signaling in long-term memory. Genetic background issues in mice.
Quirk, M., Massachusetts Institute of Technology, Cambridge: CA3-specific knockout of NMDA receptors. Disruption of LTP and pattern completion.
Reijmers, L., The Scripps Reseach Institute, La Jolla, California: ENU screen for memory mutants.
Robinson, T., University of Michigan, Ann Arbor: Drug-induced neurobehavioral plasticity.
Stephan, A., Emory University, Atlanta, Georgia: Introduction to behavioral psychology I. Introduction to behavioral psychology II.
Wiltgen, B., University of California, Los Angeles: CA1-specific GluR1 KO mice exhibit enhanced LTP and impaired hippocampal-dependent learning.
Young, L., Emory University, Atlanta, Georgia: Selective loss of social recognition in oxytocin knockout mice.

Arabidopsis Molecular Genetics

June 28–July 18

INSTRUCTORS **J. Bowman,** University of California, Davis
U. Grossniklaus, University of Zurich, Switzerland
R. Pruitt, Purdue University, West Lafayette, Indiana

ASSISTANTS **U. Akinci,** University of Zurich, Switzerland
J.-Y. Lee, University of California, Davis
J. Victor, Purdue University, West Lafayette, Indiana

This course provided an intensive overview of topics in plant growth, physiology, and development, focusing on molecular genetic approaches to understanding plant biology. It emphasized recent results from *Arabidopsis thaliana* and other model plants and provided an introduction to current methods used in *Arabidopsis* research. It was designed for scientists with experience in molecular techniques or in plant biology who wish to work with *Arabidopsis*. 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 (including development of flowers, roots, meristems, and 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 *Arabidopsis* community, and the resources provided by the *Arabidopsis* genome project to accelerating *Arabidopsis* research, were also included. Speakers provided overviews of their fields, fol-

lowed by in-depth discussions of their own work. The laboratory sessions provided an introduction to important techniques currently used in *Arabidopsis* research. These included studies of *Arabidopsis* development, mutant analysis, in situ detection of RNA, 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 included several short workshops on important themes in genetics.

PARTICIPANTS

Bullis, D., B.S., Torrey Mesa Research Institute, San Diego, California
Chakraborty, S., B.Sc., Ph.D., Jawaharlal Nehru University, New Delhi, India
Des Marais, D., B.A., Duke University, Durham, North Carolina
Farrell, G., B.Sc., University of Wales, Aberystwyth, Wales
Hark, A., B.S., Ph.D., Michigan State University, East Lansing
Havecker, E., B.S., Iowa State University, Ames
Hemsley, R., B.Sc., Ph.D., John Innes Centre, Norwich, United Kingdom
Israelsson, M., B.S., Ph.D., Swedish University of Agricultural Sciences, Umea, Sweden
Jennings, C., B.Sc., LaTrobe University, Victoria, Australia
Kaplinsky, N., B.A., Ph.D., University of California, Berkeley
Kim, M.J., B.S., M.S., Korea University, Seoul, Korea
Kumari, N., B.S., M.S., Ohio State University, Columbus
Leiber, R., DipL., The Scripps Research Institute, La Jolla, California
MacDonald, T., B.Sc., Ph.D., Goteborg University, Sweden
Ramon, M., B.S., M.S., Catholic University Leuven, Belgium
Sangster, T., B.A., Ph.D., University of Chicago, Illinois

SEMINARS

Banks, J., Purdue University, West Lafayette, Indiana: Gametophytes and evolution.
Bent, A., University of Wisconsin, Madison: Plant pathogen.
Bowman, J., University of California, Davis: Flowers.
Coupland, G., The John Innes Centre, United Kingdom: Flowering time.
Dengler, N., University of Toronto, Canada: Plant anatomy.
Ecker, J., The Salk Institute for Biological Sciences, La Jolla, California: Genomics.
Grossniklaus, U., University of Zurich, Switzerland: Female gametophyte.
Hangarter, R., Indiana University, Bloomington: Light responses.
Harper, J., The Scripps Research Institute, La Jolla, California: Calcium and signaling.
Jackson, D., Cold Spring Harbor Laboratory: Meristem and leaf.
Jorgensen, R., University of Arizona, Tucson: Epigenetics II.
Juenger, T., University of California, Berkeley: Quantitative genetics.
Kellogg, E., University of Missouri, St. Louis: Evolution.
Malamy, J., University of Chicago, Illinois: Roots.
Martienssen, R., Cold Spring Harbor Laboratory: Enhancers traps.
McCourt, P., University of Toronto, Canada: Hormone networks.
Nasrallah, J., Cornell University, Ithaca, New York: Self incompatibility.
Ogas, J., Purdue University, West Lafayette, Indiana: Embryogenesis.
Peck, S., John Innes Centre, Norwich, United Kingdom: Proteomics.
Penna, D.D., Michigan State University, East Lansing: Secondary metabolism.
Pruitt, R., Purdue University, West Lafayette, Indiana: Male gametophyte and signaling.
Quatrano, R., Washington University, St. Louis, Missouri: Cell biology.
Schaller, E., University of New Hampshire, Durham: Ethylene.
Smyth, D., Monash University, Clayton, Australia: The big picture.
Spector, D., Cold Spring Harbor Laboratory: Microscopy.
Sussex, I., Yale University, Newtown, Connecticut: Introduction.
Vance, V., University of South Carolina, Columbia: Epigenetics I.
Walbot, G., Stanford University, Stanford, California: Transposons.
Zhu, T., Torrey Mesa Research Institute, San Diego, California: Affymetrix chips.

Molecular Cloning of Neural Genes

June 28–July 18

INSTRUCTORS
J. Boulter, University of California, Los Angeles
L. Henry, Cold Spring Harbor Laboratory
K. Jensen, The Rockefeller University, New York
C. Lai, Scripps Research Institute, La Jolla, California
D. Lavery, Glaxo Wellcome Experimental Research, Lausanne, Switzerland

ASSISTANTS
J. Arjomand, University of California, Los Angeles
I. Cheung, The Scripps Research Institute, La Jolla, California
C. Cottiny, Institut de Biologie Cellulaire et Morphology, Lausanne, Switzerland
C. Ferrara, The Scripps Research Institute, La Jolla, California
T. Fischer, The Scripps Research Institute, La Jolla, California
J. LeMieux, Tufts University Medical School, Boston, Massachusetts
E. Ruchti, Institut de Biologie Cellulaire et de Morphology, Lausanne, Switzerland
M. Samson, Harvard Medical School/HHMI, Boston, Massachusetts

This intensive laboratory and lecture course taught neuroscientists current approaches to molecular neurobiology. The course consisted of daily lectures and laboratory exercises on the practice of molecular neurobiology, with an emphasis on modern approaches to cloning and analyzing the expression of neural genes. A series of evening research seminars by invited speakers focused on the ways in

which these molecular techniques have been successfully applied. In the past, evening seminar topics have included expression cloning, single-cell cloning, subtractive cDNA-cloning strategies, and genetic and mechanistic studies of neurologic disease, acquisition of cell identity, and axon guidance in the developing nervous system. The laboratory portion of the course began with instructions in a series of basic molecular biological techniques and rapidly advanced to more sophisticated methodologies. Students learned to prepare genomic, phage, and plasmid DNAs and total and poly(A)$^+$ RNAs and to generate and screen cDNA libraries. Additional topics and methods covered included restriction mapping; agarose and polyacrylamide gel electrophoresis; northern and Southern blotting; subcloning; oligonucleotide primer design; a selection of polymerase chain reaction (PCR)-based techniques; and the use of nucleotide and protein sequence databases. Gene expression studies included the production of fusion proteins in bacteria and mammalian cell transfection. The advanced techniques featured the construction of cDNA libraries from single cells and the use of subtractive cDNA methods to clone genes expressed in limited populations of cells.

PARTICIPANTS

Ango, F., Ph.D., Cold Spring Harbor Laboratory
Croft, C., B.S., University of Virginia, Charlottesville
Dulawa, S., A.B., Ph.D., Columbia University, New York
Engblom, D., B.M., University of Linkoping, Sweden
Glosmann, M., M.S., Ph.D., Harvard Medical School, Boston, Massachusetts
Jontes, J., B.A., Ph.D., Stanford University, Stanford, California
Kato, T., M.D., Ph.D., Brain Science Institute, Saitama, Japan
Klemenhagen, K., B.A., Ph.D., Columbia University, New York
Korvatska, E., M.S., Ph.D., University of California, Davis
MacInnis, B., B.Sc., Ph.D., University of Alberta, Canada
Paoletti, F, SISSA-International School for Advanced Studies, Trieste, Italy
Pongrac, J., B.Sc., Ph.D., University of Pittsburgh, Pennsylvania
Rosenstrom, H., M.Sc., University of Helsinki, Finland
Tashiro, A., B.S., Columbia University, New York

SEMINARS

Barres, B., Stanford University, Stanford, California: Neuron-glial interactions in the developing CNS.
Darnell, B., The Rockefeller University, New York: RNA-binding proteins in human neurological disease.
Eberwine, J., University of Pennsylvania, Philadelphia: Molecular analysis of dendritic funcioning: mRNA localization, mRNA transport, and local protein synthesis.
Fishell, G., Skirball Institute, New York University: Proliferation, patterning, and migration in the mouse forebrain.
Lemke, G., Salk Institute, La Jolla, California: *Vax* genes and the axial specification of the eye.
Nairn, A., Yale University, New Haven, Connecticut: Dopamine signaling in the central nervous system.
Patapoutian, A., The Scripps Institute, La Jolla, California: The sense of touch: A genomic approach.
Picciotto, M., Yale University, New Haven, Connecticut: Nicotine, addiction, and learning: Using knockout mice to go from gene to behavior.
Weinmaster, G., University of California School of Medicine, Los Angeles: On the fringes of Notch signal transduction.

Neurobiology of *Drosophila*

June 28–July 18

INSTRUCTORS **S. De Belle,** University of Nevada, Las Vegas
A. DiAntonio, Washington University School of Medicine, St. Louis, Missouri
D. Van Vactor, Harvard Medical School, Boston, Massachusetts

ASSISTANTS **Y. Ben-Shahar,** University of Illinois, Urbana
K. Johnson, Harvard Cancer Center, Boston, Massachusetts
S. Marrus, Washington University School of Medicine, St. Louis, Missouri

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 three-week course was divided into the study of development, physiology/function, and behavior. Daily seminars introduced students to a variety of research topics and developed those topics by including recent experimental contributions and outstanding questions in the field. Guest lecturers brought original preparations for viewing and discussion and direct laboratory exercises and experiments in their area of interest. The course provided students with hands-on experience using a variety of experimental preparations that were used in the investigation

of current neurobiological questions. The lectures and laboratories focused on both the development of the nervous system and its role in controlling larval and adult behaviors. In addition to an exposure to the molecular genetic approaches available in *Drosophila*, students learned a variety of techniques including embryo in situ hybridization, labeling of identified neurons, 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.

PARTICIPANTS

Belay, A., B.S., University of Toronto, Ontario, Canada
Ge, X., B.S., Tsinghua University, Beijing, Peoples Republic of China
Ghose, A., B.Sc., Ph.D., Harvard Medical School, Boston, Massachusetts
Gilestro, G., M.S., Institute of Molecular Pathology, Vienna, Austria
Krupp, J., B.S., University of Michigan, Ann Arbor
Li, C., Washington University, St. Louis, Missouri
Merrill, C., B.S., Vanderbilt University, Nashville, Tennessee
Serway, C., B.A., University of Nevada, Las Vegas
Steinhilb, M., B.S., Harvard Medical School, Boston, Massachusetts
Wakefield, S., B.Sc., King's College, London, United Kingdom
Watson, F., B.Sc., Ph.D., Dana Farber Cancer Institute, Boston, Massachusetts
Zhai, R.G., B.S., Ph.D., Baylor College of Medicine, Houston, Texas

SEMINARS

Auld, V., University of British Columbia, Vancouver, Canada: Embryonic glial development.
Budnick, V., University of Massachusetts, Amherst: Cell biology of the synapse.
De Belle, S., University of Nevada, Las Vegas: Introduction to fly behavior. Learning and mmory: hardware.
DiAntonio, A., Washington University, St. Louis, Missouri: Synaptic plasticity.
Ferrus, A., Institute Cajal, Madrid, Spain: Postembryonic peripheral nervous system.
Gaul, U., The Rockefeller University, New York: The development of the visual system.
Kernan, M., SUNY, Stony Brook, New York: Mechanotransduction.
Knoblich, J., Institute of Molecular Pathology, Vienna, Austria: Embryonic neurogenesis and asymmetric cell.
Landgraf, M., Cambridge University, Massachusetts: Determination of neuronal identity.
Murphey, R., University of Massachusetts, Amherst: Giant fiber system.
Nash, H., NIMH, National Institutes of Health, Bethesda, Maryland: Anesthesia.
Shaw, P., Neuroscience Institute, San Diego, California: Sleep.
Sokolowski, M., University of Toronto, Canada: Foraging.
Stewart, B., University of Toronto, Canada: Synaptic transmission.
Strauss, R., Theodor-Boveri-Institute, Wurzburg, Germany: Motor behavior. Visual system.
Tepass, U., University of Toronto, Canada: Introduction to the fly embryo.
Tully, T., Cold Spring Harbor Laboratory: Learning and memory: Software.
Van Vactor, D., Harvard University, Cambridge, Massachusetts: Axon guidance mechanisms in the embryo.
Vosshall, L., The Rockefeller University, New York: Olfaction.
Waters Shuler, J., Harvard University, Cambridge, Massachusetts: Fundamentals of light microscopy.

Molecular Mechanisms of Human Neurological Diseases

July 6–12

INSTRUCTORS **A. Aguzzi,** University Hospital of Zurich, Switzerland
S. Gandy, Thomas Jefferson University, Philadelphia, Pennsylvania
J. Hardy, Mayo Clinic, Jacksonville, Florida

How and why do neurons dysfunction or die in specific acute or chronic human neurological disorders? What are the molecular and biochemical manifestations of specific genetic lesions in specific neurological disorders? Do different pathological dysfunctions share common mechanisms? What practical treatments are being contemplated? This lecture course explored possible answers to these important questions. Recent advances in neurogenetics and in molecular and cell biology have begun to shed light on the mechanisms that underlie nervous system injury in disease states such as stroke, epilepsy, channelopathies, Alzheimer's disease, frontotemporal dementia, prion diseases, lissencephaly, Fragile X mental retardation, and polyglutamine repeat disorders. Taking advantage of small class size and extensive discussion, invited faculty lecturers examined critical issues in their area of expertise. Overview was provided and course participants did not need to have familiarity with neurological diseases, although a background in basic nervous system structure and organization was extremely helpful. The course focused principally on the specific hypotheses and approaches driving current research. Emphasis was placed on the highly dynamic interface between basic and clinical investigation, including the interdependence of clinical research and disease model development, and the value of disease research in understanding the function of the normal nervous system.

PARTICIPANTS

Aarum, J., M.S., Karolinska Institutet, Stockholm, Sweden
Ajit, S., M.Phil., Ph.D., Wyeth Ayerst Research, Princeton, New Jersey
Anikeeva, P., St. Petersburg State Technical University, Russia
Bark, N., M.D., M.S., Karolinsa Instituet, Stockholm, Sweden
Bergman, A., M.S., Karolinska Institutet, Stockholm, Sweden
BugajGaweda, B., M.S., Memory Pharmaceuticals Corp., Montvale, New Jersey
Cohen-Kupiec, R., B.A., M.A., Ph.D., Tel Aviv University, Israel
Genoud, N., B.S., M.S., University Hospital Zurich, Switzerland
Gerwien, J., M.S., B.S., Lundbeck A/S, Valby, Denmark
Goren, O., B.S., M.S., Tel Aviv University, Tel Aviv, Israel
Howard, V., B.A., Mayo Clinic, Jacksonville, Florida
Labunskiy, D., B.S., Institute of Neurology, Moscow, Russia
Klingeborn, M., B.S., M.S., Swedish University of Agricultural Sciences, Uppsala, Sweden
Lockhart, P., B.S., Ph.D., Mayo Clinic Jacksonville, Florida
Lopes, M., B.A., M.S., Ludwig Institute for Cancer Research, Sao Paulo, Brazil
Myre, M., B.Sc., University of Toronto, Mississauga, Canada
Pickford, F., B.A., Mayo Clinic, Jacksonville, Florida
Silverman, S., B.S., Ph.D., Wyeth Research, Princeton, New Jersey
Taylor, J., B.Sc., Mayo Clinic, Jacksonville, Florida
Venneti, S., M.D., Ph.D., University of Pittsburgh, Pennsylvania
Voisine, C., B.S., Ph.D., Massachusetts General Hospital, Charlestown
Wood, C., M.B., University of Leeds, United Kingdom

SEMINARS

Aguzzi, A., University Hospital of Zurich, Switzerland: Molecular biology of prion diseases.
Capecchi, M., University of Utah School of Medicine, Salt Lake City: The role of Hoxb8 in normal grooming behavior.
Darnell, R., The Rockefeller University, New York: Molecular biology of paraneoplastic diseases.
Fischbeck, K., NINDS, NIH, Bethesda, Maryland: Polyglutamine expansion neurodegenerative diseases.
Frackowiak, R., University College London, United Kingdom: Functional neuroimaging.
Gandy, S., Thomas Jefferson University, Philadelphia, Pennsylvania: Regulation of the metabolic fate of the Alzheimer amyloid precursor.
Hardy, J., Mayo Clinic Jacksonville, Florida: Approach to neurogenetics.
Iadecola, C., University of Minnesota, Minneapolis: Molecular pathology of stroke.
McNamara, J., Duke University Medical Center, Durham, North Carolina: Molecular pathology of epilepsy.
Murphy, D., NIND, National Institutes of Health, Rockville, Maryland: Research programs in neuroscience at NIH.
Nitsch, R., University of Zurich, Switzerland: Molecular biology of Alzheimer's disease.
Orr, H., University of Minnesota, Minneapolis: SCA1: The molecular basis of a polyglutamine disorder.
Schofield, P., The Garvan Institute of Medical Research, Sydney, Australia: The inhibitory glycine receptor: Molecular studies of structure.
Walsh, C., Beth Israel Deaconess Medical Center, Boston, Massachusetts: Neuronal migration disorders.
Williamson, R.A., The Scripps Research Institute, La Jolla, California: Prions: Conformations, diagnosis, and therapy; a role for antibody in multiple sclerosis.

Drosophila Genetics and Genomics

July 14–27

INSTRUCTORS **M. Ashburner,** EMBL-EBI, Cambridge, United Kingdom
K. Burtis, University of California, Davis
R.S. Hawley, Stowers Institute for Biomedical Research, Kansas City, Missouri

ASSISTANT **J.E. Van Veen,** Stowers institute for Medical Research, Kansas City, Missouri

This intensive seminar course provided an introduction to the theory and practice of methods used to analyze and manipulate the *Drosophila* genome. It was suitable for graduate students and researchers with some experience with *Drosophila* who are interested in expanding their knowledge of the wide range of genetic and genomic techniques now available for use with this organism. Topics covered included chromosome mechanics, the design and execution of genetic screens, and the use of transposable elements as genetic tools. We reviewed the current understanding of the genome sequence and discussed methodologies for genomic analysis (e.g., microarrays, gene-finding methods, comparison with the sequences of related species, and the heterochromatin genomic project) and proteomics in *Drosophila*.

PARTICIPANTS

Bennion, J., B.Sc., Friedrich Miescher Institute, Basel, Switzerland
Bettencourt-Dias, M., B.S., Ph.D., Cambridge University, United Kingdom
Blanton, H., B.A., University of North Carolina, Chapel Hill
Cabernard, C., B.S., M.S., University of Basel, Switzerland
Dandy, T., B.S., Tennessee State University, Nashville
Gong, W., B.S., Stowers Institute for Medical Research, Kansas City, Missouri
Grade, S., B.S., University of Iowa, Iowa City
Iliopoulos, O., B.S., M.D., Massachusetts General Hospital, Boston
Jin, P., B.S., Ph.D., Emory University, Atlanta, Georgia
Kenyon, K., B.A., Ph.D., MEEI/Harvard Medical School, Boston, Massachusetts
Lee, A., B.S., Harvard Medical School, Boston, Massachusetts
Lokere, J., B.S., Harvard University, Boston, Massachusetts
Oikemus, S., B.S., University of Massachusetts Medical School, Worcester
Paternostro, G., M.D., Ph.D., Burnham Institute, La Jolla, California
Qian, L., B.S., Ph.D., University of Michigan, Ann Arbor
Skjesol, A., B.S., M.S., University of Iowa, Iowa City
Wiklund, M.-L., B.S. , Ph.D., Umea University, Sweden
Williams, V., B.A., University of Alabama, Birmingham
Xie, H., B.S., Stowers Institute for Medical Research, Kansas City, Missouri

SEMINARS

Ashburner, M., EMBL-EBI, Cambridge, United Kingdom: Introduction to the biology of *Drosophila*.
Hawley, S., Stowers Institute Biomedical Research, Kansas City, Missouri: The genetics of *Drosophila* Parts I and II. The genetics of *Drosophila* Part III.
Bier, E., University of California, San Diego: *Drosophila* as a model system for neurodegenerative diseases.
Burtis, K., University of California, Davis: *Drosophila* genome biology.
Golic, K., University of Utah, Salt Lake City: Mosaic systems and targeted insertions.
Hawley, S., Stowers Institute for Biomedical Research, Kansas City, Missouri: The genetics and cell biology of meiosis.
Heberlein, U., University of California, San Francisco: Genetic analysis of behavior.
Henikoff, S., Fred Hutchinson Cancer Research, Seattle, Washington: Heterochromatin and centromeres.
Langley, C., University of California, Davis: Genome structure of *Drosophila* populations.
Lehmann, R., Skirball Institute, New York: Genetic screens.
Lewis, S., University of California, Berkeley: The fly genome: 2002 version.
Rorth, P., EMBL, Heidelberg, Germany: P-element technologies.
White, K., University of Washington, Seattle: *Drosophila* microarrays.

C. elegans

July 23–August 12

INSTRUCTORS **B. Bowerman,** University of Oregon, Eugene
A. Chisholm, University of California, Santa Cruz
R. Korswagen, Hubrecht Laboratory, Utrecht, The Netherlands

ASSISTANTS **D. Coudreuse,** Hubrecht Laboratory, Utrecht, The Netherlands
R. Harrington, University of California, Santa Cruz
T. Kurz, University of Oregon, Eugene

This course was designed to familiarize investigators with *C. elegans* as an experimental system, with an emphasis on both classical genetic analysis and reverse genetic approaches. A major goal was to teach students how to successfully exploit the information generated by the *C. elegans* genome project. The course was suited both for those who have a current training in molecular biology and some knowledge of genetics, but have no experience with *C. elegans,* and for students with some prior worm experience who wished to expand their repertoire of expertise. The following topics were covered both in the laboratory and by lectures from experts in the field: worm pushing, *C. elegans* databases and worm bioinformatics, anatomy and development, forward genetics, chemical and transposon mutagenesis, generation of transgenic animals, expression pattern analysis, reverse genetics, construction and screening of deletion libraries, and RNA inactivation. The course was designed to impart sufficient training to students in the most important attributes of the *C. elegans* system to enable students to embark on their own research projects after returning to their home institutions.

PARTICIPANTS

Chandra, A., B.Sc., Baylor College of Medicine, Houston, Texas
Chang, M.-S., D.D.S., Ph.D., New York University School of Medicine, New York
Decker, J., B.S., University of Arizona, Tucson
Deken, S., B.S., Ph.D., Washington University, St. Louis, Missouri
Ferkey, D., B.S., Massachusetts General Hospital, Charlestown
Ghazi, A., M.Sc., Ph.D., National Centre for Biological Sciences, Bangalore, India
Guarin, E., B.S., Ph.D., Hoffmann-La Roche, Basel, Switzerland
Hamza, I., B.Sc., Ph.D., Washington University School of Medicine, St. Louis, Missouri
Hu, C., M.D., Ph.D., University of Michigan, Ann Arbor
Jantti, J., B.S., Ph.D., VTT Biotechnology, Espoo, Finland
Jayasena, S., B.Sc., Ph.D., University of Colombo, Sri Lanka
Jeffries, M., B.S., M.S., Eli Lilly & Company, Greenfield, Indiana
McDonald, P., B.S., Ph.D., Vanderbilt University, Nashville, Tennessee
Parusel, C., B.S., M.S., Friedrich Miescher Institut, Basel, Switzerland
Schafer, J., B.S., University of Alabama, Birmingham
Van Binsbergen, E., M.A., University Medical Center Utrecht, The Netherlands

SEMINARS

De Bono, M., MRC Laboratory of Molecular Biology, Cambridge, United Kingdom: Using *C. elegans* to study behavior.
Driscoll, M., Rutgers, The State University of New Jersey, Piscataway: Using *C. elegans* to study neurodegeneration and cell death.
Gonczy, P., Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland: Using *C. elegans* to study cell biology.
Hall, D., Albert Einstein College of Medicine, Bronx, New York: Exploring nematode anatomy by TEM.
Harris, T., Cold Spring Harbor Laboratory: Wormbase
Hobert, O., Columbia University, New York: Transgenes: Uses and abuses.
Kim, S., Stanford University Medical Center, Stanford, California: Microarray analysis.
Koelle, M., Yale University School of Medicine, New Haven, Connecticut: Reverse genetics and G-protein signaling.
Mango, S., University of Utah, Salt Lake City: RNA interference.
Sundaram, M., University of Pennsylvania, Philadelphia: Genetic suppressor and enhancer screens.
Van den Heuvel, S., Massachusetts General Hospital Cancer Center, Charlestown: Genetics of cell division control.

Eukaryotic Gene Expression

July 23–August 12

INSTRUCTORS
L. Attardi, Stanford University, Stanford, California
B. Dynlacht, Harvard University, Cambridge, Massachusetts
W.L. Kraus, Cornell University, Ithaca, New York
M. Timmers, Utrecht University, The Netherlands

ASSISTANTS
M.L. Acevedo, Cornell University, Ithaca, New York
E. Hammond, Stanford University, Stanford, California
M. Klejman, Utrecht University, The Netherlands
J. Rayman, Harvard University, Cambridge, Massachusetts

This course was designed for students, postdocs, and principal investigators who have recently ventured into the dynamic area of gene regulation. The course focused on state-of-the-art strategies and techniques employed in the field. Students made nuclear extracts, performed in vitro transcription, and measured RNA levels using primer extension. Emphasis was placed on biochemical studies of protein-DNA and protein-protein interactions. Detailed characterizations of the DNA-binding properties of site-specific transcription factors were carried out using electrophoretic mobility shift and DNase I footprinting assays. These assays were used to study protein-DNA interactions in crude extracts and using recombinant proteins. Coimmunoprecipitation and binding assays were employed to investigate pro-

tein-protein interactions with the general transcription machinery. In addition, students learned techniques for the assembly and analysis of chromatin in vitro. Techniques using the in-vitro-assembled chromatin included transcription assays, chromatin footprinting, chromatin remodeling assays, and enzyme assays for the posttranscriptional modification of histones.

Over the past few years, the gene regulation field has begun to emphasize the importance of in vivo approaches to studying protein-DNA and protein-protein interactions. Students were therefore exposed to the chromatin immunoprecipitation technique. 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

Briones, M., B.S., Universidad Nacional Autonoma de Mexico, Mexico City
Ehmsen, J., B.S., University of Oxford, United Kingdom
Federico, M., B.S., University of Wisconsin, Madison
Heissig, F., B.A., M.S., Max-Planck Institute for Evolution, Leipzig, Germany
Henriet, P., Universite Catholique de Louvain, Brussels, Belgium
Hertzano, R., B.S., Tel Aviv University, Tel Aviv, Israel
Holmstrom, S., B.A., University of Michigan, Ann Arbor
Kalogeraki, V., B.S., Ph.D., Stanford University, Stanford, California
Lund, C., B.S., Ph.D., The Scripps Research Institute, La Jolla, California
Meriluoto, T., M.S., National Public Health Institute of Finland, Helsinki, Finland
Monesi, N., B.Sc., M.S., Ph.D., Faculdade De Ciencias Farmaceuticas, Brazil
Nisha, P., M.Sc., Carnegie Mellon University, Pittsburgh, Pennsylvania
Otteson, D., B.S., Ph.D., Johns Hopkins University School of Medicine, Baltimore, Maryland
Sanders, V., B.S., Ph.D., The Ohio State University, Columbus
Woo, E., A.B., University of California, San Francisco
Yates, P., B.A., Ph.D., Oregon Health and Science University, Portland

SEMINARS

Attardi, L., Stanford University, Stanford, California: Identification of apoptosis-specific *p53* target genes.
Burtis, K., University of California, Davis: Array-based methods for high-throughput analysis of gene expression.
Dynlacht, B., Harvard University, Cambridge, Massachusetts: Transcriptional control of the cell cycle.
Freedman, L., Memorial Sloan-Kettering Cancer Center, New York: Mechanisms of transcriptional activation by nuclear hormone receptors.
Goodrich, J., University of Colorado, Boulder: Mammalian mRNA transcription: Mechanism and regulation.
Grewal, S., Cold Spring Harbor Laboratory: Histone tail modifications, boundary elements, and epigenetic control of higher-order chromatin assembly.
Hernandez, N., Cold Spring Harbor Laboratory: Transcription of the human snRNA genes by RNA polymerases II and III.
Jenuwein, T., Institute of Molecular Pathology, Vienna, Austria: Epigenetic organization of the mammalian genome.
Joshua-Tor, L., Cold Spring Harbor Laboratory: Crystal structures of two intermediates in the assembly of the papillomavirus replication initiation complex.
Kraus, L., Cornell University, New York: Coactivators, chromatin, and epigenetic modifications in estrogen-dependent gene regulation.
Lees, J., Massachusetts Institute of Technology, Cambridge: The role of the E2F transcription factors in development and tumorigenesis.
Lis, J., Cornell University, Ithaca, New York: Heat shock genes of *Drosophila:* A robust model for dissecting mechanisms of transcription and regulation.
Meisterernst, M., GSF-National Research Center for Environmental Health, Munich, Germany: Regulation of RNA polymerase II transcription by global transcription cofactors.
Meyer, B., University of California, Berkeley: X-chromosome dosage compensation in *C. elegans*.
Thanos, D., Columbia University, New York: Mechanisms of transcriptional regulation in higher eukaryotes.
Timmers, M., Utrecht University, The Netherlands: Dynamics of RNA polymerase II transcription.
Tsukiyama, T., Fred Hutchinson Cancer Research Center, Seattle, Washington: Functions of ISWI chromatin remodeling factors.
Yamamoto, K., University of California, San Francisco: Making and breaking transcriptional regulatory complexes.

Imaging Structure and Function in the Nervous System

July 23–August 12

INSTRUCTORS
K. Delaney, Simon Fraser University, Burnaby, B.C., Canada
F. Helmchen, MPI für Medizinsche Forschung, Heidelberg, Germany
T. Murphy, University of British Columbia, Vancouver, Canada
V. Murthy, Harvard University, Cambridge, Massachusetts
K. Svoboda, Cold Spring Harbor Laboratory

ASSISTANTS
G. Di Cristo, Cold Spring Harbor Laboratory
K. Haas, Cold Spring Harbor Laboratory
A. Kreitzer, Harvard University, Boston, Massachusetts
Y. Otsu, University of British Columbia, Vancouver, Canada
J. Waters, MPI Heidelberg, Heidelberg, Germany

Advances in light microscopy, digital image processing, and the development of a variety of powerful fluorescent probes presented expanding opportunities for investigating the nervous system, from synaptic spines to networks in the brain. This intensive laboratory and lecture course provided participants with the theoretical and practical knowledge to utilize emerging imaging technologies. The primary emphasis of the course was on vital light microscopy. Students learned the principles of light microscopy, as well as the use of different types of electronic cameras, laser-scanning systems, functional fluorophores, delivery techniques, and digital image-processing software. In addition to transmit-

ted light microscopy for viewing cellular motility, the course examined a variety of molecular probes of cell function, including calcium-sensitive dyes, voltage-sensitive dyes, photo-activated ("caged") compounds, and exocytosis tracers. Issues arising in the combination of imaging with electrophysiological methods were covered. Particular weight was given to multi-photon laser-scanning microscopy and to newly available biological fluorophores, especially green fluorescent protein (GFP) and its variants. We used a spectrum of neural and cell biological systems, including living animals, brain slices, and cultured cells. Applicants had a strong background in the neurosciences or in cell biology.

PARTICIPANTS

Engel, U., Diplom., Ph.D., Harvard Medical School, Boston, Massachusetts
Fall, C., B.S., Ph.D., New York University, New York
Gerencser, A., M.D., M.Sc., Semmelweis University, Budapest, Hungary
Kawasaki, F., B.S., Ph.D., Pennsylvania State University, University Park
Kerr, J., Dip.Sci., Ph.D., National Institute of Mental Health, Rockville, Maryland
Lambe, E., A.B., Ph.D., Yale University, New Haven, Connecticut
Lee, W.-C., S.B., Massachusetts Institute for Technology, Cambridge
Murthy, M., B.S., Harvard Medical School, Boston, Massachusetts
Solecki, D., B.S., Ph.D., The Rockefeller University, New York
Stutzmann, G., B.A., Ph.D., University of California, Irvine
Tyler, W., B.S., University of Alabama, Birmingham
Zhu, Y., B.S., M.S., Ph.D., The Salk Institute for Biological Studies, La Jolla, California

SEMINARS

Ahlmers, W., Oregon Health and Science University, Portland: TIRF.
Betz, B., University of Colorado, Denver: Imaging secretion, FM-1-43.
Cline, H., Cold Spring Harbor Laboratory: In vivo imaging of morphogenesis.
Coppey-Moisan, M., Institut Jacques Monod, Paris, France: FLIM microscopy.
Denk, W., Max-Planck Institute for Medical Research, Heidelberg, Germany: Imaging, past and future.
Fetcho, J., SUNY, Stony Brook, New York: In vivo imaging zebrafish.
Forscher, P., Yale University, New Haven, Connecticut: DIC and multimodal microscopy.
Helmchen, F., Max-Planck Institute, Heidelberg, Germany: Ca imaging: Applications of 2-photon microscopy.
Keller, E., Zeiss Co., Thorwood, New York: Basics of microscopy.
Kleinfeld, D., University of California, San Diego, La Jolla: In vivo imaging of blood flow.
Lanni, F., Carnegie-Mellon University, Pittsburgh, Pennsylvania: Fluorescence microscopy.
Lichtman, J., Washington University, St. Louis, Missouri: Confocal microscopy.
Maruno, T., Hamamatsu Corporation, Bridgewater, New Jersey: CCD cameras.
Mertz, J., Ecole Supérieure de Physique et de Chimie Industrielles, Paris, France: Nonlinear microscopies.
Moomaw, B., Hamamatsu Photonic Systems, Spring Branch, Texas: CCD cameras.
Murphy, T., University of British Columbia, Vancouver, Canada: Imaging synaptic structure and function.
Murthy, V., Harvard University, Cambridge, Massachusetts: Imaging secretion, FM-1-43. GFP probes to image secretion.
Shepherd, G., Cold Spring Harbor Laboratory: Glutamate uncaging.
Sorkin, A., University of Colorado Health Sciences Center, Denver: GFP basics: FRED basics and applications.
Svoboda, K., Cold Spring Harbor Laboratory: Basic optics. 2-photon microscopy.
Tsien, R., University of California, San Diego, La Jolla: Bapta and synthetic Ca^{++} indicators. Imaging intracellular signaling.
Ziv, N., Technion Faculty of Medicine, Haifa, Israel: Immunofluorescence. Imaging synaptic proteins.
Zucker, R., University of California, Berkeley: Ca^{++} uncaging.

Yeast Genetics

July 23–August 12

INSTRUCTORS **D. Amberg,** SUNY, Syracuse, New York
D. Burke, University of Virginia, Charlottesville
O. Cohen-Fix, NIDDK, National Institutes of Health, Bethesda, Maryland

ASSISTANTS **A. Allison,** University of Virginia, Charlottesville
B. Bettinger, SUNY Upstate Medical University, Syracuse, New York
M. Ng, NIDDK, National Institutes of Health, Bethesda, Maryland

The Yeast Genetics course is a modern, state-of-the-art laboratory course designed to teach the students the full repertoire of genetic approaches needed to dissect complex problems in the yeast *Saccharomyces cerevisiae*. Combinations of classical genetic approaches were emphasized, including the isolation and characterization of mutants, tetrad analysis, complementation, and mitotic recombination. Molecular genetic techniques, including various types of yeast transformation, gene replacement with plasmids and polymerase chain reaction (PCR), construction and analysis of gene fusions, and generation of mutations in cloned genes, were also emphasized. Students used classical and molecular approaches to gain experience in identifying and interpreting various kinds of genetic interactions, including suppression and synthetic lethality. Students were immersed in yeast genomics and performed and interpreted experiments with DNA arrays. Students gained first-hand experience in modern cytological approaches such as epitope tagging and imaging yeast cells using indirect immunofluorescence, green fluorescent protein (GFP)-protein fusions, and a variety of fluorescent indicators for various subcellular organelles. Lectures on fundamental aspects of yeast genetics were presented along with seminars given by outside speakers on topics of current interest.

PARTICIPANTS

Bellows, D., B.S., Samuel Lunenfeld Research Institute, Toronto, Canada
Brace, J., B.S., Ph.D., University of Chicago, Illinois
Buainain, L., B.A., Katholieke Universiteit Leuven, Belgium
D'Agostino, J., B.S., Brandeis University, Waltham, Massachusetts
Dai, J., B.S., M.S., Iowa State University, Ames
Hamilton, C., National Institutes of Health, Bethesda, Maryland
Ewing, D., Jr., A.S., B.S., Uniformed Services University of Health Sciences, Bethesda, Maryland
Lorenz, A., Mag., University of Vienna, Austria
Lundkvist, P., M.S., Ph.D., University of Stockholm, Sweden
Ramanathan, S., M.Sc., Ph.D., Lucent Technologies, Murray Hill, New Jersey
Swain, P., B.A., Ph.D., The Rockefeller University, New York
Torres-Ramos, C., B.S., M.S., Ph.D., University Central del Caribe, Bayamon, Puerto Rico
Van Oevelen, C., M.S., Ph.D., University Medical Centre, Utrecht, The Netherlands
Waters, L., B.A., Massachusetts Institute of Technology, Cambridge
Williams, B., B.S., Howard Hughes Medical Institute and University of Wisconsin, Madison
Zakrzewska, A., M.S., University of Amsterdam, The Netherlands

SEMINARS

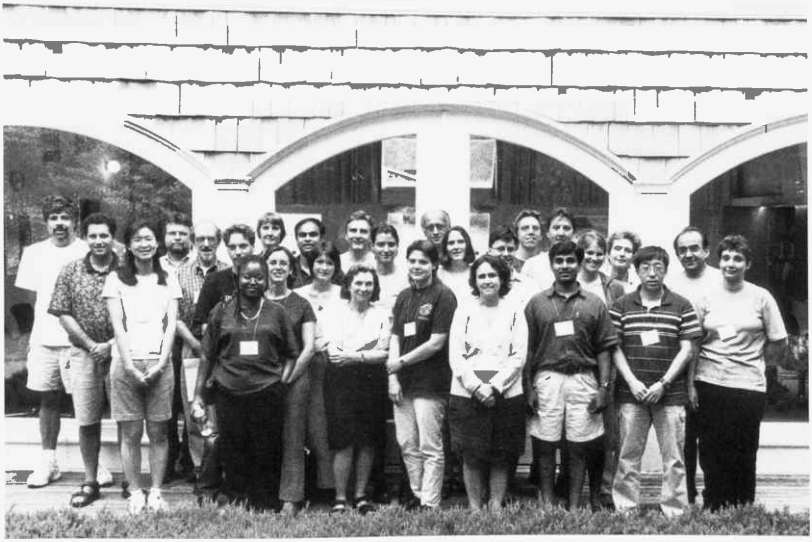
Amon, A., Massachusetts Institute of Technology, Cambridge: Regulation of mitosis and meiosis by the protein phosphatase Cdc14.
Bi, E., University of Pennsylvania School of Medicine, Philadelphia: The Rab GTPase-activating proteins Msb3p and Msb4p are involved in coupling actin organization to polarized secretion.
Cunningham, K., Johns Hopkins University, Baltimore, Maryland: Calcium regulation in yeast.
Dawson, D., Tufts University, Boston, Massachusetts: Finding your partner without losing your sister: Challenges of the meiotic chromosome.
Hampton, R., University of California, San Diego, La Jolla: Mechanisms of ER protein degradation.
Konopka, J., SUNY, Stony Brook, New York: Mating pheromone receptor signaling: Classical genetics to chemical genetics.
Kron, S., University of Chicago, Illinois: Genetic analysis of yeast dimorphism.
Luca, F., University of Pennsylvania, Philadelphia: MOB control of the yeast cell cycle.
Meluh, P., Memorial Sloan-Kettering Cancer Center, New York: Of yeast and man: 21st century centromeres.
Smith, M., University of Virginia, Charlottesville: Histones: Deviants studying variants.
Stearns, T., Stanford University, Stanford, California: Making mutants the old-fashioned way: Earning them!
Toczyski, D., University of California, San Francisco: Control of mitotic progression.
Tyers, M., Lunenfeld Research Institute, Toronto, Canada; Boone, C., University of Toronto, Canada: Large-scale methods to solve a small problem: Toward a global genetic interaction map for yeast.

Stem Cells

July 30–August 12

INSTRUCTORS **B. Hogan,** Vanderbilt University Medical Center /HHMI, Nashville, Tennessee
G. Keller, Mount Sinai School of Medicine, New York
R. McKay, National Institutes of Health, Bethesda, Maryland

This two-week 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, stem cell technology promises to become a major new element in biomedical science. This lecture and discussion course covered a series of subjects including the methods that allow stem cells to be identified and grown in the laboratory; the mechanisms that control the number of stem cells and their longevity; the stability and transformation of cell types; and the clinical potential and difficulties of stem cell technology. This material was presented in depth by invited speakers. The course instructors and the invited lecturers were accessible for informal discussion. The purpose of the course was to provide participants with a background in medicine or biology an advanced understanding of the scientific and clinical importance of stem cells.



PARTICIPANTS

Andersson, E., B.Sc., M.Sc., Lund University, Lund, Sweden
Batista, C., B.S., Ph.D., Univesidade Federal De Rio de Janiero, Brazil
Desponts, C., B.S., University of South Florida, Tampa
Dimos, J., B.S., Princeton University, Princeton, New Jersey
Drew, C., B.A., University of California, Los Angeles
Ferri, A., Laurea, University of Milano-Bicocca, Milan, Italy
Galande, S., B.Sc., Ph.D., National Centre for Cell Science, Pune, India
Hansson, M., B.S., M.S., Hagedorn Research Institute, Gentofte, Denmark
Kotton, D., B.A., M.D., Boston University School of Medicine, Massachusetts
Kumar, R., M.Sc., Ph.D., Cold Spring Harbor Laboratory
Liang, Y., B.S., Ph.D., University of Kentucky Medical Center, Lexington
McGiffert, C., B.S., M.S., University of California, San Diego
Mira, H., M.S., Ph.D., Karolinska Institute, Stockholm, Sweden
Pirvola, U., B.S., University of Helsinki, Finland
Roth, M., A.B., Ph.D., University of Medicine and Dentistry of New Jersey, Piscataway
Sivertsson, M., M.S., Ph.D., Royal Institute of Technology, Stockholm, Sweden
Tiscornia, G., Ph.D., The Salk Institute, La Jolla, California
Yin, Y., M.D., M.S., National University Medical Institutes, Singapore
Zettervall, C., M.A., Ph.D., Umea University, Sweden

SEMINARS

Alison, M., Imperial College, London, United Kingdom: The stem cell repertoire in the gastrointestinal tract and liver.
Cossu, G., University of Rome"La Sapienza: Mesoangioblasts, vessel-associated, pluripotent stem cells.
Downing, G., National Institutes of Health, Bethesda, Maryland: The politics and policy of stem cells in the United States of America.
Edelberg, J., Cornell University, New York: The vascular system.
Hochedlinger, K., Whitehead Institute, Massachusetts Institute of Technology, Cambridge: The biology and applications of nuclear cloning.
Hogan, B., Howard Hughes Medical Institute/Vanderbilt University Medical Center, Nashville, Tennessee: Branch, bud, and stem: The embryonic mouse lung as a model system for organogenesis.
Iscove, N., The Ontario Cancer Institute, Toronto, Canada: Hematopoietic stem cells.
Itescu, S., Columbia University, New York: Hemangioblasts and the heart vascular system.
Keller, G., Mount Sinai School of Medicine, New York: Mesoderm commitment to the hematopoietic system.
Krause, D., Yale University, New Haven, Connecticut: Plasticity of bone-marrow-derived stem cells.
Krumlauf, R., Stowers Institute for Medical Research, Kansas City, Missouri: Plasticity and patterning of cell populations in vertebrate craniofacial development.
Kulesa, P., California Institute of Technology, Pasadena: Intravital imaging of neural crest cells and neural progenitors.
Lemischka, I., Princeton University, Princeton, New Jersey: Specific gene expression in stem cells.
Marvin, M., Harvard Medical School, Boston, Massachusetts: The stem cell repertoire in the gastrointestinal tract and liver.
McKay, R., National Institutes of Health, Bethesda, Maryland: Stem cells in development and disease.
McLaren, A., University of Cambridge, United Kingdom: Germ cells.
Millar, S., University of Pennsylvania, Philadelphia: Stem cells of the skin and its appendages.
Nusse, R., Stanford University, Stanford, California: Wnt signaling and stem cells.
Pappaioannou, G., Columbia University, New York: Stem cells of the early embryo.
Sanchez, A., University of Utah School of Medicine, Salt Lake City: The planarian neoblasts: An experimentally accessible stem cell population.
Sorrentino, B., St. Jude Children's Research Hospital, Memphis, Tennessee: Hematopoiesis: The side population and gene therapy.
Spradling, A., Carnegie Institute of Washington, Baltimore, Maryland: Stem cells in their natural context: The stem cell niche.
Stoffel, M., The Rockefeller University, New York: The endocrine pancreas.
Van der Kooy, D., University of Toronto, Canada: The life history of neural stem cells.
Verfaillie, C., University of Minnesota, Minneapolis: Unexpected potential of multipotent adult stem cells.
Wright, C., Vanderbilt University, Nashville, Tennessee: Gene inactivation and lineage-labeling studies of pancreas formation.
Zhong, W., Yale University, New Haven, Connecticut: Asymmetric cell division and its potential roles in stem cell maintenance and differentiation.

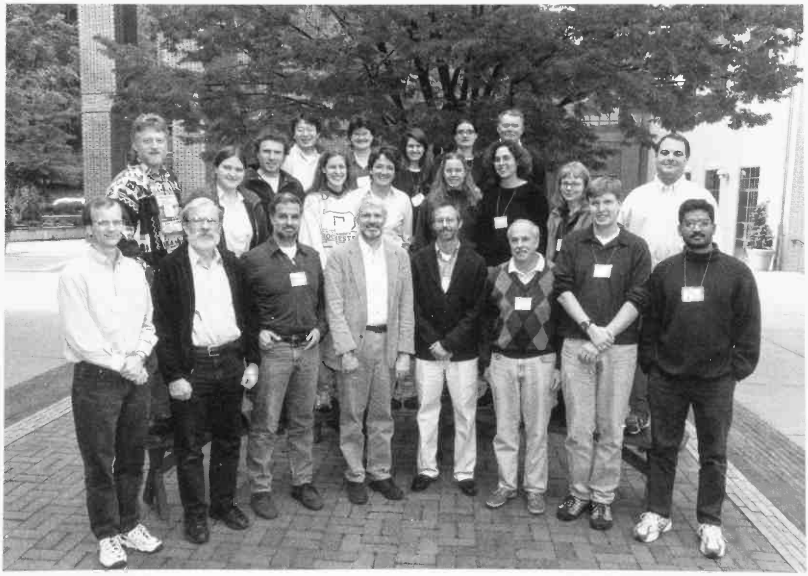
Macromolecular Crystallography

October 16–29

INSTRUCTORS **W. Furey,** V.A. Medical Center, Pittsburgh, Pennsylvania
G. Gilliland, National Institute of Standards and Technology, Gaithersburg, Maryland
A. McPherson, University of California, Irvine
J. Pflugrath, Rigaku/MSC. Inc, The Woodlands, Texas

ASSISTANT **D. Kuzmanovic,** National Institute of Standards and Technology, Gaithersburg, Maryland

Crystallography and X-ray diffraction yield a wealth of structural information unobtainable through other methods. This intensive laboratory/computational course focused on the major techniques used to determine the three-dimensional structures of macromolecules. It was designed for scientists with a working knowledge of protein structure and function, but who were new to macromolecular crystallography. Topics covered included 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 phase determination, solvent flattening, molecular replacement and averaging, electron density interpretation, structure refine-



ment, molecular graphics, noncrystallographic summary, simulated annealing, and coordinate deposition. Participants learned through extensive hands-on experiments. They crystallized and determined a protein structure, along with lectures on the theory and informal discussions behind the techniques. Applicants were familiar with the creation and editing of simple text files on UNIX workstations, using a screen-based editor such as vi, emacs, or jot.

PARTICIPANTS

Chakravarthy, S., B.Sc., M.Sc., Colorado State University, Fort Collins
Chmiel, N., Ph.D., University of California, Berkeley
Deaconescu, A., B.E., The Rockefeller University, New York
Finkielstein, C., Ph.D., University of Colorado, Denver
Golan, G., B.Sc., Hebrew University, Jerusalem, Israel
Kennedy, M., Ph.D., Pacific Northwest National Laboratory, Richland, Washington
Langley, D., Ph.D., University of Sydney, Australia
Loewen, M., Ph.D., National Research Council of Canada, Saskatchewan
Loschi, L., Ph.D., The University of British Columbia, Vancouver, Canada
Lubahn, B., B.A., Ph.D., University of Missouri, Columbia
Murray, D., Ph.D., Weill Medical College, New York
Ogawa, H., B.S., Ph.D., Cleveland Clinic Foundation, Ohio
Prat Gay, G., Ph.D., University of Buenos Aires, Argentina
Schroeder, S., Ph.D., Yale University, New Haven, Connecticut
Thomas, S., Ph.D., University of Texas Health Sciences Center, San Antonio
Waugh, D., Ph.D., National Cancer Institute, Frederick, Maryland

SEMINARS

Adams, P., Lawrence Berkeley Laboratory, Berkeley, California: Introduction to CNS. CNS macromolecular refinement.
Furey, W., V.A. Medical Center, Pittsburgh, Pennsylvania: Patterson group therapy. Isomorphous replacement and anomalous scattering I. Isomorphous replacement and anomalous scattering II. Phase improvement by solvent flattening/negative density truncation. Noncrystallographic symmetry averaging. Crystallographic symmetry operations and their application.
Hendrickson, W., Columbia University, New York: MAD phasing.Theory and practice.
Joshua-Tor, L., Cold Spring Harbor Laboratory: Two intermediates in the assembly of the papillomavirus replication initiation complex. Structure presentation.
Kjeldgaard, M., Aarhus University, Denmark: Electron density fitting from A to 0.
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 macrmolecules I. Crystallization of macromolecules II. Vectors, waves, symmetry, and planes. Introduction to X-ray crystallography. Preliminary crystal characterization crystallographic symmetry and unit cells. Fundamental diffraction relationships I. Fundamental diffraction relationships II. Fourier transforms and the electron density equations. Patterson techniques. Heavy atoms and anomalous scatterers.
Pflugrath, J., Rigaku/MSC, Inc., The Woodlands, Texas: X-ray beams, images, and reflections. Data collection. Design and setup. Cryocrystallography. Crystallization review and optimization. Application of anomalous scattering from sulfur atoms.
Richardson, D., Duke University Medical Center, Durham, North Carolina: Detection and repair of model errors using all atom contacts.
Terwilliger, T., Los Alamos National Laboratory, New Mexico: Automated structure solution, density modification, and model building for M.A.D, SAD, and MIR.
Tronrud, D., University of Oregon, Eugene: Macromolecular refinement I. TNT tutorial.
Westbrook, T., University of Rochester School of Medicine, New York: The protein data bank.
Xu, R.-M., Cold Spring Harbor Laboratory: Structural studies of histone methyltransferases.

Bioinformatics: Writing Software for Genome Research

October 16–29

INSTRUCTORS **S. Lewis,** University of California, Berkeley
L. Stein, Cold Spring Harbor Laboratory

ASSISTANTS

J. Barnett, Massachusetts Institute of Technology, Cambridge
K. Clark, Cold Spring Harbor Laboratory
A. Day, University of California, Los Angeles
R. Halgren, Michigan State University, East Lansing
S. Lembark, Workhouse Computing, Chicago, Illinois
B. Marshall, University of California, Berkeley
V. Mootha, Whitehead Institute, Cambridge, Massachusetts
C. Mungall, Lawrence Berkeley Laboratories, Berkeley, California
L. Palmer, Cold Spring Harbor Laboratory
S. Prochnik, University of California, Berkeley
L. Teytelman, Cold Spring Harbor Laboratory

The desktop computer is rapidly becoming an indispensable tool in the biologist's toolchest. The success of the human genome project created an explosion of information: Billions of bits of biological information stashed electronically in databases around the globe just waiting for the right key to unlock them. New technologies such as DNA microarrays and high-throughput genotyping are creating an information overload that the traditional laboratory notebook cannot handle. To exploit the information

revolution in biology, biologists must move beyond canned Web interfaces and Excel spreadsheets. They must take charge of the data by creating their own software to fetch, manage, and integrate it. The goal of this course was to provide biologists with the tools needed to deal with this changing landscape. Designed for students and researchers with little prior knowledge of programming, this two-week course taught the fundamentals of the Unix operating system, Perl scripting, dynamic Web page development with the CGI protocol, and database design. The course combined formal lectures with hands-on experience in which students worked to solve a series of problem sets drawn from common scenarios in biological data acquisition, integration, and laboratory workflow management. For their final projects, students were posed problems using their own data and worked with each other and the faculty to solve them. Note that the primary focus of this course was to provide students with the practical aspects of software development, rather than to present a detailed description of the algorithms used in computational biology.

PARTICIPANTS

Barrasa, I.M., B.S., Ph.D., University of Pennsylvania, Philadelphia
Birkeland, M., B.A., Ph.D., GlaxoSmithKline, King of Prussia, Pennsylvania
Bultrini, E., B.S., Istituto Superiore di Sanita, Rome, Italy
Caenepeel, S., B.S., Sugen, South San Francisco, California
Costain, W., M.Sc., Ph.D., Dalhousie University, Halifax, Nova Scotia, Canada
Deng, D., B.S., Ph.D., Alabama A&M University, Normal
Doniger, S., B.S., Gladstone Institute, San Francisco, California
Girke, T., M.A., Ph.D., University of California, Riverside
Gutierrez, R., B.S., M.S., MSU-Plant Research Laboratory, East Lansing, Michigan
Hue, S., B.Sc., M.Sc., Central Public Health Laboratory, London, United Kingdom
Hur, J., B.S., Ph.D., Massachusetts General Hospital Cancer Center, Charlestown
Koch, B., B.S., University of Heidelberg, Heidelberg, Germany
McKay, S., M.Sc., Ph.D., British Columbia Cancer Agency, Vancouver, Canada
Qi, W., B.S., Ph.D., University of Maine, Orono
Ringquist, S., B.A., Ph.D., University of Pittsburgh, Pennsylvania
Robb, S., B.S., University of Utah, Salt Lake City
Smith, J., B.S., Ph.D., University of Alabama, Birmingham
Somerville, C., B.S., Ph.D., Marshall University, Huntington, West Virginia
Tang, J., B.S., Ph.D., Delaware Biotechnology Institute, Newark, Delaware
Tanguay, D., B.S., Ph.D., Zycos Pharmaceuticals, Lexington, Massachusetts
Taylor, J., B.S., Ph.D., University of Victoria, Canada
Tello-Ruiz, M., B.S., Cold Spring Harbor Laboratory
Tewari, M., B.A., Ph.D., Dana-Farber Cancer Institute, Boston, Massachusetts
Yamada, C., B.Sc., University of Cambridge, United Kingdom
Yang, X., B.S., Ph.D., University of Texas, Dallas

SEMINARS

Gilman, B., Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Managing large genotyping projects.
Hide, W., University Western Cape, SANBI, South Africa: EST clustering techniques.
Hughey, R., University of California, Santa Cruz: Hidden Markov models.
Jamison, C., George Mason University, Manassas, Virginia: Using genome databases effectively.
Marth, G., NIH/National Center for Biotechnology Information, Bethesda, Maryland: Pipelines for nucleotide sequence analysis.
Pearson, W., University of Virginia, Charlottesville: Sequence alignment.
Peitzsch, R., Pfizer Global Research and Development Bioinformatics, Groton, Connecticut: Practical and flexible database design.
Slonim, D., Genetics Institute, Wyeth-Ayerst Research, Cambridge, Massachusetts: Expression chips.
Stajich, J., Duke University, Durham, North Carolina: Using BioPerl 1. Using BioPerl 2.

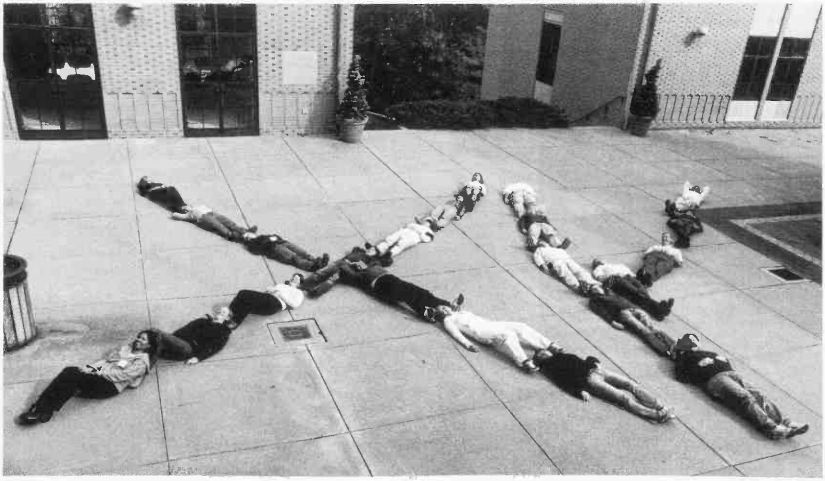
Immunocytochemistry, In Situ Hybridization, and Live Cell Imaging

October 19–November 1

INSTRUCTORS **A. Dernburg,** Lawrence Berkeley National Laboratory, Berkeley, California
J. Murray, University of Pennsylvania, Philadelphia
J. Swedlow, University of Dundee, United Kingdom

ASSISTANTS **T. Howard,** University of New Mexico, Albuquerque
K. Hu, University of Pennsylvania, Philadelphia
M. Johnson, University of California, Berkeley
M. Platani, University of Dundee, United Kingdom

This course focused on specialized techniques in microscopy, in-situ hybridization, immunocytochemistry, and live-cell imaging related to localizing DNA, RNA, and proteins in fixed cells as well as protein and RNA dynamics in living cells. The course emphasized the use of the latest equipment and techniques in fluorescence microscopy, including confocal laser-scanning microscopy, deconvolution methods, digital image processing, and time-lapse imaging of living specimens. The course was designed to present students with state-of-the-art technology and scientific expertise in the use of light microscopy to address basic questions in cellular and molecular biology. The course was designed for



the molecular biologist who was in need of microscopic approaches and for the cell biologist who was 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 green fluorescent protein (GFP) variants to study protein expression, localization and dynamics. In each method, several experimental protocols were presented that allowed 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 that were presented.

PARTICIPANTS

Al-Rashid, S., M.S., University of Toronto, Canada
Carter, R., B.S., University of Alabama, Birmingham
Castelo Branco, P., B.S., Ph.D., Oxford University, United Kingdom
Drea, S., B.A., Ph.D., John Innes Centre, Norwich, United Kingdom
Ehmsen, J., B.S., Oxford University, United Kingdom
Gustafsson, M., B.A., Goteborg University, Sweden
Holter, E., Karolinska Institute, Huddinge, Sweden
Hance, N., Ph.D., Karolinska Institute, Huddinge, Sweden
Hodges, E., B.A., Karolinska Institute, Huddinge, Sweden
Ku, C.-C., Ph.D., Stanford University, Stanford, California
Martins, R., M.S., Instituto de Biofisica, Rio de Janeiro, Brazil
Sansam, C., B.S., Vanderbilt University, Nashville, Tennessee
Soutoglou, E., PhD., IGBMC, Strasbourg, France
Uno, E., B.S., The Rockefeller University, New York
Usborne, A., D.V.M., Wisconsin National Primate Research Center, Madison
Wadum, M., B.A., University of Southern Denmark, Odense

SEMINARS

Day, R., University of Virginia, Charlottesville: Seeing colors: Applications and limitations of the fluorescent proteins.
Dernburg, A., Lawrence Berkeley National Laboratory, Berkeley, California: Basic of FISH.
Hostetter, G., National Institutes of Health, Bethesda, Maryland: Applications and challenges of interphase FISH.
Murray, J., University of Pennsylvania School of Medicine, Philadelphia: Basic introduction to light and fluorescence microscopy. Immunocytochemistry. Cameras and digital imaging fundamentals. Principles of confocal microscopy and deconvolution techniques.
Ried, T., National Cancer Institute/NIH, Bethesda, Maryland: Mechanisms and consequences of chromosomal aberrations in cancer cells.
Rieder, C., New York State Department of Health, Albany: Laser microbeam studies on how mitosis works in mammals.
Spector, D., Cold Spring Harbor Laboratory: Localization of gene expression by FISH and in living cells.
Swedlow, J., University of Dundee, United Kingdom: Live-cell imaging. Quantitative fluorescence microscopy. When and how to use F-words.

Phage Display of Combinatorial Antibody Libraries

November 5–18

INSTRUCTORS
C. Barbas, Scripps Research Institute, La Jolla, California
D. Siegel, University of Pennsylvania School of Medicine, Philadelphia
G. Silverman, University of California, San Diego, La Jolla

ASSISTANTS
R. Fuller, Scripps Research Institute, La Jolla, California
C. Goodyear, University of California, San Diego, La Jolla
J. Struthers, University of California, San Diego, La Jolla
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 prac-

tical 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 instructors and a number of invited speakers, emphasized polymerase chain reaction (PCR) of immunoglobulin genes, the biology of filamentous phage and the utility of surface expression libraries, expression of antibodies in *E. coli* and mammalian cells, antibody structure and function, catalytic antibodies, directed protein evolution, retroviral and cell display libraries, the immunobiology of the antibody response, and recent results on the use of antibodies for diagnostics and therapy. The theory and practical implications for selection from phage displayed libraries of random peptides, cDNA products, and semisynthetic proteins were also explored.

PARTICIPANTS

Astatke, M., Ph.D., Kirkegaard and Perry Laboratories, Cessna, Connecticut
Crowley, J.M. Centeno M.S., Institute of Biochemical Research, Buenos Aires, Argentina
Duchala, C., Ph.D., Cleveland Clinic Foundation, Ohio
Edwards, B., Ph.D., Wayne State University, Detroit, Michigan
Fermer, C., Ph.D., CanAg Diagnostics AB, Gothenburg, Sweden
Finlay, W., Ph.D., Food and Drug Administration, Bethesda, Maryland
Gearhart, D., Ph.D., Medical College of Georgia, Augusta
Habib, A., B.S., Medical Research Council, London, United Kingdom
Healy, S., Ph.D., University of California, San Francisco
Hilgers, M., Ph.D., Syrrx, San Diego, California
Huff, I., B.S., Center of Excellence for Structural Biology, Knoxville, Tennessee
Poersch, C., B.S., Molecular Biology Institute of Parana, Curitiba, Brazil
Schulz, R., B.A., Louisiana State University, Baton Rouge
Sousa, R., M.S., National Institute of Health, Portugal
Stubbs, D., M.S., Georgia Institute of Technology, Atlanta
Wang, Y., MD, Ph.D., Harvard Medical School, Boston, Massachusetts

SEMINARS

Arap, W., University of Texas/M.D. Anderson Cancer Center, Houston: In vivo panning.
Barbas, C., Scripps Research Institute, La Jolla, California: Software and hardware for genomes: Polydactyl zinc finger proteins and the control of endogenous genes.
Lowman, H., Genentech, Inc., San Francisco, California: SAR of peptides using phage.
McHeyzer-Williams, M., The Scripps Research Institute, La Jolla, California: Regulation and development of antibody responses in vivo.
Nolan, G., Stanford University School of Medicine, Stanford, California: Retroviral libraries.
Noren, C., New England Biolabs, Beverly, Massachusetts: Phage peptide libraries: The Ph.D. for peptides.
Sidho, S., Genentech, Inc., San Francisco, California: Use of antibody-phage libraries to develop antibodies as reagents and potential therapeutics.
Siegel, D., University of Pennsylvania Medical Center, Philadelphia: Cell surface selection of combinatorial Fab libraries.
Silverman, G., University of California, San Diego, La Jolla: Repertoire cloning of SLE autoantibodies.
Webster, R., Duke University, Durham, North Carolina: The biology of filamentous phage.
Wilson, I., Scripps Research Institute, La Jolla, California: Structural biology of the immune system.

Proteomics

November 5–18

INSTRUCTORS
P. Andrews, University of Michigan Medical School, Ann Arbor
J. La Baer, Harvard Medical School, Boston, Massachusetts
A. Link, Vanderbilt University School of Medicine, Nashville, Tennessee

ASSISTANTS
P. Braun, Harvard Medical School, Boston, Massachusetts
M. Fernandez, Harvard Medical School, Boston, Massachusetts
J. Jennings, Vanderbilt University School of Medicine, Nashville, Tennessee
S. Vazquez, University of Michigan, Ann Arbor

This intensive laboratory and lecture course focused on two major themes in proteomics. In the profiling section of the course, students learned methodologies of protein preparation from diverse samples, sample analysis by two-dimensional gel electrophoresis, high-sensitivity mass spectrometric analysis of proteins utilizing both peptide mass mapping and tandem mass spectrometry, and the application of bioinformatics tools to identify proteins and posttranslational modifications and assess their relative abundance. In the functional proteomics section of the course, students learned the use of recombinational cloning to move many genes simultaneously to different expression vectors, how to apply

robotics to high-throughput methods, and how to perform high-throughput expression, purification, and characterization of proteins. Students learned approaches to identify protein interactions utilizing affinity isolation techniques coupled with mass spectrometric protein identification. The overall aim of the course provided students with the fundamental knowledge and hands-on experience necessary to be able to perform and analyze proteomics experiments, and to learn to identify new opportunities in applying proteomics approaches to their own research.

PARTICIPANTS

Alexander, J., B.S., Ph.D., Virginia Commonwealth University, Richard, Virginia
Baars, L., B.S., M.S., Stockholm University, Sweden
Burgess, S., Ph.D., Mississippi State University, Starkville
Fast, B., B.S., M.S., University of Bergen, Norway
Francis, M., B.S., University of California, Los Angeles
Gardiner, K., B.S., Ph.D., Eleanor Roosevelt Institute, Denver, Colorado
Karpuj, M., M.S., Ph.D., University of California, San Francisco
Kimbro, K., B.A., Ph.D., Clark Atlanta University, Atlanta, Georgia
Kulkarni, R., B.S., Ph.D., North Carolina State University, Raleigh
McPherson, J., B.S., Ph.D., Washington University School of Medicine, St. Louis, Missouri
Methe, B., B.S., Ph.D., The Institute for Genomic Research, Rockville, Maryland
Puthanveettil, S., B.Sc., Ph.D., Columbia University, New York
Rogers, A., B.Sc., Ph.D., British Antarctic Survey, Cambridge, United Kingdom
Sorin, M., B.S., Albert Einstein College of Medicine, Bronx, New York
Tsai, Y.C., B.S., MSE, Johns Hopkins University, Baltimore, Maryland
Zhang, S., B.S., Columbia University, New York

SEMINARS

Bachland, P., National Institutes of Health, Bethesda, Maryland: Interpretation of MS/MS spectra: De novo sequence analysis.
Chait, B., The Rockefeller University, New York: Identification of posttranslational modifications by MS/MS.
Clauser, K., Millennium Pharmaceuticals, Inc., Cambridge, Massachusetts: Applications of proteomics/protein Informatics.
Goodlett, D., Proteomics, Seattle, Washington: Applications of ICAT for protein quantitation.
Jebanthirajah, J., Harvard Medical Center, Boston, Massachusetts: Quantitative proteomics: ICAT and beyond.
MacBeath, G., Harvard University, Cambridge, Massachusetts: Protein microarrays.
Tasto, J., Vanderbilt University, Nashville, Tennessee: Tandem affinity purifications (TAP) of protein complexes.
Weil, T., Vanderbilt University, Nashville, Tennessee: Protein purification/transcriptional complexes.

Computational Genomics

November 6–11

INSTRUCTORS **W. Pearson,** University of Virginia, Charlottesville
R. Smith, GlaxoSmithKline, King of Prussia, Pennsylvania

ASSISTANT **A. Mackey,** 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 include 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 made extensive use of local WWW pages to present problem sets and the computing tools to solve them. Students used Windows

and Mac workstations attached to a UNIX server; participants had to be comfortable using the Unix operating system and a Unix text editor. The course was designed for biologists seeking advanced training in biological sequence analysis, for computational biology core resource directors and staff, and for 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 software development were encouraged to apply to the *Bioinformatics: Writing Software for Genome Research* course.

PARTICIPANTS

Adams, R., B.A., Ph.D., Edinburgh University, United Kingdom
Bangarusamy, D., B.S., M.S., Genome Institute of Singapore
Bates, E., B.Sc., Ph.D., University of Dentistry and Medicine of New Jersey/New Jersey Medical School, Newark
Belfiore, N., B.A., Ph.D., Purdue University, West Lafayette, Indiana
Bordo, D., B.S., Ph.D., National Cancer Research Institute, Genova, Italy
Carroll, M., B.S., Ph.D., Xavier University of Louisiana, New Orleans
Chen, C., B.S., Ph.D., National Yang-Ming University, Taipei, Taiwan
Cheung, K.-H., B.S., Ph.D., Yale Center for Medical Information, New Haven, Connecticut
Engel, A., Ph.D., Roche Diagnostics GmbH, Penzberg, Germany
Fjelistrom, R., B.S., Ph.D., USDA-Agricultural Research Service, Beaumont, Texas
Gilchrist, M., B.A., Ph.D., Cambridge University, United Kingdom
Holford, I., B.A., Ohio State University, Wooster
Kim, S.-W., B.A., Ph.D., Brigham & Women's Hospital, Boston, Massachusetts
Korpelainen, E., B.S., Ph.D., Center of Scientific Computing, Espoo, Finland
Landers, M., B.Sc., Ph.D., University of Connecticut Health Center, Farmington
Li, J., B.S., Ph.D., GlaxoSmithKline, Bioinformatics, Research Triangle Park, North Carolina
Lin, Y., B.S., M.S., Institute of Botany, Taipei, Taiwan
Meneely, P., B.S., Ph.D., Haverford College, Haverford, Pennsylvania
Rohacs, T., M.D., Ph.D., Mount Sinai School of Medicine, New York
Rose, A., Ph.D., Ohio State University, Columbus
Schmuth, M., M.D., University of Innsbruck, Austria
Silverman, G., B.S., Ph.D., University of Massachusetts, Worcester
Vaughn, M., B.S., Ph.D., Cold Spring Harbor Laboratory
Wu, J., B.S., Human Genome Sequencing Center, Houston, Texas

SEMINARS

Altschul, S., National Institutes of Health, Bethesda, Maryland: Statistics of sequence similarity scores. Iterated protein database searches with PSI-BLAST.
Cooper, P., National Institutes of Health, Bethesda, Maryland: Introduction to the databases and tools at the National Center for Biotechnology Information. Genome resources of the NCBI.
Hardison, R., Pennsylvania State University, University Park: Mammalian genome comparison. Databases and browers for mammalian genomes.
Lewis, S., University of California, Berkeley: Gene ontologies: From structure to function.
Yandell, M., University of California, Berkeley: Eukaryotic gene identification.

The Genome Access Course

February 6–7 (internal), April 30–May 1, August 27–28, October 1–2, December 4–5

TRAINERS **J. Gergel, M. Katari, and L. Palmer,** Cold Spring Harbor Laboratory
U. Hilgert, Dolan DNA Learning Center

The Genome Access Course is an intensive two-day introduction to bioinformatics that was held multiple times in 2002 and trained almost 150 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: 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. The curriculum of The Genome Access Course was developed in conjunction with staff at the Wellcome Trust Sanger Centre and the European Bioinformatics Institute (Hinxton, United Kingdom) who separately have been teaching a parallel series of courses in the United Kingdom (the open-door workshops).

Genome Access Course, February 2002

April 30–May 1

Arulnayagam, L., B.Sc., GlaxoSmithKline, RTP, North Carolina
Balkovski, E., Ph.D., Rutgers University, Piscataway, New Jersey
Chen, C.-R., Ph.D., Memorial Sloan-Kettering Cancer Center, New York
Collins, A., Ph.D., Johnson & Johnson Research and Development, Raritan, New Jersey
Derham, B., Ph.D., University of Oxford, United Kingdom
Dong, M., B.Sc., Novartis Pharmaceutics, Summit, New Jersey
Flockhart, I., B.Sc., Alphagene, Woburn, Massachusetts
Foster, J., Ph.D., New England Biolabs, Beverly, Massachusetts
Huang, X., B.Sc., Bristol-Myers Squibb, Pennington, New Jersey
Jeng, A., Ph.D., Novartis Pharmaceuticals Corporation, Summit, New Jersey
Kaushal, S., Ph.D., University of Minnesota, Minneapolis
Kim, M.-Y., B.Sc., Otsuka Maryland Research Institute, Rockville
Kuo, F.-S., B.Sc., Orchid BioSciences, Inc., Princeton, New Jersey
Kustka, A., Ph.D., SUNY, Stony Brook, New York
Laszlo, A., Ph.D., Washington University School of Medicine, St. Louis, Missouri
Meyer, S., Ph.D., Cephalon, Inc., West Chester, Pennsylvania
Nollen, E., Ph.D., Northwestern University, Evanston, Illinois
Noorwez, S., Ph.D., University of Minnesota, Minneapolis
Nykanen, P., B.Sc., Åbo Akademi University, Turku, Finland
Petti, F., B.Sc., OSI Pharmaceuticals Inc., Uniondale, New York
Pollard, H., Ph.D., Uniformed Services University, Bethesda, Maryland
Preston, R., B.Sc., Rensselaer, Hartford, Connecticut
Scott, K., B.Sc., Orchid BioSciences, Inc., Princeton, New Jersey
Thierse, H.-J., Ph.D., Max-Planck Institute for Immunobiology, Freiburg, Germany
Xu, D., Ph.D., Johnson & Johnson Research and Development, Raritan, New Jersey

Genome Access Course, April 2002

August 27–28

Briggs, J., Ph.D., National Institutes of Health, Bethesda, Maryland
Chang, D., B.Sc., Baylor College of Medicine, Houston, Texas
Chen, X., B.Sc., Bristol-Myers-Squibb, Princeton, New Jersey
Cheng, K., Ph.D., Pennsylvania State College of Medicine, Hershey
D'adamio, L., Ph.D., Albert Einstein College of Medicine, Bronx, New York
De Blas, A., Ph.D., University of Connecticut, Storrs
Denney, N., B.Sc., Merck & Co., Inc., West Point, Pennsylvania
Dunne, C., B.Sc., The Rockefeller University, New York
Jim, S., B.Sc., The Rockefeller University, New York
Lee, T.-L. (Cindy), B.Sc., Cold Spring Harbor Laboratory
Levine, A., Ph.D., The Rockefeller University, New York
Michels, C., Ph.D., Queens College of CUNY, Flushing, New York
Miralles, C., B.Sc., University of Connecticut, Storrs
Parnassa, A., B.Sc., Weill Comell School of Medicine, New York
Pollak, E., Ph.D., University of Pennsylvania Medical Center, Philadelphia
Qi, Zhenhao, Ph.D., Boehringer Ingelheim Pharmaceutical Inc., Danbury, Connecticut
Rios, C., Ph.D., Bristol-Myers-Squibb, Pennington, New Jersey
Roccanova, Louis, Ph.D., New York Institute of Technology, Old Westbury
Rush, J., Ph.D., Howard Hughes Medical Institute and Yale School of Medicine, New Haven, Connecticut
Sadeghi, N., B.Sc., Mount Sinai School of Medicine, New York
Salero, E., Ph.D., The Rockefeller University, New York
Sepulveda, M., B.Sc., Albert Einstein College of Medicine, Bronx, New York
Singh, K., Ph.D., VA Medical Center (New York University School of Medicine), New York
Smyth, B., Ph.D., University of Pennsylvania, Philadelphia
Wakeland, E., Ph.D., University of Texas Southwestern Medical Center, Dallas
Wang, C., Ph.D., Bristol-Myers-Squibb, Lawrenceville, New Jersey
Webb, E., Ph.D., Woods Hole Oceanographic Institution, Massachusetts
Yang, C., Ph.D., The Rockefeller University, New York

October 1–2

Balinsky, C., B.Sc., USDA, Agricultural Research Service, Greenport, New York
Bennett, J., B.Sc., Skirball Institute, New York University School of Medicine
Bhatt, A., Ph.D., Howard Hughes Medical Institute/Albert Einstein College of Medicine, Bronx, New York
Canaan, A., Ph.D., Yale University, Medical School, New Haven, Connecticut
Caradonna, S., Ph.D., University of Medicine and Dentistry of New Jersey, Stratford
Dackour, R., B.Sc., Long Island Jewish Medical Center, New Hyde Park, New York
Eastman, S., B.Sc., Lehigh University, Bethlehem, Pennsylvania
Euskirchen, G., B.Sc., Yale University, New Haven, Connecticut
Fischer, J., Ph.D., University of Dentistry and Medicine of New Jersey-Graduate School of Biomedical Sciences, Stratford
Gadura, N., B.Sc., Queens College, CUNY, Flushing, New York
Henegariu, O., Ph.D., Yale University, New Haven, Connecticut
Holman, A., B.Sc., Tufts University, Boston, Massachusetts
Jacobs, W., Ph.D., Albert Einstein College of Medicine/ Howard Hughes Medical Institute, Bronx, New York
Li, X.-C., Ph.D., The Rockefeller University, New York
McMahan, L., Ph.D., The Rockefeller University, New York
Murphy, K., Ph.D., Children's Hospital of Philadelphia, Pennsylvania
Pongrac, J., Ph.D., University of Pittsburgh, Pennsylvania
Pumfery, A., Ph.D., The George Washington University, Washington, D.C.
Rinn, J., B.Sc., Yale University, New Haven, Connecticut
Severinova, E., Ph.D., Rutgers University, Piscataway, New Jersey
Silver, J., Ph.D., North Shore University Hospital, Manhasset, New York
Simha, R., Prof., The George Washington University, Washington, DC
Svoboda, K., Ph.D., Howard Hughes Medical Institute/Cold Spring Harbor Laboratory
Tulman, E., B.Sc., Plum Island Animal Disease Center, USDA, Greenport, New York
Tyurina, O., B.Sc., University of Massachusetts, Amherst
Unniraman, S., Ph.D., Yale University School of Medicine, New Haven, Connecticut
Wang, X., B.Sc., Queens College, CUNY, Flushing, New York
Xu, Ks, Ph.D., NIAAA, National Institutes of Health, Rockville, Maryland
Zuk, D., Ph.D., Cell Press, Cambridge, Massachusetts

Genome Access Course, October 2002

December 4–5

Ascherio, A., Ph.D., Harvard School of Public Health, Boston, Massachusetts
Beg, A., Ph.D., Columbia University, New York
Cheung, K.-H., Ph.D., Yale Center for Medical Informatics, New Haven, Connecticut
Dranginis, A., Ph.D., St. Johns University, Jamaica, New York
Freire-Moar, J., Ph.D., Forest Laboratories, New York
Friedman, C., B.Sc., College of Staten Island-CUNY, New York
Gerstbrein, B., B.Sc., Rutgers University, Piscataway, New Jersey
Grabowski, P., Ph.D., University of Pittsburgh/Howard Hughes Medical Institute, Pennsylvania
Grech, A., B.Sc., Yale University, New Haven, Connecticut
Gunderson, S., Ph.D., Rutgers University, Piscataway, New Jersey
Hong, P., Ph.D., Harvard University, Cambridge, Massachusetts
Hopkins, N., Ph.D., Massachusetts Institute of Technology,

Cambridge
Laal, S., Ph.D., New York University School of Medicine, New York
Lai, K., B.Sc., Massachusetts Institute of Technology, Cambridge
Li, L., B.Sc., St. John's University, Jamaica, New York
Marasco, W., Ph.D., Dana Farber Cancer Institute, Boston, Massachusetts
Maston, G., Ph.D., University of Massachusetts Medical School, Worcester
Monteiro, A., Ph.D., Cornell Medical College, New York
Murray, D., Ph.D., Weill Medical College of Cornell University, New York
Nickas, M., Ph.D., SUNY, Stony Brook, New York
Oliveira, M.B., B.Sc., Tufts University School of Medicine, Boston, Massachusetts
Rivlin, P., Ph.D., Cornell University, Ithaca, New York
Rossi, P., Ph.D., Columbia University, New York
Royal, D., Ph.D., Rutgers, The State University of New Jersey, Piscataway
Scamborova, P., B.Sc., Yale University, New Haven, Connecticut
Tallarico, A., Ph.D., Dana-Farber Cancer Institute, Boston, Massachusetts
Vilinsky, I., B.Sc., Cornell University, Ithaca, New York
Wang, H., Ph.D., Thomas Jefferson University, Philadelphia, Pennsylvania
Wormsley, S., Ph.D., Yale University, New Haven, Connectcut
Yarilina, A., B.Sc., Columbia University, New York

Genome Access Course, December 2002

The Laboratory would like to acknowledge the generosity of the following companies who loaned equipment and reagents to the various courses:

Actimetrics
Affymetrix Inc.
Agencourt Bioscience Corporation
Agilent Technologies, Inc.
Ambion Inc.
Amersham Biosciences
A.M.P.I.
Applied Biosystems
Applied Precision Inc.
Astro-Med Inc.
Axon Instruments
Becton Dickinson Labware
Berthold Technologies USA, LLC
Biocrest Manufacturing
Bio-Rad Laboratories
Bioptechs Inc.
BioVision Technologies
BioWhittaker Molecular Applications
Brinkmann Instruments
Burleigh Instruments, Inc.
Carl Zeiss, Inc.
Cell and Molecular Technologies, Inc.
Charles River Laboratories, Inc.
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Diagnostic Instruments, Inc.
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Dynal
Eastman Kodak Company
Erie Scientific Company
Fisher Scientific
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Hamamatsu Photonic Systems
Hampton Research
Harvard Apparatus, Inc.
Hitachi Denski America
Improvision Inc.
Instrutech Corp.
Integrated DNA Technologies, Inc.
Intelligent Imaging Innovations Inc.
Invitrogen Corporation
Jackson Immunoresearch Laboratories
The Jackson Laboratory
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Med Associates Inc.
Micro Video Instruments
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Modulation Optics, Inc.
Molecular Devices Corporation
Molecular Dynamics
Molecular Probes Inc.
Nalge Nunc International
Narishige International USA, Inc.
New England Biolabs, Inc.
Newport Corp.
Nikon Inc.
NovagenInc.
Olympus America Inc.
Operon Technologies Inc.
Optronics
PE Biosystems
Perkin Elmer Life Sciences
Pierce Chemical Co.
Promega Corp.
Qiagen Inc.
Robbins Scientific Corporation
Roche Diagnostics
SD Instruments Inc.
Santa Cruz Biotechnology Inc.
Schleicher & Schuell Inc.
Sigma-Aldrich
Sutter Instruments
T.I.L.L. Photonics Gmbh
Universal Imaging Corporation
VWR Scientific Products
Vector Laboratories
Vincent Associates
Warner Instruments
Waters Corp.

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. In addtion to the Invited Speaker Program, there are many other speakers presenting seminars at Cold Spring Harbor each year.

January

Dr. Ralph Greenspan, The Neurosciences Institute, San Diego. Gene networks and the subtleties of behavior in *Drosophila*. (Host: Tim Tully)

Dr. Angelika Amon, Howard Hughes Medical Institute, Massachusetts Institute of Technology. Cell cycle control in yeast. (Host: Nick Tonks)

Dr. John Kuriyan, Molecular and Cell Biology, University of California, Berkeley. Structural studies on *E. coli* DNA polymerase clamp loading. (Host: Leemor Joshua-Tor)

February

Dr. Titia de Lange, The Rockefeller University. The telomerase tumor suppressor pathway. (Host: Scott Lowe)

Dr. Martin Heisenberg, Theodor-Boveri-Institut fuer Biowissenschaften Lehrstuhl fuer Genetik and Neurobiologie. Behavioral analysis and genetic intervention in a small brain. (Host: Jerry Yin)

Dr. Joe Gall, Department of Embryology, Carnegie Institution. Cajal bodies: Their role in transcription and processing of RNA. (Host: David Spector)

March

Dr. Frank McCormick, Department of Microbiology & Immunology, University of California, San Francisco. Cancer therapy based on Ras and p53. (Host: Scott Lowe)

Dr. Dennis Bray, Department of Zoology, University of Cambridge. Molecular events in a small volume of cytoplasm. (Host: Yuri Lazebnik)

Dr. Cynthia Kenyon, Department of Biochemistry, University of California, San Francisco. Genes and cells that regulate the aging of C. *elegans*. (Host: Winship Herr)

Dr. Tom Ellenberger, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School. The anatomy of infidelity. Structures of error-prone and accurate DNA polymerases. (Host: Leemor Joshua-Tor)

April

Dr. Pietro De Camilli, Howard Hughes Medical Institute, Yale University School of Medicine. Phosphoinositides in the morphogenesis of the plasma membrane at the synapse and in muscle. (Host: Jacek Skowronski)

Dr. Roderick MacKinnon, Howard Hughes Medical Institute, The Rockefeller University. Ion channels. (Host: Karel Svoboda)

Dr. David J. Anderson, Howard Hughes Medical Institute, California Institute of Technology. Molecular mechanisms of neural cell fate determination. (Host: Grisha Enikolopov)

October

Dr. David Baulcombe, The Sainsbury Laboratory. Roles, mechanisms, and applications of RNA silencing. (Host: David Jackson)

Dr. Mike Tyers, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Canada. Ultrasensitivity and commitment to cell division. (Host: Bruce Stillman)

Dr. Linda Buck, Howard Hughes Medical Institute, Fred Hutchinson Cancer Research Center. Deconstructing smell. (Host: Yi Zhong)

Dr. Martin Nowak, Institute for Advanced Study, Princeton, New Jersey. Evolution of cooperation. (Host: Mitya Chklovskii)

November

Dr. W. James Nelson, Stanford University School of Medicine. Role of spatial cues in generating cell polarity. (Host: Senthil Muthuswamy)

Dr. Stephen P. Bell, Howard Hughes Medical Institute, Massachusetts Institute of Technology. The duplication of eukaryotic chromosomes. (Host: Bruce Stillman)

December

Dr. Carl Wu, National Cancer Institute, NIH. ATP-dependent chromatin remodeling protein complexes for transcription. (Host: Winship Herr)

Dr. Craig C. Mello, Howard Hughes Medical Institute, University of Massachusetts Medical School. RNA interference and development in C. *elegans*. (Host: Greg Hannon)

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.

January

Juan Mendez (Stillman Lab): Control of initiation of DNA replication in human cells.
Eli Hatchwell: Sporadic human genetic disease.
Tomek Swigut (Skowronski Lab): Mechanisms for down-regulation of cell surface proteins by Nef.

February

Erik Vollbrecht (Martienssen Lab): Regulation of meristem activity and inflorescence architecture in maize.
Adrian Krainer: Listening to silence and understanding nonsense: Coding-sequence mutations that affect splicing.
Bud Mishra: A random walk down the genomes: A case study of DNA evolution in VALIS.

March

Xuemei Zhao (Herr Lab): Mechanisms of TATA-box-binding protein function: Teaching an old dog new tricks.
Esther Nimchinsky (Svoboda Lab): Optical studies of individual synapses with single receptor resolution.
Greg Hannon: RNAi: Mechanisms and applications.

April

Yuri Lazebnik: Assisted suicide, promiscuous cells, and the joy of the unexpected.
Tatsuya Hirano: Molecular basis of chromosome condensation and cohension.
Linda Van Aelst: The rap on Ras and Rho signaling.

October

Amy Caudy (Hannon Lab): Thinking about RNAi: Fragile X mental retardation protein and RNA interference.
Ping Hu (Hernandez Lab): A minimum set of factors for RNA polymerase III transcription from the human U6 promoter.
MaryJane Gething: Unraveling the unfolded protein response.
Takuya Takahashi (Malinow Lab): Experience strengthens transmission by driving AMPA receptors into synapses.

November

Jordan Fridman (Lowe Lab): Dissecting the Mdm2 oncogene in vivo: Is full length the full story?
Zach Mainen: What the rat's nose knows: Strategies and mechanisms for odor perception.
Terence Strick: Single-molecule observation of DNA denaturation fluctuations and RNA polymerase-induced promoter melting.

December

Tom Volpe (Martienssen Lab): RNAi in *S. pombe:* Getting to the core of chromatin.
John Mignone (Enikolopov Lab): The identification of stem cells using fluorescent transgenics.
Tim Tully: An attempt to understand signal-to-noise from DNA microarray data.

BANBURY CENTER

BANBURY CENTER DIRECTOR'S REPORT

The Banbury Center continues to be used throughout the year, with the exception of the few weeks during the depth of the winter. There were 24 meetings in 2002 and Laboratory scientists used the Center for four group meetings. The Watson School of Biological Sciences held two week-long "Topics in Biology" courses, and there were the usual five neuroscience lecture courses. Finally, two local groups made use of the Center.

There were 654 participants in Banbury Center meetings, 75% of whom came from the United States, drawn from 36 states. As usual, New York, Maryland, California, and Massachusetts supplied most visitors; these four states accounted for almost 50% of the U.S. participants. 164 visitors came from around the world—from no fewer than 22 countries, demonstrating the high international reputation of the Center.

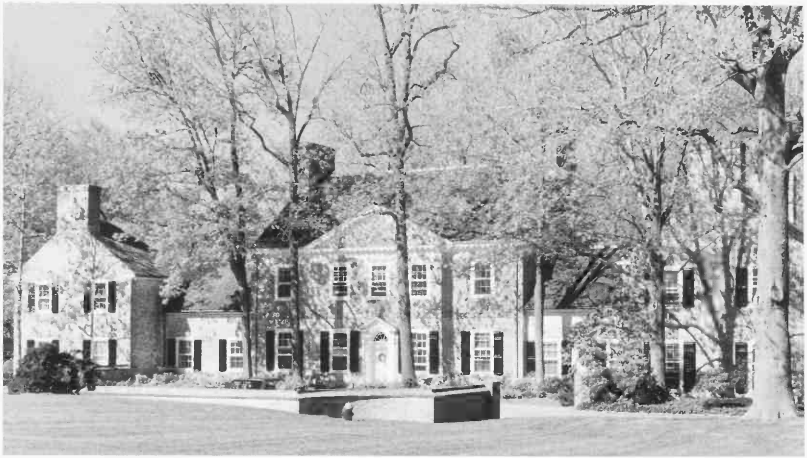
The topics for the meetings were varied as always, although this year, the program was largely concerned with genetics and cancer, and there were fewer neuroscience meetings than usual.

Genomics

Banbury Center's 24 years encompasses the revolution in human genetics brought about by gene mapping and sequencing. Now, with the human genome sequence available for nucleotide-by-nucleotide examination, new strategies should be devised to exploit this remarkable resource. *Sequenced-based Disease Gene Hunts*, organized by Aravinda Chakravarti (April 28–May 1), discussed how this might be done. It is especially important for complex traits such as schizophrenia or asthma, where progress has been slow using standard family linkage methods. New tools that allow the scanning of the entire genome, for expression differences or sequence variation, promise to change this situation.

The impact of genome sequences is not limited, of course, to human beings. Plant scientists have been at the forefront of sequencing efforts, and crop geneticists are making intensive use of the data. John Doebley and Antoni Rafalski organized the meeting *Sequence Diversity in Crop Plants: Results,*

Graduate School Course held at Banbury Center



Robertson House provides housing accommodations at Banbury Center.

Interpretations, and Applications (November 3–6) to examine new information coming from sequence comparisons of crop plants and wild species. These are being used to examine crop plant domestication, the evolution of polyploidy, and associations between traits and alleles. Some of the results have been surprising and some conflicting, and the meeting was intended to increase collaborations between experimentalists and theoreticians, as well as encourage the exchange of information between those interested in crop plants and those working with wild species or model systems.

Human Genetics

Fragile X is an example of a genetic disorder that has progressed from the gene-hunting stage to the functional analysis of the gene and its protein. Banbury Center has held annual meetings on Fragile X, and the advances in understanding the disorder are remarkable. *RNA Metabolism and the Fragile X Syndrome*, organized by Robert Darnell, Steve Warren, and David Nelson (April 7–10), continued the analysis of the role of the key protein—FMRP—that appears to bind to mRNA in the dendritic spines of neurons. The working hypothesis is that FMRP regulates specific mRNA transcripts that are critical in mediating communication between neurons. Such studies require the interactions of scientists drawn from diverse fields of research, and Banbury, as always, provides an ideal environment in which to promote collaborations.

Collaborations are very powerful ways of getting ahead through the pooling of resources and ideas. The Huntington's Disease Society of America and the Amyotrophic Lateral Sclerosis Association jointly sponsored the meeting on *Neurodegenerative Disease Models: From Pathogenesis to Therapeutics* (Christopher Ross and Lucie Bruijn; February 10–13). The theme of the meeting was that a set of neurodegenerative diseases including Huntington disease and amyotrophic lateral sclerosis seem to result from protein misfolding and aggregation. Perhaps, then, there are commonalities of mechanisms and questions to be answered: What are the roles of misfolding and aggregation in pathogenesis? Which are likely to be advantageous models for screening for therapeutics? Why are certain cells differential-

ly vulnerable in the different diseases? Answers were not found at the meeting, but new collaborations were fostered.

Gene Therapy

It is not often that a Banbury Center meeting attracts the attention of *Sports Illustrated*, but the meeting, *Genetic Enhancement of Athletic Performance* (Ted Friedmann, Gary Wadler, and Jan Witkowski; March 17–20), did so. Gene therapy has yet to live up to its promise, and it has suffered repeated setbacks, most recently the development of leukemia in children treated with retroviral vectors. However, the pressures and financial rewards of success in sport are so great that athletes may use gene therapy to introduce "desirable" performance-promoting genes while ignoring the dangers of gene therapy. Participants in this meeting, funded by the World Anti-Doping Agency (WADA), reviewed the current means by which athletes use medical treatments to promote performance and the current state of gene therapy, and discussed the future risks. It was hoped that the meeting would assist in the development of appropriate policy to anticipate and regulate extension of genetic therapy techniques and other emerging technologies to sports. The meeting was followed by a press conference in the New York City.

Infectious Diseases

Two meetings were prompted by the heightened awareness of the dangers of biological terrorism. The first, organized by Bud Mishra, was called *Designer Molecules for Biosensor Applications* (August 12–14) and discussed the potential of current state-of-the-art and possible short-term and long-term technology for rapid detection of pathogenic microorganisms. The relative merits of various technologies, based on genomic expression (mRNA), genome structure (DNA/RNA), protein structure, and other physical and geometric properties, were reviewed. Participants discussed the requirements of sensors for an advanced warning system against a biochemical attack, quick diagnosis of bio-warfare agents involved in the attack, and the forensics needed to determine the source of the attack.

The second meeting, *Microbial Forensics* (Steven Schutzer, Bruce Budowle, and Roger Breeze; November 10–13) was, in some ways, a descendant of the historic Banbury Center meeting on DNA fingerprinting held in 1989. Indeed, some of the participants in this meeting had been here in 1989. Participants discussed how to detect and identify pathogens, in particular using what might be called DNA signatures for pathogens. The meeting was initiated in part by new legislation that will change dramatically how certain dangerous pathogens are handled in the United States and that will subject academic laboratories to special physical, personnel, and pathogen security measures. The meeting was notable for the mix of scientists from academia and from government agencies—it set a new record for the number of acronyms appearing in a program.

The third meeting on infectious diseases—*Phage Therapy: Potential and Challenges* (Janakiraman Ramachandran, Gary Schoolnik, and Suresh Subramani; November 13–15)—discussed an antibacterial strategy that has been available for more than 80 years. Bacteriophages are viruses that attack bacteria, and their use as antibacterial agents was urged by Félix d'Herelle, codiscover, with Frederick Twort, of bacteriophage in the early part of the 20th century. However, phage therapy was ineffective because of the poor understanding of the biology of phages, especially their specificity, by the early practitioners. Recent global emergence of antibiotic-resistant bacterial pathogens, especially in hospitals, has led to the reevaluation of the potential of bacteriophages for the treatment of these infections. Phage therapy also has enormous potential for medical care in the developing world.

Cancer

The four meetings on cancer ranged from fundamental research through clinical trials of therapeutics to an evaluation of possible therapies. *Cell Immortalization and Transformation* (Gordon Peters and

John Sedivy; September 22–25) reviewed critically the current data on human cell immortalization and transformation, events that set a normal cell on the path to cancer. Here, an understanding of the pathways involved has been confused by the differing responses of rodent and human cells in assays; for example, two cooperating oncogenes are sufficient for transformation of primary rodent cells but not for transformation of human cells. Participants reviewed the data from different cell types and different species and tried to come to some consensus on what is going on.

The therapy of advanced melanoma remains a great challenge to researchers and physicians. Although there has been considerable progress in our understanding of the immune response in melanoma, clinical results based on this knowledge have not yet realized their full potential. Nonimmunological therapies may hold much promise, but relatively little has been done in this area, and it is not clear what these therapies might be. Meenhard Herlyn and Scott Lowe organized the meeting on *A Critical Review of Melanoma: Biology and Therapy* (September 29 to October 2) to bring together investigators working on the fundamental biology of melanomas, physicians developing and using therapies, and scientists working on related topics but not directly on melanoma. It was hoped that this combination of participants would foster critical reviews of current research and therapies for melanoma and suggest new strategies to attack this intractable cancer.

Control of angiogenesis as a strategy for controlling cancer has received intense study over the past few years. In particular, a considerable amount of research—laboratory and clinical—is taking place on endostatin, and it seemed the right time to examine what is known of endostatin and its activities. Judah Folkman organized the meeting *A Critical Review of Endostatin and Its Biology* (March 10–13) that focused on topics such as the production of recombinant endostatin, studies of its mechanism of action in vitro and in animals, endostatin gene therapy, and clinical trials.

Innumerable "folk" remedies have been used for centuries to treat the all manner of illnesses. Green tea is said to have protective effects against cancer, but although some laboratory studies in vitro and in animals have found evidence that green tea contains cancer-preventing chemicals, these results are at variance with epidemiological studies that find no effect or are themselves inconsistent. Banbury Center excels in this sort of situation, bringing together researchers who have conflicting data to discuss—rationally one hopes—what is going on. Chung Yang undertook to bring the relevant parties together for a fascinating meeting *Green Tea and Cancer: A Critical Review* (May 12–15), which included a public lecture and demonstration of the Japanese tea ceremony in Grace Auditorium.

Cell Biology

Two meetings followed a rather unusual format, pioneered some years ago by Winship Herr, Robert Kingston, and Keith Yamamoto in a meeting on transcription factors. Instead of having a meeting with 36, 30-minute presentations, each of five sessions was devoted to a specific topic, introduced by one or two short talks that set the background for the topic. Anyone who had data relevant to the topic could then contribute, and could do so several times in the same session or in different sessions. To encourage short and to-the-point contributions, participants could use no visual aids other than the chalkboard or the overhead projector.

DNA Recombination and Repair (James Haber and Scott Hawley; October 20–23) reviewed the flood of new information about the proteins that carry out recombination, and the new methods being used to look in vivo at how DNA strands are being manipulated. The goal of the meeting was to see whether a consensus could be reached about the essential elements in the several types of homologous and nonhomologous recombination pathways.

The second meeting—*Glucocorticoid Regulatory Mechanisms and Pathophysiology* (Daryl Granner and Keith Yamamoto; December 8–11)—also looked at a well-established field. The corticosteroid hormones and their agonist and antagonist derivatives are among the most widely used therapeutic agents, and the glucocorticoid receptor is one of the best-understood eukaryotic transcriptional regulatory factors. The meeting brought together investigators working across the spectrum of studies of

Meier House Provides housing accommodations for meeting participants at Banbury Center.

glucocorticoid action, as well as several experts in related areas, to integrate knowledge in the field, identify specific challenges and opportunities, and bridge intellectual gaps.

Meetings of this format are much harder work for the participants, but they can be very rewarding. Indeed, both meetings worked extremely well and participants were enthusiastic, once the shock of not using PowerPoint presentations had worn off!

Gary Bokoch and Ulla Knaus organized a meeting on *Oxidases in Inflammation and Cellular Signaling* (November 17–20). Reactive oxygen species were recognized nearly 30 years ago as products generated by phagocytic leukocytes for the purpose of bacterial killing. During subsequent years, the system responsible for generating oxidants in a controlled fashion has been defined, and it is now apparent that oxidants may also have roles as intracellular signaling molecules or second messengers. Here again, disparate areas of research find common elements, and a discussion meeting of investigators drawn from these different fields can help promote new research.

Neuroscience

The neuroscience meeting took as its subject a topic that goes all the way from basic neurological processes to the highest levels of psychology. Understanding the neural and psychological basis of emotions is essential since emotional responses pervade all aspects of cognition and behavior and can give insight into psychiatric illnesses, including depression, anxiety, and aspects of drug addiction. Emotion has been a neglected topic for scientific discussion, but there is now a revival of interest from several different viewpoints, not least the advances in neurobiology and the increasing convergence of animal and human studies.

Psychobiology of Emotion (Ray Dolan and Lewis Wolpert; October 27–30) included biologists, imaging scientists, experimental psychologists, and pharmacologists, all attempting to understand how we come to experience these most personal experiences.

Vaccines

Banbury Center has hosted a series of meetings for the Albert B. Sabin Vaccine Institute devoted to many aspects of vaccines, scientific as well as social. *Global Vaccine Shortage: The Threat to Children and What to Do About It* (Lance Gordon, Lewis A. Miller, and Nancy Tomich; October 23–25) was one that dealt with social and policy issues. The participants, drawn from academia, government, and industry, discussed the most feasible approaches to solving the recurring vaccine supply problems in the United States and in developing nations.

Education

The Dolan DNA Learning Center and Banbury Center continue to work together on the eugenics Web Site (http://www.eugenicsarchive.org/eugenics/), funded by a grant from the ELSI program of the National Human Genome Research Institute. The most recent grant includes an education component—funding for a series of workshops on *American Eugenics and the New Biology: Perspectives and Parallels* (David Micklos and Jan Witkowski; April 14–16). This workshop, which was targeted at opinion leaders and policy makers from government, science, healthcare, education, and the mass media, provided an opportunity to learn about America's past involvement in eugenics from leading experts and to interact with them in the context of a small meeting.

Watson School of Biological Sciences

This year, for the first time, we held two *Topics in Biology* courses so as to keep the class size small. The first was the return visit, by popular demand, of Hidde Ploegh from Harvard to teach an immunology course. The second was a new course on microbial pathogenesis, taught by Stan Malloy and Ron Taylor. They teach the summer Bacterial Genetics course at the main campus and are wonderful teachers. By all accounts, their course was a tremendous success.

Acknowledgments

As always, Bea Toliver, Ellie Sidorenko, and Katya Davey ensure that the meetings ran properly; Chris McEvoy, Andy Sauer, Joe Ellis, and Craig Campbell keep the estate looking beautiful; Ed Campodonico, Bill Dickerson, and Jon Parsons coped masterfully with participants' audiovisual needs; and Claudia Schmid keeps our buildings pristine.

Jan Witkowski

MEETINGS

DNA Interactive Advisory Panel

January 17–19

FUNDED BY **Howard Hughes Medical Institute**

ARRANGED BY **D. Micklos,** Dolan DNA Learning Center, Cold Spring Harbor Laboratory
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1

J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory: Welcome and procedures.

D. Micklos, Dolan DNA Learning Center, Cold Spring Harbor Laboratory: Purpose and overview of the Genetic Journey/ *DNAi* project.

Introductions: Participants, CSHL, HHMI, Windfall Films Ltd., and RGB Company Ltd.

P. Bruns and D. Liu, Howard Hughes Medical Institute, Chevy Chase, Maryland: Funding perspective and *DNAi* mini-courses.

J.D. Watson, Cold Spring Harbor Laboratory: Genetic Journey, evolution of the project, thoughts in the DNA structure, and key points of the series.

D. Dugan, Windfall Films Ltd., London, United Kingdom, and Max Whitby, The Red Green & Blue Company Ltd., London, United Kingdom: Production perspective.

D. Micklos, Dolan DNA Learning Center, Cold Spring Harbor Laboratory: Concept for *DNAi.*

SESSION 2

D. Micklos and DNALC Staff, Dolan DNA Learning Center, Cold Spring Harbor Laboratory: Tour of Dolan DNALC.

D. Micklos and DNALC Staff, Dolan DNA Learning Center, Cold Spring Harbor Laboratory: Discussion of *DNAi* template and Technology I.

Exploration of Innovative WWW Sites and Technology

D. Micklos and DNALC Staff, Dolan DNA Learning Center, Cold Spring Harbor Laboratory: Discussion of *DNAi* template and Technology II.

SESSION 3

J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory: Introduction to five content areas of Genetic Journey.

Small Group Brainstorming on Five Content Areas, Concepts, and Innovative Approaches

Summary of group findings

Next Steps: WWW Site as Interaction Tool and Summer Internships

Dolan DNALC Focus Group

Neurodegenerative Disease Models: From Pathogenesis to Therapeutics

February 10–13

FUNDED BY **Huntington's Disease Society of America and the Amyotrophic Lateral Sclerosis Association**

ARRANGED BY **C.A. Ross,** Johns Hopkins University School of Medicine and Huntington's Disease Society of America, Baltimore, Maryland
L. Bruijn, Amyotrophic Lateral Sclerosis Association, Guilford, Connecticut

Welcome: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
Opening Remarks: C.A. Ross, Johns Hopkins University School of Medicine, Baltimore, Maryland and Huntington's Disease Society of America
L. Bruijn, Amyotrophic Lateral Sclerosis Association, Guilford, Connecticut

SESSION 1: Biochemical Systems
Chairperson: S.L. Lindquist, Whitehead Institute, Cambridge, Massachusetts

R. Wetzel, University of Tennessee Medical Center, Knoxville: Polyglutamine aggregation and interactions.
A. Ciechanover, Technion-Israel Institute of Technology, Haifa, Israel: Ubiquitin activation of transcriptions: NF-κB as a paradigm.
T.M. Dawson, Johns Hopkins University School of Medicine, Baltimore, Maryland: The syns of PD.
J.F. Gusella, Massachusetts General Hospital, Charlestown: Genetic criteria for evaluating HD models.
R.H. Brown, Massachusetts General Hospital, Charlestown: ALS genetics and pathogenesis: Insights from studies of SOD1.

SESSION 2: Cell Systems
Chairperson: K. Duff, Nathan Kline Institute, Orangeburg, New York

Y. Lazebnik, Cold Spring Harbor Laboratory: Cell death.
R.R. Kopito, Stanford University, California: Protein aggregation and the ubiquitin proteasome system.
M.E. MacDonald, Massachusetts General Hospital, Charlestown: Early events in HD.
D. Sulzer, Columbia University, New York: Is macroautophagy a desperate attempt at neuroprotection?
H.D. Durham, Montreal Neurological Institute, Quebec, Canada: Physiology of aggregation in motor neurons.

R. Morimoto, M. MacDonald

SESSION 3: Invertebrate Model Systems
Chairperson: R.R. Kopito, Stanford University, California

M. Feany, Brigham and Women's Hospital, Boston, Massachusetts: Fly models for neurodegeneration.
L.M. Thompson, University of California, Irvine: Therapeutic implications of transcriptional dysregulation and aggregation in HD.
S.L. Lindquist, Whitehead Institute, Cambridge, Massachusetts: Neurodegenerative disease models: Lessons from yeast.
R.I. Morimoto, Northwestern University, Evanston, Illinois: Stressing worms by polyglutamines: Effects of aging on aggregation and toxicity.
C. Link, University of Colorado, Boulder: β amyloid-chaperone interactions in a transgenic *C. elegans* model.

SESSION 4: Vertebrate Model Systems
Chairperson: L.M. Thompson, University of California, Irvine

S.B. Prusiner, University of California, San Francisco: Transgenic studies of prion diseases.
M. Hutton, Mayo Clinic, Jacksonville, Florida: *Tau* in neurodegenerative disease.
C.A. Ross, Johns Hopkins University School of Medicine, Baltimore, Maryland and Huntington's disease Society of America: Polyglutamine pathogenesis: Proteolysis, aggregation, and transcription.
J.D. Rothstein, Johns Hopkins University School of Medicine, Baltimore, Maryland: Astroglia, glutamate, and transporters: Pathway for cell death and target for neuroprotection.

SESSION 5: Models and Therapeutics
Chairperson: J.D. Rothstein, Johns Hopkins University School of Medicine, Baltimore, Maryland

K. Duff, Nathan Kline Institute, Orangeburg, New York: Sequestration of peripheral A-beta as a therapeutic approach.
D.R. Borchelt, Johns Hopkins Medical Institutions, Baltimore, Maryland: Protein aggregation in neurodegenerative disease.
Q. Liu, Harvard Medical School, Boston, Massachusetts: Chemical screens for compounds that affect mutant SOD1-induced aggresome formation.
P.T. Lansbury, Brigham and Women's Hospital, Boston, Massachusetts: From Parkinson's genes to new therapeutic strategies.

R. Abendroth, S. Lindquist

R. Brown, T. Maniatis

A Critical Review of Endostatin and Its Biology

March 10–13

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **J. Folkman,** Children's Hospital, Boston, Massachusetts

SESSION 1: Discovery and Structure-Function of Endostatin
Chairperson: J. Folkman, Children's Hospital, Boston, Massachusetts

M.S. O'Reilly, MD Anderson Cancer Center, Houston, Texas: The discovery and characterization of endostatin.
Y.-H. Ding, Pfizer Inc., Cambridge, Massachusetts: Crystal structure of human endostatin and its implications.
R. Kalluri, Beth Israel Deaconess Medical Center, Boston, Massachusetts: Cross-talk between endogenous inhibitors of angiogenesis.
A.W. Griffioen, University Hospital Maastricht, The Netherlands: Development of novel angiostatics by peptide design based on the structure of known angiogenesis inhibitors.

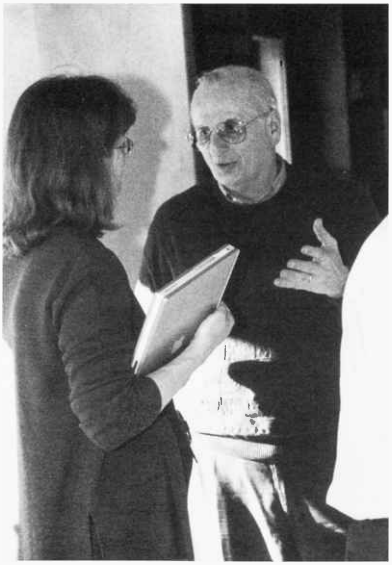
Summary and Discussion

SESSION 2: Production of Recombinant Endostatin
Chairperson: S. Libutti, National Cancer Institute, Bethesda, Maryland

J. Shiloach, National Institutes of Health, Bethesda, Maryland: Production and recovery of recombinant endostatin from *pichiapastoris*.
I.S. Chung, Kyung Hee University, Suwon, Korea: Production of recombinant endostatin from stably transformed lepidopteran and dipteran insect cells.

SESSION 3: In Vitro Biology Mechanism of Action
Chairperson: S. Libutti, National Cancer Institute, Bethesda, Maryland

T. Pihlajaniemi, University of Oulu, Finland: Diverse roles of the endostatin precursor, type XVIII collagen, and its homologue, type XV collage.
L. Claesson-Welsh, Uppsala University, Sweden: Is endostatin's effect on endothelial cell migration receptor-independent and is it critical in anti-angiogenesis?
D.S. Milstone, Brigham & Women's Hospital, Boston, Massachusetts: E-selectin and the antiangiogenic activity of endostatin.
B.K. Lee Sim, Entremed, Inc., Rockville, Maryland: Endostatin interacts with tropomyosin and actin: A potential modulator of the antitumor activity of endostatin.
W.D. Figg, National Cancer Institute, Bethesda, Maryland: Comparison of murine and human endostatin in pre-clinical models.



L. Claesson-Welch, J. Folkman

SESSION 4: In Vivo: Laboratory Experiments I
Chairperson: M.S. O'Reilly, MD Anderson Cancer Center, Houston, Texas

B.D. Ackley, University of California, Santa Cruz: The *C. elegans* homologue of collagen XVIII/endostatin regulates aspects of cell motility and neurogenesis.

F. Bertolini, European Institute of Oncology, Milan, Italy: Effect of endostatin on mobilization, clonogenic potential, and differentiation of endothelial progenitors.

B.R. Olsen, Harvard Medical School, Boston, Massachusetts: Phenotypic abnormalities of collagen VIII/endostatin null mice: Implications for biological function.

O. Kisker, University Hospital Marburg, Germany: Continuous administration of endostatin improves the efficacy and potency of therapy in a mouse xenograft tumor model.

S. Soker, Children's Hospital, Boston, Massachusetts: Novel functions of endostatin, or what have we missed in vitro that can explain the antitumor activity of endostatin in vivo.

M.R. Passos-Bueno, Universidade de Sao Paulo, San Paulo, Brazil: Characterization of novel SNPs (single nucleotide polymorphisms) in endostatins derived from collagens XV and XVIII and their impact in the susceptibility of cancer.

SESSION 5: In Vivo: Laboratory Experiments II
Chairperson: H.M. Pinedo, VU Medical Center, Amsterdam, The Netherlands

K. Moulton, Children's Hospital, Boston, Massachusetts: Endostatin inhibition of angiogenesis in atherosclerotic plaques.

B. Fenton, University of Rochester Medical Center, New York: Disparate effects of endostatin on tumor vascular perfusion and hypoxia in two murine mammary carcinomas.

R. Bjerkvig, University of Bergen, Norway: Delivery of endostatin to brain tumors from engineered cells encapsulated in alginate.

S. Libutti, National Cancer Institute, Bethesda, Maryland: Antiangiogenic gene therapy and endothelial cell gene expression profiling.

R.M. Blaese, National Cancer Institute, Bethesda, Maryland: Commentary.

SESSION 6: Preclinical and Clinical Translation
Chairperson: W.D. Figg, National Cancer Institute, Bethesda, Maryland

J.G. McArthur, Cell Genesys, Inc., Foster City, California: The impact of vector 5 decisions on antiangiogenic gene therapy.

H.M. Pinedo, VU Medical Center, Amsterdam, The Netherlands: Intrapatient comparison of pharmacokinetics following subcutaneous and intravenous administration of endostatin.

C. Sidor, EntreMed, Inc., Rockville, Maryland: The issues in designing trials using endostatin.

R.S. Herbst and M.S. O'Reilly, MD Anderson Cancer Center, Houston, Texas: The MD Anderson Phase I endostatin study.

J. Heymach, Children's Hospital, Boston, Massachusetts: Use of circulating endothelial cells as a surrogate marker for endostatin therapy in patients.

J.P. Eder, Dana-Farber Cancer Institute, Boston, Massachusetts: Clinical trials of endostatin.

Banbury grounds

Genetic Enhancement of Athletic Performance

March 17–20

FUNDED BY **WADA Health, Medical & Research Committee**

ARRANGED BY **T. Friedmann,** University of California, San Diego
G.I. Wadler, New York University School of Medicine, Manhasset
J. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1
Chairperson: T. Friedmann, University of California, San Diego

J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory: Introduction.
T. Friedmann, University of California, San Diego: Background.
A. Ljungqvist, International Amateur Athletic Federation, Enebyberg, Sweden: Welcome.
R. Pound, World Anti-Doping Agency, Montreal, Quebec, Canada: Introduction.
G.I. Wadler, New York University School of Medicine, Manhasset, New York: The history of the nature of doping.
J.O. Koss, WADA Health, Medical & Research Committee, Aurora, Ontario, Canada: Athlete's perspective.
R.K. Mueller, Leipzig University, Germany: Current methods of screening.

SESSION 2
Chairperson: G. Goldspink, Royal Free & University College Medical School, London, United Kingdom

T. Friedmann, University of California, San Diego: Principles of gene therapy: History, current state, and directions.
H.L. Sweeney, University of Pennsylvania, Philadelphia: Target tissues, muscle.
J. Glorioso, University of Pittsburgh, Pennsylvania: Detection of gene transfer and genetic approaches to pain control.
C. Evans, Brigham and Women's Hospital, Boston, Massachusetts: Stem cells, injury, and tissue repair.
O. Cohen-Haguenauer, Hospital Saint-Louis, Paris, France: Regulatory issues.

Questions and General Discussion

J.-C. Mbanya, P. Verbiest, R. Pound

SESSION 3: Genetic Targets: Metabolism, Muscle Function and Growth Factors, Oxygen Carrying Capacity, Energy Utilization
Chairperson: G.I. Wadler, New York University School of Medicine, Manhasset, New York

C. Sundberg, Karolinska Institute, Stockholm, Sweden: Muscle physiology.
C. Sundberg, Karolinska Institute, Stockholm, Sweden: Metabolism of exercising muscle.
G. Goldspink, Royal Free & University College Medical School, London, United Kingdom: IGF-1.
B.J. Byrne, University of Florida School of Medicine, Gainesville: EPO.
D.C. Wallace, Emory University School of Medicine, Atlanta, Georgia: Mitochondrial energy production and performance.
J. Glorioso, University of Pittsburgh, Pennsylvania: Metabolic changes: Microarrays.

SESSION 4: Roundtable Discussion
Chairperson: T. Friedmann, University of California, San Diego

H.L. Sweeney, University of Pennsylvania, Philadelphia
B.J. Byrne, University of Florida School of Medicine, Gainesville
D.C. Wallace, Emory University School of Medicine, Atlanta, Georgia
B. Saltin, The Copenhagen Muscle Research Centre, Denmark
G.I. Wadler, New York University School of Medicine, Manhasset

Legal and Ethical Aspects of Gene-based Enhancement in Sport

Chairperson: R.R. Young, Holme Roberts & Owen LLP, Colorado Springs, Colorado
B.-M. Knoppers, Universite de Montreal, Quebec, Canada: Legal, medical perspective.
E.T. Juengst, Case Western Reserve University, Cleveland, Ohio: Biomedical ethics of enhancement.
A. Schneider, The University of Western Ontario, London, Canada: The ethics of sport.
R.R. Young, Holme Roberts & Owen LLP, Colorado Springs, Colorado: Sport and the law.

SESSION 5: Open Discussion, WADA Statement, and Communique
Chairpersons: A. Ljungqvist, International Amateur Athletic Federation, Enebyberg, Sweden; **R. Pound,** World Anti-Doping Agency, Montreal, Quebec, Canada; **G.I. Wadler,** New York University School of Medicine, Manhasset, New York; and **T. Friedmann,** University of California, San Diego

B. Drinkwater, A. Schneider

RNA Metabolism and the Fragile X Syndrome

April 7–10

FUNDED BY **National Institute of Mental Health, NIH, and National Institute of Child Health and Human Development, NIH**

ARRANGED BY **R.B. Darnell,** The Rockefeller University, New York
S.T. Warren, HHMI, Emory University School of Medicine, Atlanta, Georgia
D.L. Nelson, Baylor College of Medicine, Houston, Texas

Introduction: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
R.B. Darnell, The Rockefeller University, New York

SESSION 1: FMRP and the Regulation of RNA and Protein Metabolism
Chairperson: R.B. Darnell, The Rockefeller University, New York

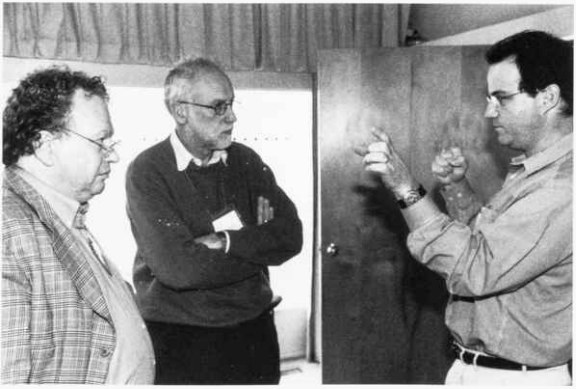
S. Ceman, Emory University School of Medicine, Atlanta, Georgia: Regulation of FMRP function by posttranslational modifications.
B. Oostra, Erasmus Universiteit Rotterdam, The Netherlands: Transport of FMRP in PC12 cells.
Y. Feng, Emory University, Atlanta, Georgia: FMRP in developing neural cells.
K. Jensen, The Rockefeller University, New York: Identifying in vivo RNA targets of vertebrate RNA-binding proteins.
H.T. Orr, University of Minnesota, Minneapolis: The SCA1 protein, ataxin-1, and RNA processing.
D.L. Black, HHMI, University of California, Los Angeles: Neuronal regulation of pre-mRNA splicing.

Overview and Questions

SESSION 2: FMR RNA Targets
Chairperson: S.T. Warren, HHMI, Emory University School of Medicine, Atlanta, Georgia

J. Darnell, The Rockefeller University, New York: Unique RNA targets of the RGG box and KH domains of FMRP.
W.T. Greenough, University of Illinois, Urbana: Possible phenotype contributions of some FMRP-interacting mRNAs.
H. Moine, Institut de Genetique et de Biologie Moleculaire et Cellulaire, Strasbourg, France: The interaction of FMRP with RNAs containing G-quartets.
L.J. Regan, Yale University, New Haven, Connecticut: Specific RNA recognition by FMRP: The role of the KH domains.

Overview and Questions



J.L. Mandel, B. Oostra, G. Bassell

SESSION 3: RNA Trafficking and Translational Control
Chairperson: D.L. Nelson, Baylor College of Medicine, Houston, Texas

O. Steward, University of California, Irvine: Targeting mRNA to synaptic sites on dendrites.
G.J. Bassell, Albert Einstein College, Bronx, New York: Regulation and function of FMRP and FMR1 mRNA trafficking in developing neurons.
M.W. Hentze, European Molecular Biology Laboratory, Heidelberg, Germany: Translational regulation by mRNA-binding proteins.
J. Richter, University of Massachusetts Medical School, Worcester: CPEB-mediated translational control.
J.R. Fallon, Brown University, Providence, Rhode Island: Regulation of FMR1 mRNA translation in neurons.

Overview and Questions

SESSION 4: FMR in Flies
Chairperson: W.T. Greenough, University of Illinois, Urbana

T.A. Jongens, University of Pennsylvania School of Medicine, Philadelphia: Analysis of behavioral and germ line defects of *dfmr1* mutant *Drosophila*.
A. Costa, Princeton University, New Jersey: dFMR-Orb interactions and mRNA localization/translation.
J. Morales, Baylor College of Medicine, Houston, Texas: DFXR regulates brain morphology and function in the CNS.
H. Matthies, University of Utah, Salt Lake City: Does *Drosophila* FMRP regulate microtubule dynamics/stability?
K. Moses, Emory University School of Medicine, Atlanta, Georgia: Genetic screen for dominant modifiers of *dFMRp*.
J.-L. Mandel, Institut Genetique et de Biologie Moleculaire et Cellulaire, Illkirch, France: FMRP interactors and *Drosophila* results.

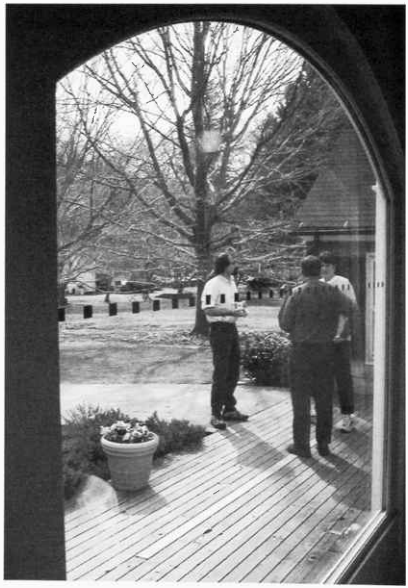
Overview and Questions

SESSION 5: FMR RNA Physiology
Chairperson: R.B. Darnell, The Rockefeller University, New York

K.M. Huber, University of Texas Southwestern Medical Center, Dallas: Role for ERK in mGluR and protein-synthesis-dependent LTD.
M.F. Bear, HHMI, Brown University, Providence, Rhode Island: Fragile X: The LTD connection.
R. Malinow, Cold Spring Harbor Laboratory: AMPA receptor trafficking during synaptic plasticity.
K. Zito, Cold Spring Harbor Laboratory: Identification of genes differentially expressed in wild-type and *fmr1* knockout mouse barrel cortex.

Summing Up: Current Issues, Future Plans

R.B. Darnell, The Rockefeller University, New York
D.L. Nelson, Baylor College of Medicine, Houston, Texas
S.T. Warren, HHMI, Emory University School of Medicine, Atlanta, Georgia



Coffee break

American Eugenics and the New Biology: Perspectives and Parallels

April 14–16

FUNDED BY **National Human Genome Research Institute**

ARRANGED BY **D. Micklos,** Dolan DNA Learning Center, Cold Spring Harbor Laboratory
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: History

J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory: Introduction
G. Allen, Washington University, St. Louis, Missouri: Progressive origins of eugenics and the Eugenics Record Office.
E. Carlson, SUNY, Stony Brook: Badseed, corrupted germ plasm, prized pedigrees, and eugenic worth.

SESSION 2: Impacts

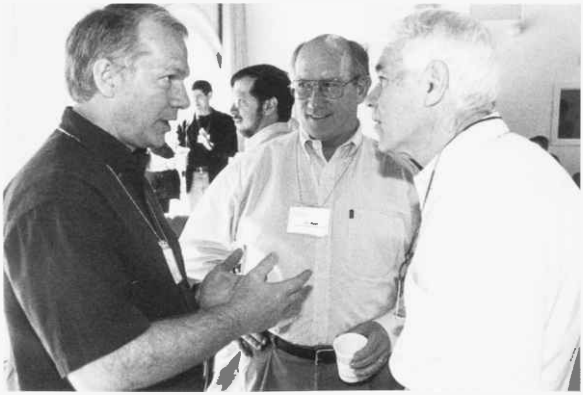
S. Selden, University of Maryland, College Park: Fitter Families for Future Firesides: State fairs and the construction of merit and race in America, 1913–1930.
P.A. Lombardo, University of Virginia, Charlottesville: Immigration and sterilization in the United States.

SESSION 3: Resources: Dolan DNA Learning Center

D. Micklos and J.A. Witkowski, Cold Spring Harbor Laboratory: Introduction to the Image Archive on the American Eugenics Movement.

SESSION 4: Lessons

B. Biesecker, National Human Genome Research Institute, Bethesda, Maryland: Use of genetic information: Reproductive choice, risk prediction, and (ultimately) behavior change.
D. Goldman, National Institutes of Health, Bethesda, Maryland: Genetics of alcoholism.



R. Apodaca, P. Ryan, G. Allen

Sequence-based Disease Gene Hunts

April 28–May 1

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **A. Chakravarti,** Johns Hopkins University School of Medicine, Baltimore, Maryland

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
A. Chakravarti, Johns Hopkins University School of Medicine, Baltimore, Maryland

SESSION 1: Concepts of Complex Disease
Chairperson: A. Chakravarti, Johns Hopkins University School of Medicine, Baltimore, Maryland

J.K. Pritchard, University of Chicago, Illinois: Allelic heterogeneity, haplotype blocks, and linkage disequilibrium mapping.
X. Estivill, The Hospital for Sick Children, Toronto, Canada: Somatic interstitial duplications of chromosome 15 in anxiety disorders.
C.H. Langley, University of California, Davis: Association mapping of a model organism: Patterning of *Drosophila* sensory organs.

SESSION 2: Human Genomic Variation
Chairperson: C.H. Langley, University of California, Davis

J.L. Weber, Marshfield Medical Research Foundation, Wisconsin: Diallelic insertion/deletion polymorphisms.
J.C. Stephens, Genaissance Pharmaceuticals, Inc., New Haven, Connecticut: DNA variation of human genes.
D. Altshuler, Massachusetts General Hospital, Boston: A large-scale study of human haplotypes in four population samples.
D. Cutler, Johns Hopkins University School of Medicine, Baltimore, Maryland: Human genetic substructure and its implications for disease association studies..

SESSION 3: Human Genome Structure
Chairperson: F.S. Collins, National Human Genome Research Institute, Bethesda, Maryland

J.D. McPherson, Washington University, St. Louis, Missouri: Whole-genome comparative physical maps.
M. Clamp, The Sanger Institute, Cambridge, United Kingdom: Human genome annotation in Ensembl.
F.S. Collins, National Human Genome Research Institute, Bethesda, Maryland: Commentary.

SESSION 4: Genome Scanning Technology
Chairperson: D. Altshuler, Massachusetts General Hospital, Boston

C.R. Cantor, Sequenom Inc., San Diego, California: Finding disease genes in the healthy population.
M.S. Chee, Illumina, Inc., Cardiff, California: Automated large-scale SNP genotyping on randomly assembled arrays.
M. Zwick, McKusick-Nathans Institute of Genetic Medicine, Baltimore, Maryland: High-throughput genome resequencing using microarrays and the ABACUS software package.
M. Wigler, Cold Spring Harbor Laboratory: Mapping genome deletions using microarray methods.

SESSION 5: Discussion: Present and Future
Chairperson: A Chakravarti, Johns Hopkins University School of Medicine, Baltimore, Maryland

Points for Discussion Arising during Meeting

M. Clamp, E. Dawson

Green Tea and Cancer: A Critical Review

May 12–15

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **C.S. Yang,** Rutgers University, Piscataway, New Jersey

SESSION 1: Chemistry: Inhibition of Tumorigenesis in Animal Models
Chairpersons: A. Conney, Rutgers University, Piscataway, New Jersey, and **H. Fujiki,** Saitama Cancer Center Research Institute, Japan

C.-T. Ho, Rutgers University, New Brunswick, New Jersey, and D. Balentine, Unilever Research Vlaardingen, The Netherlands: Chemistry and antioxidant mechanism of green tea catechins.

H. Fujiki, Saitama Cancer Center Research Institute, Japan: Cancer prevention with green tea before cancer onset and combination cancer prevention with green tea following cancer treatment.

A.H. Conney, Rutgers University, Piscataway, New Jersey: Inhibition of skin tumorigenesis by tea: What are the mechanisms and active constituents?

F.-L. Chung, American Health Foundation, Valhalla, New York: Inhibition of lung carcinogenesis by tea.

SESSION 2: Studies In Vivo
Chairpersons: J. Weisburger, American Health Foundation, Valhalla, New York, and **H. Mukhtar,** University of Wisconsin, Madison

J. Weisburger, American Health Foundation, Valhalla, New York: Inhibition of colon carcinogenesis by tea.

R. Dashwood, Oregon State University, Corvallis: Response of *Apc*min and *A33*DNb-cat mutant mice to treatment with tea, sulindac, and 2-amino-1-methyl-6-phenylimidazo(4,5-*b*)pyridine (PhIP).

H. Mukhtar, University of Wisconsin, Madison: Green tea in prevention of prostate cancer.

Y. Hara, Tokyo Food Techno Co., Ltd., Japan: The fate of tea catechins in vivo.

X. Meng, Rutgers University, Piscataway, New Jersey: Bioavailability and biotransformation of tea polyphenols.

Public Lecture: C.S. Yang, Rutgers University, Piscataway, New Jersey: Beneficial health effects of tea: Evidence, myth, and perspectives.

A. Conney, L. Arab

SESSION 3: Mechanistic Studies In Vitro
Chairpersons: I.B. Weinstein, Columbia University, New York, and **Z. Dong,** University of Minnesota, Austin

I.B. Weinstein, Columbia University, New York: Molecular mechanisms of growth inhibition by EGCG.
Z. Dong, University of Minnesota, Austin: The effects of tea polyphenols on signal transduction pathways.
Y. Cao, Karolinska Institute, Stockholm, Sweden: Suppression of angiogenesis by green tea.
M. Egeblad, University of California, San Francisco: Green tea as a matrix metalloproteinase inhibitor: Review of the literature.
S. Garbisa, Universita degli Studi di Padova, Italy: Green tea inhibition of matrix proteases instrumental to invasion.

SESSION 4: Epidemiological Studies I
Chairpersons: L. Arab, University of North Carolina, Chapel Hill, and **Y.-T. Gao,** Shanghai Cancer Insti-tute, China.

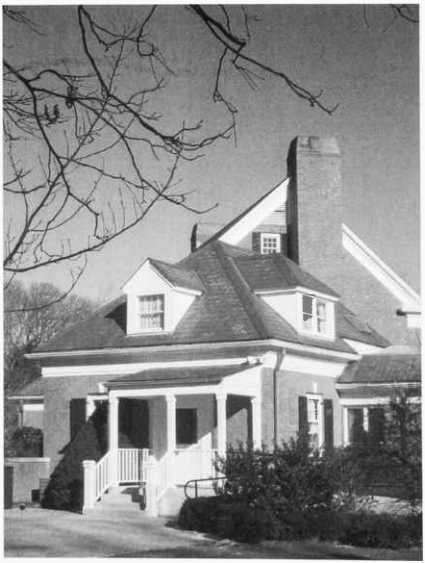
L. Arab, University of North Carolina at Chapel Hill: Tea and rectal cancer, epidemiologic studies in the U.S. and Russia.
I. Hakim, University of Arizona Health Sciences Center, Tucson: Green tea and oxidative stress among smokers: Results from a randomized clinical trial.
Y.-T. Gao, Shanghai Cancer Institute, China: Epidemiological studies on cancer and green tea drinking in Shanghai, China.
Y. Tsubono, Tohoku University, Sendai: A summary of Japanese epidemiologic studies, published and unpublished.
J.-M. Yuan, University of Southern California: Urinary tea polyphenols in relation to reduced risk of gastric and esophageal cancers: Findings from the Shanghai cohort study.
Z.-F. Zhang, University of California of Los Angeles School of Public Health: Green tea drinking and reduced risk of gastric cancer and chronic gastritis.

SESSION 5: Epidemiological Studies II
Chairperson: C.S. Yang, Rutgers University, Piscataway, New Jersey

Z.-M. Chen, Radcliffe Infirmary, Oxford, United Kingdom: Green tea and cancer mortality: A prospective study of 220,000 male adults in China.
C.-C. Hsieh, University of Massachusetts, Worcester: Green tea and cancer: Some thoughts on intervention studies.

SESSION 6: Final Assessment and Future Research

Discussion A: What are the mechanisms of inhibition of carcinogenesis by tea constituents? How do we integrate studies in vivo and in vitro?
Discussion B: Does tea consumption inhibit human carcinogenesis? How can we reconcile the different results in epidemiological studies?
Discussion C: What are the key questions to be answered on tea and cancer? How can we set about answering them?



Meier House

Designer Molecules for Biosensor Applications

August 12–14

FUNDED BY **National Science Foundation, through a grant to University of Illinois, Urbana**

ARRANGED BY **B. Mishra,** Courant Institute of Mathematical Sciences, New York University, New York

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1
Chairperson: B. Mishra, Courant Institute of Mathematical Sciences, New York University, New York

M. Desai, Program Officer, National Science Foundation, Arlington, Virginia
J.T. Schwartz, Courant Institute of Mathematical Sciences, New York University, New York
M. Wigler, Cold Spring Harbor Laboratory
J. Dahlberg, University of Wisconsin, Madison

SESSION 2: Discussion Panels I

PANEL 1: Genome-based Techniques
Chairperson and Discussion Leader: F.R. Kramer, The International Center for Public Health, Newark, New Jersey

C.R. Cantor, Sequenom, Inc., San Diego, California
J. Dahlberg, University of Wisconsin, Madison
J. Ju, Columbia Genome Center, New York
F.R. Kramer, The International Center for Public Health, Newark, New Jersey
M. Wigler, Cold Spring Harbor Laboratory

Discussion during Coffee break

SESSION 3: Discussion Panels II

PANEL 2: Protein-based Techniques
Chairperson and Discussion Leader: R. Brent, The Molecular Sciences Institute, Inc., Berkeley, California

R. Brent, The Molecular Sciences Institute, Inc., Berkeley, California
R.R. Breaker, Yale University, New Haven, Connecticut
I. Burbulis, The Molecular Sciences Institute, Inc., Berkeley, California
A. Ellington, University of Texas, Austin
M. Sitharam, University of Florida, Gainesville

SESSION 4: Discussion Panels III

PANEL 3: Miscellaneous
Chairperson and Discussion Leader: N.C. Seeman, New York University, New York

N.C. Seeman, New York University, New York
H.R. Garner, University of Texas Southwestern Medical Center, Dallas
E.M. David, The Rockefeller University, New York
B. Mishra, Courant Institute of Mathematical Sciences, New York University, New York
A. Ray, University of Rochester, New York

SESSION 5: Conclusions
Chairperson: B. Mishra, Courant Institute of Mathematical Sciences, New York University, New York

R. Brent, The Molecular Sciences Institute, Inc., Berkeley California: Personal perspective.

SESSION 6: Panel Summaries and Review

SESSION 7: Key Points for Report and Recommendations

M. Desai, National Science Foundation, Arlington, Virginia
S. Katona, Department of Health and Human Services, Washington, D.C.
J.T. Schwartz, Courant Institute of Mathematical Sciences, New York University, New York

Cell Immortalization and Transformation

September 22–25

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **G. Peters,** Cancer Research UK, London, United Kingdom
J. Sedivy, Brown University, Providence, Rhode Island

Introduction:
G. Peters, Cancer Research UK, London, United Kingdom

SESSION 1: Telomeres and Mortality
Chairperson: C.J. Marshall, Institute of Cancer Research, London, United Kingdom

T. de Lange, The Rockefeller University, New York: Telomere-directed senescence.
R.R. Reddel, Children's Medical Research Institute, Westmead, Australia: Coexistence of ALT and telomerase in cells and tumors.
M.A. Blasco, National Centre of Biotechnology, Madrid, Spain: Functional interactions at the mammalian telomere: Implications for cancer and aging.
W.E. Wright, University of Texas Southwestern Medical Center, Dallas: Telomere-based replicative aging versus damage responses in human cells.
L. Donehower, Baylor College of Medicine, Houston, Texas: Aging-associated phenotypes in p53 mutant mice.
M. Serrano, National Center of Biotechnology, Madrid, Spain: "Super p53" mice: Phenotype of transgenic mice containing supernumerary *p53* genes.

SESSION 2: Senescence versus Stasis
Chairperson: T. de Lange, The Rockefeller University, New York

S. Lowe, Cold Spring Harbor Laboratory: Initiation and maintenance of cellular senescence.
J.W. Shay, University of Texas Southwestern Medical Center, Dallas: Telomerase and human epithelial cell tumor progression.
D. Galloway, Fred Hutchinson Cancer Research Center, Seattle, Washington: Telomere-independent pathways in senescent and immortal human cells.
T.D. Tlsty, University of California San Francisco: Loss of genomic integrity in human mammary epithelial cells: Early events in breast cancer.
R. Bernards, The Netherlands Cancer Institute, Amsterdam: New tools to study immortalization and transformation.

A. Zetterberg, C. Helin, J. DeCaprio, G. Peters, N. Sharpless

SESSION 3: Cell Cycle/Myc
Chairperson: C.J. Sherr, St. Jude Children's Research Hospital, Memphis, Tennessee

A. Zetterberg, Karolinska Institute, Stockholm, Sweden: Cell growth and checkpoints in G_1.
K. Helin, European Institute of Oncology, Milano, Italy: Suppression of the pRB- or p53-mediated G_1 checkpoint is required for E2F-induced S-phase entry.
J.M. Sedivy, Brown University, Providence, Rhode Island: What does Myc do: A few new insights into the cell growth versus proliferation conundrum.
A. Trumpp, Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland: A novel role for c-Myc in stem cell self-renewal.

SESSION 4: INK4a/ARF
Chairperson: G. Peters, Cancer Research UK, London, United Kingdom

C.J. Sherr, St. Jude Children's Research Hospital, Memphis, Tennessee: *Ink4* genes and ARF.
N.E. Sharpless, Lineberger Comprehensive Center, Chapel Hill, North Carolina: The relative roles of $p16^{INK4}$ and $p19^{ARF}$ in murine cancer.
G. Peters, Cancer Research UK, London, United Kingdom: Transformation of $P16^{INK4A}$-deficient human fibroblasts.
J.A. DeCaprio, Dana-Farber Cancer Institute, Boston, Massachusetts: Genetic interactions between SV40 large T antigen and p53, Ink4a, and Arf.
M. van Lohuizen, The Netherlands Cancer Institute, Amsterdam: Senescence-bypass and transformation screens in primary mouse cells.

SESSION 5: Transformation/Tumorigenesis
Chairperson: J.M. Sedivy, Brown University, Providence, Rhode Island

W.C. Hahn, Dana-Farber Cancer Institute, Boston, Massachusetts: Human cell transformation: Cooperation among telomerase, tumor suppressor proteins, and oncogenes.
C. Counter, Duke University Medical Center, Durham, North Carolina: Distinct requirements for Ras oncogenesis in human versus mouse cells.
M. Frame, Beatson Institute for Cancer Research, Glasgow, United Kingdom: Transformation and cancer behavior controlled by Src kinase.
C.J. Marshall, Institute of Cancer Research, London, United Kingdom: Interactions between GTPase signaling pathways in cell proliferation.
Hartmut Land, University of Rochester Medical Center, New York: Mechanisms of oncogene cooperation.

Coffee break during meeting

A Critical Review of Melanoma: Biology and Therapy

September 29–October 2

FUNDED BY **Herbert J. Siegel Fund For Cancer Pharmacogenomics**

ARRANGED BY **M. Herlyn,** The Wistar Institute, Philadelphia, Pennsylvania
S. Lowe, Cold Spring Harbor Laboratory

Introduction:
S. Lowe, Cold Spring Harbor Laboratory
M. Herlyn, The Wistar Institute, Philadelphia, Pennsylvania

SESSION 1: The Clinical Problem
Chairperson: S. Lowe, Cold Spring Harbor Laboratory

L. Schuchter, University of Pennsylvania, Philadelphia: Overview of the treatment of malignant melanoma: Limitations of our current therapeutic options.

D.L. Fraker, University of Pennsylvania, Philadelphia: Clinical results and mechanism of response of regional perfusion of melanoma.

M. Berwick, Memorial Sloan-Kettering Cancer Center, New York: Gene-environment interactions in the etiology of melanoma.

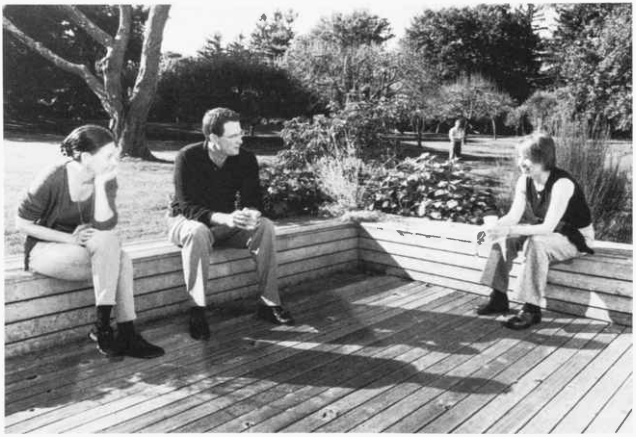
SESSION 2: Biology of Melanoma
Chairperson: M. Herlyn, The Wistar Institute, Philadelphia, Pennsylvania

D.C. Bennett, St. George's Hospital Medical School, London, United Kingdom: Melanocyte senescence, apoptosis, p16, and melanoma progression.

C. Berking, University of Munich, Germany: Induction of human melanoma by growth factors and UVB radiation.

E.E. Medrano, Baylor College of Medicine, Houston, Texas: The oncogenic protein Ski in melanoma development.

D.E. Fisher, Dana-Farber Cancer Institute, Boston, Massachusetts: MITF: Master transcriptional regulator in melanocytes and melanoma.



L. Schuchter, D. Fraker, M. Berwick

SESSION 3: Genetics of Melanoma
Chairperson: J. Sambrook, Peter MacCallum Cancer Research Institute, East Melbourne, Australia

D. Hogg, The University of Toronto, Canada: Using familial melanoma to probe mechanisms of tumor suppression.
J.M. Trent, National Human Genome Research Institute, Bethesda, Maryland: Using microarrays to dissect the genetics of melanoma.
B. Bastian, University of California, San Francisco: Genomic characteristics of melanocytic neoplasms.
L. Chin, Dana-Farber Cancer Institute, Boston, Massachusetts: Genetics, genomics, and biology of malignant melanoma.

SESSION 4: Molecular Biology of Melanoma
Chairperson: A.J. Levine, Institute for Advanced Studies, Princeton, New Jersey

Z.A. Ronai, Mount Sinai School of Medicine, New York: The transcriptional switch and melanoma resistance to apoptosis.
M.S. Soengas, University of Michigan Comprehensive Cancer Center, Ann Arbor: Bypassing cell death deficiencies in melanoma.
A. Ben-Ze'ev, Weizmann Institute of Science, Rehovot, Israel: Novel target genes of β-catenin signaling in melanoma.
M. McMahon, University of California, San Francisco: Regulation of apoptosis by Raf protein kinases.

SESSION 5: Detection and Targeting of Melanoma
Chairperson: D.E. Fisher, Dana-Farber Cancer Institute, Boston, Massachusetts

D. Becker, University of Pittsburgh Cancer Institute, Pennsylvania: Molecular and optical imaging analysis of melanoma and nevi in the context of biological therapy.
I. Hellstrom, Pacific Northwest Research Institute, Seattle, Washington: Therapeutic tumor vaccination of an MHC class I and II negative mouse melanoma.
D. Herlyn, The Wistar Institute, Philadelphia, Pennsylvania: Identification of melanoma antigen p23 using antibody phage display.
S. Ferrone, Roswell Park Cancer Institute, Buffalo, New York: HLA class I antigen abnormalities in melanoma cells: What have we learned?
P.S. Huang, GlaxoSmithKline, King of Prussia, Pennsylvania: Application of the IL-18 cytokine as an antimelanoma therapy.

Walking to lunch at Robertson House

DNA Interactive Advisory Panel—Second Meeting

October 6–8

FUNDED BY **Howard Hughes Medical Institute**

ARRANGED BY **D. Micklos,** Dolan DNA Learning Center, Cold Spring Harbor Laboratory
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Banbury Center

J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory: Welcome and procedures.
D. Micklos, Dolan DNA Learning Center, Cold Spring Harbor Laboratory: Project overview and review.

Prototype DNA Interactive Modules
WWW page: C.-H. Yang
Timeline: S. Chan and E.-S. Jeong
DNA: S. Chan and C.-H. Yang
Genome: S. Chan and W.-B. Wu
WWW Site: D. Micklos and A. Arva
Lesson Builder: D. Micklos and A. Arva
Summer Fellows: C. Gough, L. Fletcher, M. Colvard
Classroom demo of lesson builder
Teacher Guide for Anastasia
DVD and Animation Resources: M. Whitby and D. Berry

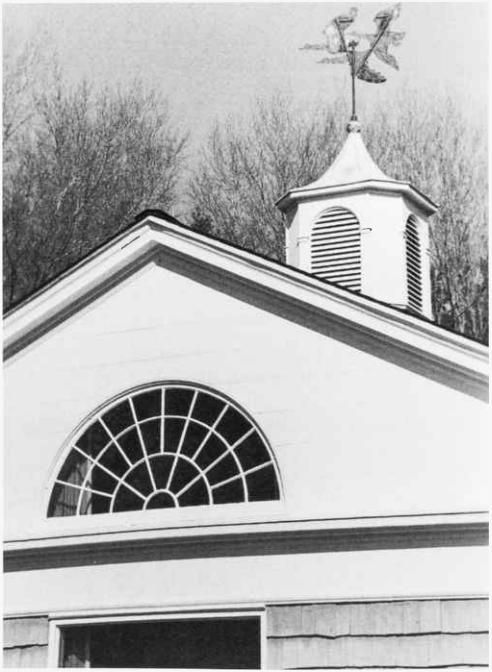
SESSION 2: Dolan DNA Learning Center

Session A: Design and Functionality
Customizable WWW site
Lesson Builder
DVD

Session B: Design and Functionality
Timeline
DNA
Genome

Session C: Content and Teacher Resources
Timeline
DNA
Genome

Session D: Content and Teacher Resources
Manipulation
Applications
Implications
Session Reports
Future Activities
Keeping Connected
Classroom Testing
Dissemination and Workshops



Banbury Conference Center

DNA Recombination and Repair

October 20–23

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **J. Haber,** Brandeis University, Waltham, Massachusetts
S. Hawley, Stowers Institute for Medical Research, Kansas City, Missouri

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1

Overviews:
J. Petrini, Memorial Sloan-Kettering Cancer Center, New York, and **P. Sung,** University of Texas Health Science Center, San Antonio:

What does the MRX (MRN) complex do? Does it directly act as an exonuclease in vivo or does it control an unknown exonuclease (or endonuclease)? Does it bridge DNA ends or span sister chromatids similar to other SMC proteins? Does it play a role in end-joining other than in budding yeast?

S.C. Kowalczykowski, University of California, Davis:

How does Rad51p assemble on to a filament and what do other recombination proteins do during this process of stand invasion?

SESSION 2

Overviews:
D. Bishop, University of Chicago, Illinois:

What does Dmc1 do that Rad51 doesn't do? How might Dmc1 and Rad1 act at opposite ends of a DSB? How are the two ends of a DSB coordinated and why doesn't BIR occur if both ends of a DSB share homology to the donor?

F.W. Stahl, University of Oregon, Eugene, and **M. Lichten,** National Cancer Institute, NIH, Bethesda, Maryland:

Are there two distinct crossover-generating pathways in meiosis, one using only Rad51 (but also Msh4/5-independent) and one using both Dmc1 and Rad51? How many non-crossover pathways are there and how do they relate to crossover-generating events?

SESSION 3

Overviews:
R. Rothstein, Columbia University, New York, and **R. Kanaar,** Erasmus University Rotterdam, The Netherlands:

Are there "recombination centers" where many independent repair events occur? How do donor and recipient sequences assemble at these places?

S. Takeda, Kyoto University Medical School, Japan, and **M. Jasin,** Sloan-Kettering Institute, New York:

What distinguishes vertebrate/mammalian DSB repair from yeast? What do the BRAC proteins do? Why is Rad52 dispensable in worms and flies and not very important in vertebrate cells, even though its overall properties appear to be preserved?

T. Petes, F. Stahl

SESSION 4

Overviews:

R.S. Hawley, Stowers Institute for Medical Research, Kansas City, Missouri, and **N. Kleckner,** Harvard University, Cambridge, Massachusetts:

What is the role of known SC components in facilitating meiotic exchange and/or synapsis?

B. Michel, Institut National de la Recherche Agronomique, Jouy-en-Josas, France, and **R.G. Lloyd,** University of Nottingham, United Kingdom:

How does recombination lead to replication restart? In eukaryotes, what proteins carry out the functions assigned to RecG and RuvABC?

SESSION 5

Overviews:

S.C. West, Clare Hall Laboratories Cancer Research UK, Herts, **N.M. Hollingsworth,** SUNY, Stony Brook, and **A. Shinohara,** Osaka University, Osaka, Japan:

Is Mus81/Mms4 (Eme1) THE Holliday junction resolvase, or A HJ resolvase, or does it act predominantly to cleave other types of branched molecules? Budding versus fission yeast. What other HJ-resolving activities are found in eukaryotes.

J. Haber, Brandeis University, Waltham, Massachusetts, and **F. Fabre**, Commissariat a l'Energie Atomique, Fontenay-aux-Roses, France:

How many distinct roles are played by the helicases Sgs1and Srs2? Do they act early/middle/late or after recombination is complete? What is the significance that overexpressing SGS1 suppresses *srs2* but not vice versa?

Grand Summing Up

F. Fabre, N. Kleckner

M. Lichten, L. Symington, J. Haber

Global Vaccine Shortage: The Threat to Children and What to Do About It

October 23–25

FUNDED BY **Bill & Melinda Gates Foundation, through a grant to Albert B. Sabin Vaccine Institute, Inc.**

ARRANGED BY **L.K. Gordon,** VaxGen, Inc., Brisbane, California
H. Larson, UNICEF, New York
N.E. Tomich, U.S. Medicine Institute, Bethesda, Maryland
L. Miller, Intermedica, Darien, Connecticut

SESSION 1: Keynote Speeches
C. Bellamy, UNICEF, New York
K. Reilly, Wyeth Global Vaccines, St. Davids, Pennsylvania

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
L.K. Gordon, VaxGen, Inc., Brisbane, California
H. Larson, UNICEF, New York

N.E. Tomich, U.S. Medicine Institute, Bethesda, Maryland: Charge to the conference.
Piers Whitehead, VaxGen Inc., Brisbane, California: 2002 Mercer Report. The worldwide problem.

SESSION 2: Return on Investment in the Vaccine Industry

Overview:
D. Braga, Aventis, USA, Swiftwater, Pennsylvania

Discussants:
K. Reilly, Wyeth Global Vaccines, St. Davids, Pennsylvania
J. Heinrich, U.S. General Accounting Office, Washington, D.C.
S. Jarrett, UNICEF, New York

Round Table Discussions

Reports from Round Tables

SESSION 3: The Regulatory Process and Vaccines I
Chairperson: L.K. Gordon, VaxGen, Inc., Brisbane, California

Overview:
W. Vandersmissen, GlaxoSmithKline Biologicals, Rixensart, Belgium

Discussants:
J.E. Fischer, Committee on Veterans' Affairs, Washington, D.C.
K. Midthun, FDA, NIH, Rockville, Maryland
J. Milstien, World Health Organization, Geneva, Switzerland

SESSION 4: The Regulatory Process and Vaccines II
Chairperson: L.K. Gordon, VaxGen, Inc., Brisbane, California

Round Table Discussions

Reports from Round Tables

SESSION 5: Adequate Vaccine Capacity
Chairperson: L.K. Gordon, VaxGen, Inc., Brisbane, California

Overview:
A. Robbins, Tufts University School of Medicine, Boston, Massachusetts

Discussants:
D. Simpson, Centers for Disease Control & Prevention, Atlanta, Georgia
L. Tan, American Medical Association, Chicago, Illinois

Round Table Discussions

Reports from Round Tables

SESSION 6: Vaccines as a National Priority

Overview:
S. Bice, Centers for Disease Control & Prevention, Atlanta, Georgia

Discussants:
R. Chalk, National Academy of Sciences, Washington, D.C.
L.Z. Cooper, American Academy of Pediatrics, New York
J.I. Santos, National Immunization Council, Mexico City, Mexico

Round Table Discussions

Reports from Round Tables

Review of Reports: Identifying Areas of Consensus
Next Steps: Need for Task Forces to Follow Up?

D. Simpson, J. Heinrich, M. Chafee

Psychobiology of Emotion

October 27–30

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **R.J. Dolan,** Institute of Neurology, London, United Kingdom
L. Wolpert, University College London, United Kingdom

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
L. Wolpert, University College London, United Kingdom

SESSION 1: Neural Basis of Emotion I
Chairperson: R.J. Dolan, Institute of Neurology, London, United Kingdom

D.J. Anderson, HHMI, California Institute of Technology, Pasadena: Neural substrates of innate fear and their regulation by anxiety.
J.H. Bachevalier, University of Texas Health Science Center, Houston: Neural correlates of emotion from the perspective of primate neuropsychological studies.
A. Calder, MRC Cognition & Brain Science Unit, Cambridge, United Kingdom: The neuropsychology of disgust and anger.
R.J. Davidson, University of Wisconsin, Madison: The neuroscience of affective style.
W.C. Drevets, National Institutes of Health, NIMH/MIB, Bethesda, Maryland: The neurobiology of major depression.

SESSION 2: Neural Basis of Emotion II
Chairperson: J.H. Bachevalier, University of Texas Health Science Center, Houston

R. Garcia, Universite Nice-Sophia Antipolis, Nice, France: Neural basis of emotional perseveration.
K. Nader, McGill University, Montreal, Canada: Reconsolidation: New opportunities for treatment of psychiatric disorders.
T. Ono, Toyama Medical & Pharmaceutical University, Sugitani, Japan: Neural representation of emotion in the prefrontal cortex, limbic system, and nucleus accumbens.
D. Pare, Rutgers University, Newark, New Jersey: Activity-dependent synaptic plasticity in intercalated neurons of the amygdala.
J.A. Parkinson, University of Cambridge, United Kingdom: Psychological representations and neural mechanisms underlying appetitive emotional conditioning.
P.J. Whalen, University of Wisconsin, Madison: The human dorsal amygdaloid region in facial expression processing.

D. Dennett, J. Parkinson, J. Winston

SESSION 3: Emotion and Cognition
Chairperson: L. Wolpert, University College London, United Kingdom

A. Bechara, University of Iowa College of Medicine, Iowa City: Is emotion beneficial or disruptive to judgment and decision-making?
A. Dickinson, University of Cambridge, United Kingdom: The function of affect: The interface between cognition and motivation.
R.J. Dolan, Institute of Neurology, London, United Kingdom: Emotion, cognition, and behavior.
M. Gallagher, Johns Hopkins University, Baltimore, Maryland: Amygdala/orbitofrontal interactions for goal-directed behavior.
E.A. Phelps, New York University, New York: The human amygdala and episodic memory or interaction of cognition and emotion.

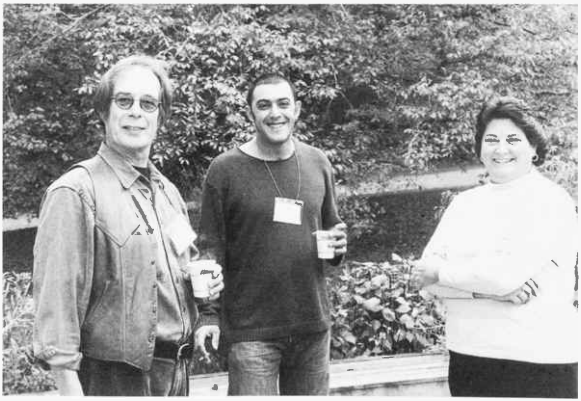
SESSION 4: Emotion, Mood, and Personality
Chairperson: L. Wolpert, University College London, United Kingdom

D. Evans, University of Bath, United Kingdom: Emotions and physical health: A biological mechanisms for the placebo response.
J.J. Gross, Stanford University, California: Emotion regulation.
T. Heatherton, Dartmouth College, Hanover, New Hampshire: Self-regulation of emotion.
A. Holmes, National Institutes of Health, Bethesda, Maryland: Analysis of emotional behavior in genetically modified mice.
A. Ohman, Karolinska Hospital, Stockholm, Sweden: The overlap of emotion activation and attention capture.

SESSION 5: Emotion, Mood, and Society
Chairperson: D. Dennett, Tufts University, Medford, Massachusetts

D. Fessler, University of California at Los Angeles: The evolutionary psychology of human emotions.
A. Hopfensitz, CREED, University of Amsterdam, The Netherlands: Emotions in economics.
A.R. Hariri, National Institute of Mental Health, Bethesda, Maryland: Genetic variation and the response of the human amygdala.
J.S. Winston, University College London, United Kingdom: Brain regions responding to social and emotional information in faces.

SESSION 6: General Discussion and Summary
Chairpersons: R.J. Dolan, Institute of Neurology, London, United Kingdom, and **L. Wolpert,** University College London, United Kingdom



A. Dickerson, K. Nader, J. Bachevalier

Sequence Diversity in Crop Plants: Results, Interpretations, and Applications

November 3–6

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **J. Doebley,** University of Wisconsin, Madison
J. Antoni Rafalski, DuPont Agricultural Enterprise, Newark, Delaware

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Theoretical/General
Chairperson: B. Burr, Brookhaven National Laboratory, Upton, New York

B.S. Weir, North Carolina State University, Raleigh, North Carolina: Recent methods for characterizing population structure and association mapping.
A. Long, University of California, Irvine: Using linkage disequilibrium to dissect complex traits.
E. Thompson, North Carolina State University, Raleigh: The information available in the pedigree relationships among inbred lines.
M. Nordborg, University of Southern California, Los Angeles: A genomic-wide survey of polymorphism in *Arabidopsis*.
H.K. Dooner, Rutgers University, Piscataway, New Jersey: The polymorphic organization of the *bz* genomic region in maize.

SESSION 2: Learning from Flies and Humans
Chairperson: J. Doebley, University of Wisconsin, Madison

A.G. Clark, Cornell University, Ithaca, New York: LD patterns and haplotypes in humans.
W. Stephan, University of Munich, Germany: Species and recombination effects on DNA sequence variation in the tomato genus.
C.F. Aquadro, Cornell University, Ithaca, New York: Finding novel genes based on footprints of natural selection.
C. Schlotterer, Universitat für Veterinarmedizin, Vienna, Austria: Local selective sweeps: What can be learned from *Drosophila*?

S. Tingey, T. Mitchell-Olds

SESSION 3: Sequence Diversity in Plants I
Chairperson: S. Tingey, DuPont Company, Newark, Delaware

M.D. Purugganan, North Carolina State University, Raleigh: Selection in the *Arabidopsis* genome.
T. Mitchell-Olds, Max-Planck Institute for Chemical Ecology, Jena, Germany: Functional nucleotide polymorphisms within and between species.
K. Schmid, Max-Planck Institute for Chemical Ecology, Jena, Germany: Population genomics in *Arabidopsis* Identification and analysis of rapidly evolving genes.
P.B. Cregan, USDA, Agricultural Research Service, Beltsville, Maryland: Nucleotide and haplotype diversity and linkage disequilibrium in cultured and wild soybean.
M. Aquade, Universitat de Barcelona, Spain: Variation in phenylpropanoid genes in cruciferae.

SESSION 4: Sequence Diversity in Plants II
Chairperson: J.A. Rafalski, DuPont Agricultural Enterprise, Newark, Delaware

O. Savolainen, University of Oulu, Finland: Sequence diversity in species at different stages of domestication.
B.S. Gaut, University of California, Irvine: DNA sequence diversity in maize and its wild relatives.
M. Morgante, DuPont Crop Genetics, Newark, Delaware: Sequence conservation in conifers.
O. Smith, Pioneer Hi-Bred International, Inc., Johnston, Iowa: Marker diversity and preliminary LD results among lines in elite breeding populations.

SESSION 5: Linking Phenotypes and Sequences
Chairperson: D.T. Tomes, Pioneer Hi-Bred International, Inc., Johnston, Iowa

J.A. Rafalski, DuPont Agricultural Enterprise, Newark, Delaware: Sequence diversity selection and linkage disequilibrium in maize elite germ plasm.
E. Buckler, North Carolina State University, Raleigh: Candidate gene associations across diverse maize germ plasm.
J. Liu, Cornell University, Ithaca, New York: Genetic basis of the evolution of crop plant morphology.
M. Cooper, Pioneer Hi-Bred International Inc., Johnston, Iowa: Modeling breeding strategies for complex traits.
J. Doebley, University of Wisconsin, Madison: What microsatellites tell us about maize and its genome.

Back view of Banbury Conference Center

Microbial Forensics

November 10–13

FUNDED BY **National Institute of Justice of the U.S. Department of Justice and the U.S. Department of Energy (NNSA, CNBP)**

ARRANGED BY **S.E. Schutzer**, UMDNJ-New Jersey Medical School, Newark
B. Budowle, Federal Bureau of Investigation, Washington, D.C.
R. Breeze, USDA, Agricultural Research Service, Washington, D.C.

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
S.E. Schutzer, UMDNJ-New Jersey Medical School, Newark

SESSION 1: Issues
Chairperson: R. Breeze, USDA, Agricultural Research Service, Washington, D.C.

B. Budowle, FBI Academy, Washington, D.C.: What is the big picture? What are FBI needs and how can they be in concert with those of HHS, USDA, DOD, DOE, EPA, etc.? What are the court needs?

R.P. Harmon, Alameda County Attorney's Office, Oakland, California: Rules of evidence for the courts.

J. Smith, FBI Laboratory, Washington, D.C.: How did DNA testing begin in U.S. Courts? What problems were encountered? How did this grow into the CODIS system? What can be learned for microbial forensics?

R. Breeze, USDA, Agricultural Research Service, Washington, D.C.: Developments in physical security, personnel assurance, and pathogen surety for select human and agricultural pathogens: How guns, guards, and gates relate to microbial forensics.

SESSION 2: High-consequence Pathogens for the FBI
Chairperson: R. Breeze, USDA, Agricultural Research Service, Washington, D.C.

FBI's Human, Animal, and Plant Concerns:
B. Budowle, FBI, Washington, D.C., and **L. Collins Kelley**, USDA, Agricultural Research Service, Athens, Georgia

SESSION 3: What Is The State of the Art for "Signatures?" I
Chairperson: A.D. Steinberg, Mitretek Systems, McLean, Virginia

General Issues and Specific Cases:
Key differences between classes of pathogens
Evidence for laboratory of origin and/or recent culture
Distinguishing from background, close neighbors, and mixed populations

D.L. Rock, USDA, Agricultural Research Service, Greenport, New York: Viruses.

P. Keim, Northern Arizona University, Flagstaff: Bacteria.

G.A. Payne, North Carolina State University, Raleigh: Fungi.

J. Marks, University of California, San Francisco: Botulinum toxins.

P.J. Jackson, Los Alamos National Laboratory, New Mexico: Geo-location.

P. Keim, R. Okinaka

SESSION 4: What Have We Learned About Microbial Forensics Over the Past Year? Including What Should Have Been in Place for Anthrax?
Chairperson: S.E. Schutzer, UMDNJ-New Jersey Medical School, Newark

D. Beecher, FBI Academy, Quantico, Virginia: Microbiological sampling at the scene of a covert biological release.
J.W. Ezzell, USAMRIID, Ft. Detrick, Maryland: Sample analysis: Meeting a forensic standard.
P. Keim, Northern Arizona University, Flagstaff: High-resolution DNA typing for precise identification of bacterial pathogens.
T.D. Read, The Institute for Genomic Research, Rockville, Maryland: Genomics of *Bacillus anthracis*.

SESSION 5: What Is the State of the Art for "Signatures?" II
Chairperson: P. Keim, Northern Arizona University, Flagstaff

S. Salzberg, The Institute for Genomic Research, Rockville, Maryland, and John J. Dunn, Brookhaven National Laboratory, Upton, New York: Foreign genes: Identification, function, origin, natural, or engineered.
W.D. Wilson, Lawrence Livermore National Laboratory, California, and C.M. Schaldach, Lawrence Livermore National Laboratory, California: Nongenomic signatures.
A.D. Steinberg, Mitretek Systems, McLean, Virginia: Host-pathogen interactions.
S.E. Schutzer, UMDNJ-New Jersey Medical School, Newark: Components of the immune response distinguishing perpetrator from victim.
B.J. Luft, SUNY, Stony Brook: Protective measures: Antibiotic half-life vaccines.
D.L. Wilson, FBI, Washington, D.C., and M. Wilson, FBI, Washington, D.C.: Matrices and trace substances.

SESSION 6: Validating Signatures I
Chairperson: D.L. Rock, USDA, Agricultural Research Service, Greenport, New York

C.L. Cook, Defense Threat Reduction Agency, Ft. Belvoir, Virginia, and L. Collins Kelley, USDA, Agricultural Research Service, Athens, Georgia: The DTRA Microbial Forensics Initiative.

SESSION 7: Validating Signatures II
Chairperson: D.L. Rock, USDA, Agricultural Research Service, Greenport, New York

C. Carrillo, USDA, Agricultural Research Service, NASS, Greenport, New York: Pathogen evolution: Behavior in culture vs. interspecies and intraspecies infections.
J.J. Dunn, Brookhaven National Laboratory, Upton, New York: The unexpected signature.

SESSION 8: General Discussion: What Do These Signature Techniques Mean?
Discussion Leader: R. Breeze, USDA, Agricultural Research Service, Washington, D.C.

What do they not tell us?
How do we interpret them?
What do we need to do to get the degree of attribution we require? And can we reach it?
Can the signatures be forged?
How do we get automated technologies for high throughput?

SESSION 9: Can We Have a Systematic Approach to an Unknown Sample?
Discussion Leader: R. Breeze, USDA, Agricultural Research Service, Washington, D.C

E.S. Raveche, UMDNJ-New Jersey Medical School, Newark: An algorithmic approach.

SESSION 10: Outline of a U.S. Microbial Forensics System I
Discussion Leader: R. Breeze, USDA, Agricultural Research Service, Washington, D.C

P.J. Jackson, Los Alamos National Laboratory, New Mexico, and P. Keim, Northern Arizona University, Flagstaff: Repositories.
N.D. Zinder, The Rockefeller University, New York: Personal perspective: Academia and chemical and biological defense.

SESSION 11: Outline of a U.S. Microbial Forensics System II
Chairpersons: B. Budowle, Federal Bureau of Investigation, Washington, D.C., and **S.E. Schutzer,** UMDNJ-New Jersey Medical School, Newark

T. Slezak, Lawrence Livermore National Laboratory, California, and N. Kahn, CIA/DST/ODDST/ITIC, Washington, D.C.: Databases.
R.T. Okinaka, Los Alamos National Laboratory, New Mexico: Sample issues/near neighbors.
S.A. Morse, CDC/NCID/BPRP, Atlanta, Georgia: Validation.

QA/QC Proficiency Tests

Research Needs

G. Parker, U.S. Army Medical Research and Material Command, Fort Detrick, Maryland: For each threat class, how can we get the capacity to obtain the information we need and the degree of attribution we require?

Discussion and Recommendations for Action Agencies
Discussion Leaders: B. Budowle, Federal Bureau of Investigation, Washington D.C., and **S.E. Schutzer,** UMDNJ-New Jersey Medical School, Newark

Phage Therapy: Potential and Challenges

November 13–15

FUNDED BY **ICF Ventures**

ARRANGED BY **J. Ramachandran,** GangaGen, Inc., Palo Alto, California
G.K. Schoolnik, Stanford University Medical Center, California
S. Subramani, University of California, San Diego

Introduction: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
J. Ramachandran, GangaGen, Inc., Palo Alto, California

SESSION 1: The New Phage Biology
Chairperson: S. Adhya, National Cancer Institute, NIH, Bethesda, Maryland

R. Young, Texas A&M University, College Station: Timing is everything; optimizing phage lysis for any growth condition.
P. Garcia, Centro de Investigaciones Biologicas, Madrid, Spain: Pneumococcal phages and their lytic enzyme.
M.J. Loessner, Technical University of Munich, Germany: The weapons of the enemy: Viral enzymes for selective targeting of pathogenic bacteria.
M. Waldor, New England Medical Center, Boston, Massachusetts: Mechanisms controlling the horizontal and vertical transmission of the cholera toxin genes.
D. Fitzgerald, National Cancer Institute, Bethesda, Maryland: Pseudomonas and phage.

SESSION 2: Phage Therapy I
Chairperson: G.K. Schoolnik, Stanford University Medical Center, California

S. Adhya, National Cancer Institute, NIH, Bethesda, Maryland: Bacteriophage therapy of experimental bacterimia .
V.A. Fischetti, The Rockefeller University, New York: Using phage lytic enzymes to control bacterial infections.
C.R. Merril, National Institute of Mental Health, NIH, Bethesda, Maryland: Phage interactions with mammalian systems: Pharmacokinetic effects and other considerations for antibacterial phage therapy.
E. Kutter, Evergreen State College, Olympia, Washington: Virulent phage infection under conditions reflecting their natural habitats.
J.A. Fralick, Texas Tech University Health Sciences Center, Lubbock: Phage therapy: The treatment of *P. aeruginosa* infections of burn wounds and *C. difficile* associated disease in animal model systems.

SESSION 3: Phage Therapy II
Chairperson: R. Young, Texas A&M University, College Station

W.C. Summers, Yale University, New Haven, Connecticut: Historical origins of phage therapy.
R. Adamia, United Nations, New York: Tbilisi experience.
G.K. Schoolnik, Stanford University Medical Center, California: Clinical results from Poland.
J.M. Manur, GangaGen Biotechnologies Pvt. Ltd., Bangalore, India: Investigation of potential limitations of phage therapy.
K. Murthy, GangaGen Life Sciences Inc., Ottawa, Canada: Regulatory issues and challenges.

Oxidases in Inflammation and Cellular Signaling

November 17–20

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **G.M. Bokoch,** The Scripps Research Institute, La Jolla, California
U.G. Knaus, The Scripps Research Institute, La Jolla, California

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
G.M. Bokoch, The Scripps Research Institute, La Jolla, California

SESSION 1: Neutrophil NADPH Oxidase (Phox I)
Chairperson: A.R. Cross, The Scripps Research Institute, La Jolla, California

K. Rittinger, National Institute for Medical Research, London, United Kingdom: NADPH oxidase assembly: A structural perspective.

A. Jesaitis, Montana State University, Bozeman: Structural changes induced in human neurtrophil cytochrome *b* by NADPH oxidase activators.

E. Pick, Tel Aviv University Sackler School of Medicine, Israel: Deconstructing the oxidase.

G.M. Bokoch, The Scripps Research Institute, La Jolla, California: Mechanism of NADPH oxidase regulation by Rac GTPase.

M. Dinauer, Indiana University School of Medicine, Indianapolis: Superoxide production by phagocytes: NADPH oxidase and regulation by RhoGTPase Rac2.

M.-C. Dagher, Laboratoire Biochimie et Biophysique des Systèmes Intégrés, Département Réponse et Dynamique Cellulaire, CEA, Grenoble, France: Insights into differential reactivity of G12V and Q61L *rac* mutants.

SESSION 2: Neutrophil NADPH Oxidase (Phox II)
Chairperson: E. Pick, Tel Aviv University Sackler School of Medicine, Israel

A.R. Cross, The Scripps Research Institute, La Jolla, California: Electron transport in NADPH oxidase.

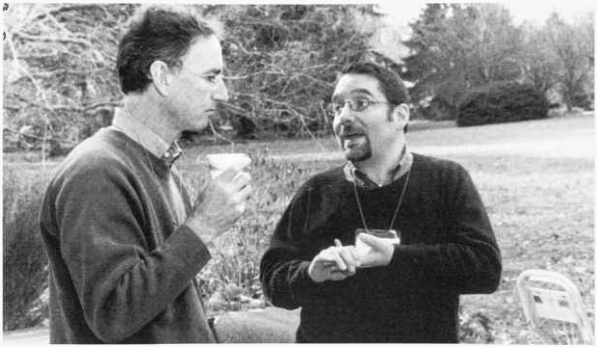
T.E. DeCoursey, Rush Medical Center, Chicago, Illinois: Interactions between voltage-gated proton channels and NADPH oxidase.

L. Henderson, University of Bristol, United Kingdom: Expression of Nox homologues in gp91phox knockout PLB-985 cells: An explanation for the presence of voltage-gated proton currents.

A.W. Segal, University College London, United Kingdom: The influence of NADPH oxidase induced K+ movement into the phagocytic vacuole on protease activity and microbial killing.

M.T. Quinn, Montana State University, Bozeman: Transcriptional regulation of p67*phox* gene expression.

H. Sumimoto, Kyushu University, Fukuoka, Japan: The adaptor protein p40*phox* as a positive regulator of the phagocyte NADPH.



J. Jones, T. Leto

SESSION 3: Noxel Oxidases (Nox I)
Chairperson: R. Clark, University of Texas Health Science Center, San Antonio

R. Clark, University of Texas Health Science Center, San Antonio: Regulation and function of NADPH oxidases: NOX1 versus the phagocyte oxidase.

K.-H. Krause, Geneva University Hospital, Switzerland: Nox1 in humans and mice.

T.L. Leto, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland: The Nox/Duox family of NAD(P)H oxidases: Potential mediators of host defense and inflammation.

T. Kawahara, University of Tokushima, Japan: Roles of Nox1 in innate immune responses of the gastrointestinal tract.

M. Geiszt, Semmelweis University, Budapest: Functional characterization of NADPH oxidase: Remarkable similarities to the phox systems.

U.G. Knaus, The Scripps Research Institute, La Jolla, California: Regulation and functions of Nox proteins.

SESSION 4: Novel Oxidases (Nox II)
Chairperson: W.M. Nauseef, University of Iowa, Iowa City

R. Arnold, Emory University, Atlanta, Georgia: The Nox family of NADPH oxidases: Regulation and cancer associations.

B. Banfi, Geneva University Hospital, Switzerland: Activation of NOX1 by two novel subunits.

W.M. Nauseef, University of Iowa, Iowa City: Biosynthesis of NOX proteins: Work in progress.

R. Fluhr, Weizmann Institute of Science, Rehovot, Israel: Multifunctional ROS signaling plants.

J.D.G. Jones, John Innes Centre, Norwich, United Kingdom: Diverse roles of plant respiratory burst oxidase homologs in cellular signaling.

SESSION 5: ROS Signaling and Cellular Consequences
Chairperson: S.G. Rhee, National Heart, Lung & Blood Institute, NIH, Bethesda, Maryland

S.G. Rhee, National heart, Lung & Blood Institute, NIH, Bethesda, Maryland: Intracellular messenger function of hydrogen peroxide.

N.K. Tonks, Cold Spring Harbor Laboratory: Harnessing ligand-induced reversible oxidation for elucidating the signaling function of protein tyrosine phosphatases.

E. Werner, Emory University, Atlanta, Georgia: A novel mechanism for *ras*-dependent ROS generation.

P. Hordijk, Sanquin Research at CLB, Amsterdam, The Netherlands: Role of endothelial ROS in the control of cell-cell adhesion and leukocyte transmigration.

P.J. Pagano, Case Western Reserve University, Cleveland, Ohio: Adenoviral targeting of NADPH oxidase inhibitors to the vasculature.

S. Nemoto, National Heart, Lung & Blood Institute, NIH, Bethesda, Maryland: Strategies to isolate novel regulators of the intracellular redox state.

Glucocorticoid Regulatory Mechanisms and Pathophysiology

December 8–11

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **K. Yamamoto,** University of California, San Francisco
D.K. Granner, Vanderbilt University School of Medicine, Nashville, Tennessee

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1

Overview: M. Dallman, University of California, San Francisco
Discussants:
S. Davis, Vanderbilt University, Nashville Tennessee
D.B. DeFranco, University of Pittsburgh School of Medicine, Pennsylvania
D.K. Granner, Vanderbilt University School of Medicine, Nashville, Tennessee
G. Schutz, German Cancer Research Center, Heidelberg

What are the key actions and coupling networks for corticosteroids in metabolism, stress, and the HPA axis?

Overview: J. Funder, Prince Henry's Institute of Medical Research, Clayton, Australia
Discussants
E.R. de Kloet, Gorlaeus Laboratory, Leiden, The Netherlands
D. Pearce, University of California, San Francisco

What are the key actions and coupling networks for corticosteroids in cardiovascular and renal physiology?

G. Schutz, B. Thompson

SESSION 2

Overview: J.N. Miner, Ligand Pharmaceuticals, San Diego, California
Discussants:
H. Samuels, New York University, New York
A. Shiau, Tularik, Inc., South San Francisco, California
S. Simons, National Institute of Diabetes and Digestive and Kidney Diseases, NIH
H.E. Xu, Van Andel Research Institute, Grand Rapids, Michigan

How can we begin to move toward rational design of selective glucocorticoid agonists?

Overview: M. Garabedian, New York University School of Medicine, New York
Discussants:
J. Funder, Prince Henry's Institute of Medical Research, Clayton, Australia
P. Herrlich, Institute of Toxicology and Genetics, Karlsruhe, Germany
D. Pearce, University of California, San Francisco
G. Schutz, German Cancer Research Center, Heidelberg

How do receptor modifications, such as phosphorylation, methylation, sumoylation, couple receptor action to other signaling pathways, and what cross-talk circuits are most significant physiologically?

SESSION 3

Overview: P. Herrlich, Institute of Toxicology and Genetics, Karlsruhe, Germany
Discussants:
A.C.B. Cato, Institute of Toxicology and Genetics, Karlsruhe, Germany
G. Haegeman, University of Gent, Belgium
T. Heinzel, Institute for Biomedical Research, Frankfurt, Germany
B. Thompson, University of Texas Medical Branch, Galveston

What are the key actions and coupling networks for corticosteroids in immune cell and inflammatory development, physiology, and pathophysiology?

Overview: L. Freedman, Merck Research Labs, West Point, Pennsylvania
Discussants:
R. Derynck, University of California, San Francisco
M. Garabedian, New York University School of Medicine, New York

What are the key actions and coupling networks for corticosteroids in bone pathophysiology?

SESSION 4

Overview: K. Yamamoto, University of California, San Francisco
Discussants:
M. Brown, Dana-Farber Cancer Institute, Boston, Massachusetts
G.L. Hager, National Cancer Institute, NIH, Bethesda, Maryland
T. Heinzel, Institute for Medical Research, Frankfurt, Germany
D.K. Granner, Vanderbilt University School of Medicine, Nashville, Tennessee
H. Samuels, New York University, New York

How can we understand combinatorial assembly of transcriptional regulatory complexes?

Overview: D.K. Granner, Vanderbilt University School of Medicine, Nashville, Tennessee
Discussants:
T.K. Archer, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North Carolina
M. Brown, Dana-Farber Cancer Institute, Boston, Massachusetts
T. Heinzel, Institute for Medical Research, Frankfurt, Germany
S.S. Simons, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, Maryland

What are the activities and actions of receptor cofactors, and how do they signal specific changes in polymerase activity?

SESSION 5

Overview: T. K. Archer, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North Carolina
Discussants:
D.B. DeFranco, University of Pittsburgh School of Medicine, Pennsylvania, *and others.*

What are the signals, mechanisms, and physiological significance of receptor proteolysis?

Overview: G.L. Hager, National Cancer Institute, NIH, Bethesda, Maryland
Discussants:
D.B. DeFranco, University of Pittsburgh School of Medicine, Pennsylvania
K. Yamamoto, University of California, San Francisco

What are the mechanisms and significance of receptor dynamics and intracellular localization?

Grand Summing Up

DOLAN DNA LEARNING CENTER

DOLAN DNA LEARNING CENTER

ADMINISTRATION

Judy Cumella-Korabik
Nancy Daidola
David Micklos
Erin Wahlgren

INSTRUCTION

Elna Carrasco
Kimberly Kessler
Erin Maroney
Tricia Maskiell
Amanda McBrien
Michael O'Brien
Danielle Sixsmith

BIOMEDIA

Shirley Chan
Darius Farraye
Eun-Sook Jeong
Susan Lauter
Karwai Pun
Bronwyn Terrill
Beverly Tomov
Chun-hua Yang

TECHNOLOGY DEVELOPMENT

Jennifer Aizenman
Adrian Arva
Scott Bronson
Uwe Hilgert

With the entire human DNA sequence now "in the bag," ahead lies the task of translating this trove of information into healthier and happier lives. We still need to find the genes behind many common, chronic diseases that stealthily sap productivity and shorten lifespan—including asthma, noninsulin-dependent diabetes, schizophrenia, and bipolar disorder (manic depression). Each of these "complex" disorders appears to involve multiple genes whose expression is further modified by environmental factors. Thus, we cannot expect a single "magic bullet" to treat these diseases. Rather, different patients will require different treatments to counter the specific gene changes that are at the root of their illness.

Prescribing the right gene-based remedy may require a precision that is generally absent from medicine today. Everyone must have taken pause at the paradox of a physician asking us if we are allergic to a particular drug. After all, shouldn't the doctor be the one to inform us of a potential problem? Unfortunately, trial and error is the only way to determine a patient's response to many drugs—it takes an allergic reaction to know you are allergic! Thus, the endgame of genetic medicine is pharmacogenetics—predicting drug response and tailoring treatment to each person's genetic makeup.

The term "pharmacogenetics" was first coined in 1959 by Friedrich Vogel to describe inherited drug responses that vary between population groups. For example, some African American soldiers serving in Italy during World War II had severe reactions to the antimalaria drug primaquine. Later work showed that inherited defects in so-called metabolic enzymes—the cytochrome P450 monooxygenases (CPY450s)—are responsible for many adverse drug responses. In the liver, the CPY450 enzymes convert many drugs to their bioactive forms. It is estimated that mutations in CPY450 genes lead to poor or toxic metabolism of more than half of the common drugs—including albuterol, codeine derivatives, β-blockers, monoamine oxidase inhibitors, antihypertensives, tricyclic antidepressants, antipsychotics, neuroleptics, and Prozac—as well as a similar proportion of anticancer drugs. Prevalence of mutations in different CPY450 genes varies greatly, affecting as few as several percent of people in one population or as many as 20% in another.

Affymetrix produces a GeneChip® that can assess common mutations in all of the major CPY450 genes. However, until such assays become standard in medical care, people will continue to be guinea pigs in experiments with increasingly powerful drugs. This is especially troubling in the case of psychoactive drugs, whose improper metabolism can send patients even deeper into psychosis.

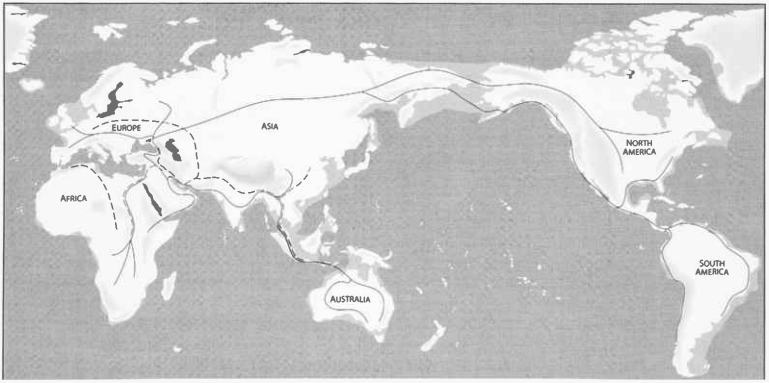
Each person's unique disease susceptibilities and responses to drugs are, in large part, the balance between our uniqueness as individuals and similarities we share with others in our historical population groups. Written in each person's DNA is a record of our shared ancestry and our species' struggle to populate the earth. Our ancient ancestors moved around, and eventually out of, Africa. They moved in small groups, following river valleys and coastlines, reaching Asia and Europe. Land bridges that appeared during recurring Ice Ages later allowed them to reach Australia and the Americas.

As these early people wandered, their DNA accumulated mutations. Some provided advantages

that allowed these pioneers to adapt to new homes and ways of living. Others were nonessential. Mutations are the grist of evolution, producing gene and protein variations that have allowed humans to adapt to a variety of environments—and to become the most far-ranging mammal on the planet. The same mutational processes that generated human diversity—point mutations, insertions/deletions, transposition, and chromosome rearrangements—also generated disease.

It may be hard to see from our current vantage point, but the entire industrial revolution has occupied only about 0.1% of our 150,000-year history as a species. The cradles of western civilization—classical Greece and Rome—take us back into only 2% of our history. The earliest city-states of Mesopotamia, Babylonia, Assyria, and China take us back only 4% of the way into our past. At 7%, we reach the watershed of agriculture, which changed forever the way humans would live and work. After language, the domestication of plants and animals is the single greatest civilizing factor in human history. Increased production and performance of domesticated organisms made possible urbanization and task specialization in human society. Thus, the labor of fewer and fewer farmers produced enough food and clothing materials to satisfy the needs of growing numbers of nonfarmers—artisans, engineers, scribes, and merchants—freeing them to develop other elements of culture. Reaching back the remaining 93% of our history, to the dawn of the human species, we lived only as hunter-gatherers.

Throughout most of human history, the hunter-gatherer group was the basic population unit upon which evolution acted. These small populations were subject to the founder effect, inbreeding, and genetic drift (a random fluctuation of nonessential alleles). Over millennia, these effects join with selection to concentrate particular gene variations within different population groups. The fastest evolving part of our genome, the mitochondrial control region, accumulates about one new mutation every 20,000 years. Mutations are five- to tenfold less frequent in most regions of the nuclear chromosomes. Thus, virtually every gene in our genome is, on average, only one or two mutation events away from our hunter-gatherer heritage. Our genomes preserve the genetic residue of a time when all human beings lived in small, cohesive groups. Our basic anatomy, physiology, and many aspects of behavior are essentially identical to the hunter-gatherers who ranged through the ancient landscapes of Africa, Europe, Asia, Australia, and the Americas. These ideas can substantially broaden our understanding of the genetic basis and treatment of human disease.

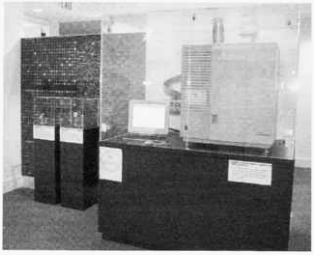


Ancient humans of the species *Homo erectus* left Africa 1.7 million years ago, reaching Europe and Asia (dashed lines). Groups of *Homo sapiens* left Africa about 70,000 years ago (solid lines). These groups replaced any remaining ancient populations, reaching Asia and Australia about 60,000 years ago and entering Europe about 45,000 years ago.

Enabling Students to Explore Our Shared Genetic Heritage

Over the past four years, we have developed a unique program that allows students to use their own DNA as a starting point to investigate the related concepts of pharmacogenetics and human variation. Using a laboratory kit developed by the DNA Learning Center (DNALC) and distributed nationwide by the Carolina Biological Supply Company, students isolate DNA from hair roots or cheek cells. Their DNA is mixed with freeze-dried polymerase chain reaction (PCR) reagents to amplify (clone) a highly variable region of their mitochondrial genome. The amplified samples are then shipped to the DNALC, where student interns perform the final DNA sequencing reactions. In addition to our national program, we now offer DNA sequencing as a lab field trip for local school districts at both the Cold Spring Harbor DNALC and the DNALC *West* in Lake Success.

During the year, our *DNA Sequencing Service* processed over 3400 samples submitted by 95 high schools, 44 universities/colleges, and nine community colleges. As a testament to the growing popularity of this free service, the number of samples processed this year has grown over 70% from last year. DNA sequences are uploaded to the *Sequence Server* database on our *BioServers* WWW site, where they can be used to perform a number of analyses, including BLAST searches and CLUSTAL sequence alignments. The curriculum focuses on evolutionary comparisons with other students' DNA, modern human DNA from around the world, ancient human DNA, and Neandertal DNA. Visits to the *BioServers* WWW site tripled from 30,332 in 2001 to 89,677 in 2002.



The ABI 377 DNA sequencer is a working exhibit at the DNALC.

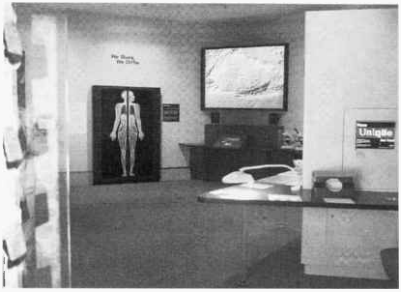
We are grateful to Frank Stephenson at Applied Biosystems for allowing the DNALC to increase the size of our program while continuing to provide the service free of charge. Applied Biosystems provides costly experimental reagents and critical technical assistance for maintaining our ABI 377 DNA sequencing machine. Lori Grady and Jodi Barditch in Tim Tully's lab and Ray Preston at the CSHL Cancer Genome Research Center provide technical support for the program.

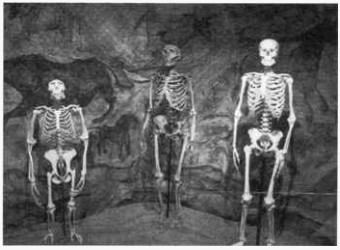
The Genes We Share

In September, we launched our tribute to the Human Genome Project, a new exhibition entitled *The Genes We Share*. The 2000-square-foot exhibit takes a global look at the incredible genetic similarity of all human beings, as well as the differences that make each person unique. Visitors are encouraged to view the human genome as a record of our shared ancestry, an instruction manual for our bodies, and a source of information that can foreshadow a person's future health.

In our main hall, visitors have the opportunity to look at their similarities and differences on both an individual and a population level. An interactive area encourages visitors to compare characteristics that make them unique—such as tongue-rolling ability, hairiness, eye color, and other physical traits. Shifting focus even closer, microscopic footage and high-resolution animations have been used to highlight the body structures and biological processes that we all share. DNA and personality profiles of identical twins from Long Island, Matt and Danny, explore the relationship between nature (DNA) and nurture (environment).

After this personal view, the focus shifts to the level of human populations and how genetic differences have evolved between them. A wall-sized, interactive world map depicts the migration paths of our earliest ancestors and illustrates some of the environmental factors that influenced human evolution. An interactive table in the shape of a mitochondrion allows visitors to explore the use of DNA to study human evolution and trace our ancestry. An adjacent gallery is given over to a recreation of a Paleolithic cave in southern Europe. Here, skeletons of a chimpanzee, modern human, and Neandertal





The Genes We Share exhibition.

encourage visitors to consider the genetic and anatomical changes that set humans apart from other primates. Prehistoric paintings, as well as Neandertal and Cro-Magnon burials, encourage visitors to think about the earliest evidence of unique human behaviors and self awareness.

The interpretation of the human DNA sequence is introduced by an interactive exhibit, "Stories in Our Genes," in which Matt Ridley presents a guided tour through the human chromosomes—based on his popular book, *Genome: Autobiography of a Species in 24 Chapters*. An eight-foot-tall adaptation of the original metal DNA model constructed in 1953 by Francis Crick and James Watson represents the beginning of our quest to understand the "book of life." A working DNA sequencer, operating daily to sequence the DNA submitted by student classes from around the United States, illustrates more recent advances in DNA technology. Finally, exhibits on DNA "chips" and gene therapy give the visitor an opportunity to ponder how DNA will impact their future lives and health.

The exhibit received notable visitors in 2002, including Prince Andrew, the Duke of York, and the actor William Hurt. In October, scientists attending the *Human Origins and Disease* meeting at Cold Spring Harbor Laboratory visited the exhibit, and were particularly keen to see the Neandertal skeleton reconstruction, the first ever displayed in any museum. Experts included Dr. Chris Stringer from the Natural History Museum in London, and Dr. Svante Pääbo, whose team isolated the first DNA from ancient Neandertal bones. Funding for *The Genes We Share* was provided by the Richard Lounsbery Foundation, The William A. Haseltine Foundation for Medical Sciences and the Arts, Tularik Inc., and Laurie J. Landeau, V.M.D.

DNA Interactive for DNA's 50th Birthday

As part of the many activities that will mark the 50th anniversary of the discovery of the structure of DNA, the DNALC took on the challenge of developing a WWW site to parallel a five-part television series,

DNA: The Secret of Life, due to air in spring 2003. The WWW site, *DNA Interactive (DNAi)*, is funded by a grant from the Howard Hughes Medical Institute. *DNAi* offers visitors a unique multimedia resource through which to learn about the history and impact of DNA science.

www.dnai.org

Many of the 18 instructional modules in *DNAi* make use of a "4-P" structure, which allows students to "discover" key principles of DNA science. ***Problem*** introduces the question at hand and provides key background information. ***Players*** uses video clips to introduce the views and approaches of the principal scientists working on the problem. ***Pieces of the Puzzle*** uses animations to illustrate experiments that provide clues to solving the problem. ***Putting It Together*** uses animations, videos, and/or interactive games to synthesize the answers to the problem.

By far the most ambitious project yet attempted by our *BioMedia* Group, the site incorporates over five hours of video footage gleaned from interviews with over 70 scientists—including ten Nobel Laureates. More than 150 animations illuminate key experiments in the history of DNA and bring to life the molecular processes that govern DNA replication and expression. A ten-person team at the DNALC coordinated with producers at the Walter and Eliza Hall Institute in Melbourne, Australia, and at three companies in London (Windfall Films, RGB Post, and The Mill). The Mill won an Academy Award for visual effects in *Gladiator* and is noted for creating much of the wizardly magic in the Harry Potter movies.

DNAi is built using Flash MX, an integrated WWW platform released by Macromedia in summer 2002. Flash MX gives us the flexibility to easily integrate different media types—text, animations, and video—into a unified page design. Flash MX has the great advantage of playing its own formatted video files, allowing for seamless integration of a video player into the web page itself. Thus, the *DNAi* site requires a single plug-in, Flash 6, for both animation and video playback, without the need for an additional RealPlayer or Quicktime plug-in. We also developed a video window that incorporates a scrollable caption—aiding the hearing-impaired, improving intelligibility of speakers, and allowing quotations to be cut and pasted into word processing documents.

Although it employs the latest technology, the site provides a satisfying multimedia experience for broadband and dial-up users alike. We devoted considerable effort to optimizing file size and video compression to minimize loading time and provide improved performance compared to sites of similar complexity. Flash formatting allowed us to achieve 100-fold compression of video files—with surprisingly little loss of quality. The video player provides three viewing options, which ensures smooth operation for all users. At an average dial-up speed of 40 kbps, the largest pages and multimedia files load in a maximum of 30 seconds, with smaller files loading in 15 seconds or less.

The site will also include a *Lesson Builder* that will allow teachers to build their own multimedia "power point"-style presentation using resources from the *DNAi* WWW site. All animations, videos, photos, and narratives will be keyword-tagged and accessioned in a database. Registered teachers then can use keywords to search the database for items of interest. After previewing material generated by the search, a lesson is created simply by dragging the desired items into a linear "filmstrip." Each lesson is stored under a teacher's profile, and a URL of the lesson can be shared with fellow teachers or students in a class.

Conclusion of Five Years of Macy Support

In the fall, we completed the final year of a five-year program to develop the companion WWW sites *DNA From the Beginning* (*DNAFTB*, http://www.dnaftb.org) and *Your Genes, Your Health* (*YGYH*,

www.dnaftb.org

www.ygyh.org

http://www.ygyh.org). These sites are specifically designed to provide students and families with the basic information they need to understand the science behind genetic disorders. Visitors to these popular sites doubled, from 804,296 in 2001 to 1.67 million in 2002.

In funding this project, the Josiah Macy, Jr. Foundation helped establish our *Biomedia* Group, a multidisciplinary team of science, communication, and design professionals. The program was among the first initiated by June Osborn, when she became Macy Foundation President after serving as Dean of the School of Public Health at the University of Michigan. Macy chairman Clarence Michalis and board member David Luke were also instrumental in a 1987 grant, which helped establish the DNALC's instructional programs.

DNAFTB was completed in November 2000 and is currently the first site listed on a search for "DNA" using Google, the web's most popular search engine. The site is organized around 41 key concepts that span one and a half centuries, beginning with basic principles of Mendelian genetics (1865) and ending with current techniques on targeted gene knockouts. The work of over 90 scientists, including 32 Nobel Laureates, is highlighted in animations and interviews.

In November 2001, we produced a CD-ROM that makes *DNAFTB* available to a much broader audience—allowing those who lack an Internet connection to use the work at school or at home, as well as providing quick operation and full-motion video to those with slow Internet connections. Sales of the CD-ROM have generated enough income to consider producing other-language *DNAFTB* CD-ROMs and *YGYH* CD-ROMs. *DNAFTB* is currently being translated into three languages: German, Traditional Chinese, and Icelandic. These translated versions will be mirrored here at the DNALC, as well as hosted at the German, Taiwanese, and Icelandic sites.

YGYH was completed in November 2002. The site is specifically targeted at patients and families who are looking for understandable information about a specific genetic disorder. *YGYH* is organized according to questions visitors may have about the disorder: What is it? What causes it? How is it inherited? How is it diagnosed? How is it treated? What is it like to have it? Where can I get more information?

The site focuses on 15 disorders, which were chosen using three criteria: high incidence rate, known genetic cause, and severity of the phenotype (symptoms). In each case, we enlisted the participation of the genetic foundation or organization for information and access to patients and/or physicians for video interviews. Each disorder comprises a number of resource pages that provide in-depth information. The first "page" provides quick facts for casual browsing. Subsequent pages include detailed animations to help visitors visualize the unseen world of genes and molecules and explain the biology of the disorder. Video interviews with researchers and patients provide insiders' views on genetic disorders. Links help users find support groups and additional information.

Eugenics Image Archive

The *Image Archive on the American Eugenics Movement* web site continues to be a popular resource for students and faculty alike. In 2002, the *Archive* received 187,263 visitors, more than double the visits in the previous year.

www.eugenicsarchive.org

Early in the year, we set to the task of editing the 1000 new images collected in the United States and England since the launch of the site in February, 2000. At a May meeting, the working group of Steve Selden, Gar Allen, Elof Carlson, and Paul Lombardo helped prepare the captions for the completed images. Pending final edits, these new images should become available in spring 2003. Additional images were collected during a visit to the Max Planck Society Historical Archives in Berlin, Germany—including images of Otmar Freiherr von Verschuer and his twin studies.

The working group, along with Barbara Biesecker (National Human Genome Research Institute) and David Goldman (National Institutes of Health), contributed their expertise by speaking in May at the second of three Banbury meetings, *American Eugenics and the New Biology: Perspectives and Parallels*. This meeting aims to familiarize "opinion leaders" about this dark saga in American science. The meet-

ing drew 26 participants from diverse fields, including family genetics, education, ethics, journalism, government, industry, and philanthropy. The third of the Banbury meetings will be held in 2003.

May 2, 2002 marked an important date in eugenics history: the 75th anniversary of the Supreme Court decision that upheld the concept of eugenic sterilization for people considered genetically "unfit." This famous court case, "Buck v. Bell," was the focus of a feature article that was included on the *Archive* web site as well as on *Gene Almanac*. In the fall, we began production on a multimedia module to provide an in-depth look at the Buck v. Bell case. Here, we enlisted extensive help from our friend Paul Lombardo, a legal historian who has devoted his career to unraveling the truth behind Buck v. Bell. With a PBS film crew, we followed Paul on a virtual "pilgrimage" of key sites in the Buck v. Bell story: the Virginia Colony for the Epileptic and Feeble Minded, in Lynchburg, where protagonist Carrie Buck and her mother Emma were incarcerated; the Amherst County Court House, where the case was first heard, before going to the U.S. Supreme Court; and the Venable School, in Charlottesville, where Carrie's child Vivian was a solid student before her early death. Dr. Lombardo's analysis of this nadir in U.S. jurisprudence was filmed against the backdrop of the rotunda of the University of Virginia, designed by Thomas Jefferson, and widely considered a symbol of the American dream for human rights. Clips from the interviews will be featured at the *Eugenics Archive* and on the new *DNA Interactive* site.

Inside Cancer

Coming in 2003.

Inside Cancer, funded by the National Institutes of Health, Science Education Partnerships Award (SEPA) program, is a multimedia web site under development that is geared toward the general public, especially teachers and students. It will be a resource for people who want authoritative information on the workings of a cancer cell. Animations and video interviews with cancer researchers and other experts will help people understand the complex science and issues of cancer.

Inside Cancer will feature five modules: *What Is Cancer?* emphasizes cancer as a disease and shows how cancers develop from a single cell; *Causes & Prevention* identifies behaviors and environmental factors that increase cancer incidence; *Diagnosis & Treatment* shows how oncologists diagnose cancers, the types of treatment options available, and how they work; *Cancer in the Laboratory* introduces major cancer researchers and the importance of their discoveries; *Pathways to Cancer* is a three-dimensional tour of a cell that focuses on the signaling pathway. Disruption of the pathway can lead to irregular cell growth and cancer. Each module is subdivided into an Overview and relevant content segments. The Overview will be the "highlight reel," and will introduce visitors to more in-depth content.

Major development effort has focused on creating high-resolution, three-dimensional animations of a cell's signaling pathway for the *Pathways to Cancer* module. A pathway initiated by platelet-derived growth factor (PDGF) was chosen because it provides an opportunity to illustrate the key points at which cellular growth control can be lost during oncogenesis. The importance of protein products of proto-oncogenes, c-*sis*, *ras*, c-*fos*, c-*jun*, and the role of phosphorylation in the regulation of protein function are illustrated. Since the PDGF receptor is a target for the new Novartis drug, Gleevec™, the pharmacological action of cell-signaling inhibitors can also be stressed. We look forward to launching *Inside Cancer* late in 2003.

Setting up Sister Institutions to the East, West, and North

East In July, we signed a three-year contract with the Ministry of Education (MOE) to use the DNALC model to support expansion of life sciences education in Singapore. The project is part of a national thrust to make biotechnology the "fourth pillar" of Singapore's future economy. With a centrally controlled school system the size of metropolitan Chicago, Singapore potentially provides the first large-scale, integrated deployment of lab-based instruction in genetics and molecular biology. Two years

The DNA Learning Center *West* opened its doors in June, 2002.

ago, there was essentially no hands-on biotechnology instruction at the precollege level in Singapore—precisely where the United States was in 1988 when we started the DNALC.

In addition to the DNALC project, significant resources are flowing into the school system through both MOE and the Agency for Science, Technology, and Research (A*STAR). Over the past two years, virtually every high school has been equipped with basic equipment for DNA analysis—including PCR machines, which have proved the stalling point for many American high schools. Recent government studies have pointed to the need to liberalize some elements of the highly structured school system. Teachers are being encouraged to experiment with new courses and enrichment activities. Thus, all the factors seem right to create in Singapore a superb model for preparing students to live, work, and fruitfully participate in the gene age.

The project got off to a fast start, with DNALC staff conducting three weeks of training in Singapore for middle- and high-school faculty. Twelve Singaporean teachers then participated in three-week "attachments" in the United States, attending DNALC workshops here and in Boston. By year's end, four teaching labs and a bioinformatics lab modeled after the DNALC were under construction at the Singapore Science Center (SSC), which will focus on student enrichment, and the National Institute of Education (NIE), which will focus on teacher training. Complete mirrors of the DNALC WWW sites were also installed at NIE (http://dnalc.nie.edu.sg) and the Singapore Bioinformatics Institute (http://dnalc.bii.a-star.edu.sg).

West With the strong support of CSHL trustee Arthur Spiro, we collaborated with the Research Institute of the North Shore–Long Island Jewish (NS–LIJ) Health System to establish a DNA Learning Center *West* in Lake Success, New York. The new center, which opened in June, includes a genetic/biochemistry lab, prep lab, and office. Lab field trips at *West* include a tour of the adjacent Clinical Core Laboratory. The objective of the new center is to extend DNALC services to schools in Western Nassau County, Brooklyn, Queens, and Manhattan. Toward this end, five weeks of student workshops were conducted during the summer, and field trips during the first half of the academic year brought total attendance to 700 during the first year of operation.

North In October, we commenced collaboration with the Science Center of Eastern Connecticut, in New London, to update their instructional activities with labs and multimedia experiences in molecular genetics. This was part of a reorganization from which emerged the Science Epicenter & DNA Learning

Center (SE DNALC). The objective here is to serve Connecticut school systems located directly across Long Island Sound from us. Together with DNALC *West*, SE DNALC potentially will extend our influence in an arch around Long Island Sound, serving much of the major population center of the Northeast. The project is of special interest to Pfizer, Inc., whose central research division is located in nearby Groton.

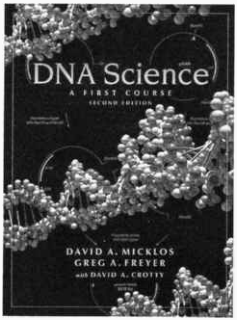
Technology transfer (including the Singapore, NS–LIJ, and Science EpiCenter collaborations; sponsored research by the Carolina Biological Supply Company; and royalties on teaching kits and CDs) contributed about $365,000 in annual revenue, or about 15% of the 2002 operating income. We are exploring additional licensing collaborations with Roberson Museum and Science Center in Binghamton, New York, and the city of Leipzig, Germany.

DNA Science, 2nd Edition

By year's end, the second edition of our popular lab text, *DNA Science*, was in press. The 2nd edition preserves the successful formula of the 1st edition: one part well-tested laboratories and one part insightful, explanatory text. First circulated in mimeographed form in 1988 and formally published in 1990, this book was largely responsible for bringing DNA experiments within reach of advanced high school and beginning college students.

The core laboratory sequence, developed by Dave Micklos and Greg Freyer in the laboratory of CSHL Nobel Laureate Rich Roberts, introduces the basic techniques of DNA restriction, transformation, isolation, and analysis—and then applies these techniques to the construction and analysis of a simple recombinant DNA molecule. We resisted the temptation to tinker very much with the laboratories, since they are the best-tested and most widely used teaching labs available on the basic techniques of gene manipulation. Two new labs focus on gene products: a colorimetric assay for the activity of β-lactamase (the enzyme produced by the ampicillin resistance gene) and expression and purification of green fluorescent protein.

The text portion has been entirely reorganized and updated, increasing from 200 to 300 pages. As before, the narrative takes students behind the scenes of modern research to show them the evolution of concepts and methods. The first three chapters cover essential principles of genetics, and DNA structure and function. The next three chapters introduce small- and large-scale methods for analyzing DNA, culminating in the race to sequence the human genome and new methods for working with hundreds of genes simultaneously. The final two chapters focus on the applications of molecular techniques to understand cancer, human variation, and our emergence as a species. The human genetics chapter also contains the first substantial treatment of American eugenics available in a general biology text.



Although much has changed in biology since the first edition, the ideas and techniques in this book are still the minimum requirements for any degree in DNA manipulation. We hope that *DNA Science* continues to provide a simple roadmap for beginning an exploration of the molecule of life—one that will take on added importance as more and more biology teachers around the world realize the value of giving students the freedom to get their hands dirty with DNA.

HHMI Bioinformatics

The free publication of genome sequences and bioinformatics tools offers students and teachers the unprecedented opportunity to work with important biological data at the same time and with the same tools as researchers. With funding provided by the Howard Hughes Medical Institute (HHMI), we continued to encourage students and teachers to make use of this trove of data. Our HHMI program intro-

duces students and faculty to genomic biology and bioinformatics using a mobile, networked computer laboratory. Central to the program is *VectorNet*, a stand-alone, portable computer laboratory consisting of 12 laptops and a laptop server. The server mirrors the entire DNALC *Gene Almanac* site, including bioinformatics tools and *GenBank* data sets.

The student component of the HHMI project, *New York City Genes*, reached 1662 primarily minority students. Working in collaboration with CUNY's *Gateway Institute for Pre-College Education*, *VectorNet* was rotated to Erasmus High School (Brooklyn), Science Skills Center High School (Brooklyn), the High School for the Humanities (Manhattan), and John F. Kennedy High School (Bronx). The availability of a set of networked computers in the biology classroom allowed students to move between lab experiments and computer analysis of their own DNA polymorphisms. *VectorNet* saw intensive use in Community School District 29, where students worked on our multimedia DNA forensics activity, *The Mystery of Anastasia*.

During the year, *VectorNet* was used to train 355 biology educators. Of these teachers, 235 attended short-term workshops at in-services and national conventions; the remaining 122 attended one of six-week-long *Vector Bioinformatics* workshops held at sites across the nation:

- Contra Costa County Office of Education, Pleasant Hill, California
- Foundation for Blood Research, Scarborough, Maine
- National Center for Biotechnology Information, Bethesda, Maryland
- Southwest Foundation for Biomedical Research, San Antonio, Texas
- Stowers Institute for Medical Research, Kansas City, Missouri
- Whitehead Institute for Biomedical Research, Cambridge, Massachusetts

During the workshop, participants make extensive use of computer tools to learn principles of gene analysis, including DNA sequence annotation, gene structure and regulatory elements, gene families and whole-gene analysis, functional genomics and DNA arrays, and gene discovery using SNPs and other markers. Participants also amplify two DNA polymorphisms and use their own data as a starting point to investigate DNA data sets, population genetics, human origins, and disease mechanisms. This illustrates the crossover between DNA experiments done *in vitro* (test tubes) and *in silico* (computers).

At the workshop WWW site, we continued development of lesson plans and user-friendly bioinformatics tools for the analysis of DNA—such as nucleotide counters, a start/stop-codon searcher, and a restriction site finder. The web site currently logs about 1000 visitors per month. DNALC staff also joined CSHL's Meetings and Courses department in developing and teaching a two-day bioinformatics course for research scientists. During 2002, 142 researchers from around the country participated in *The Genome Access Course* (*TGAC*), which was conducted five times at the CSHL Woodbury Cancer Genome Research Center.

Leadership Institute in Human and Molecular Genetics

Modeled after the intensive graduate training programs held at CSHL, the Pfizer-funded *Leadership Institute* provides high-level training for a select group of high school faculty. Known also by its nickname, *DNA Boot Camp*, the 2002 program brought together 18 exemplary high school teachers from across the United States, as well as four educators from Singapore. During the intensive three-week workshop, participants performed laboratories from the DNALC's new module in human and plant genomic biology, made extensive use of the computer laboratory as they investigated bioinformatics, and worked on independent projects. They shared lodging and dining facilities with CSHL staff and visiting scientists, and spent the weekends exploring Long Island and Manhattan. This eclectic group of lead teachers represented 16 states, from the urban Northeast, to the rural South, to the Southwest and Northwest.

New NSF Plant Initiative

Plant molecular genetic and genomic research still lag behind medically oriented research on microbes and higher animals. As a result, relatively few lab experiences that expose students to the growing insights into plants offered by genomic biology are available at the lower college level. So we were

Wild type (left) and mutant *clf-2* (right) of the model plant *Arabidopsis thaliana.*

happy when, in December, the National Science Foundation (NSF) funded our proposal to develop a laboratory- and Internet-based curriculum to bring college students up to the minute with modern plant research. This proposal clearly struck a common chord with the six reviewers, all of whom rated it as "excellent." This is the first time in 15 years that any proposal we have submitted has received such a unanimous vote of confidence from NSF reviewers.

The project is based largely on data emanating from plant research at CSHL. A comprehensive set of laboratories will be based on rapid and reproducible PCR chemistry developed under a previous NSF grant. Using the model plant *Arabidopsis* and important food crops, the laboratories illustrate key concepts of gene and genome analysis, including the relationship between phenotype and molecular genotype, genetic modification of plants and detection of transgenes in foods, and linkage and bioinformatic methods for gene mapping. Students will also have the unique opportunity to explore functional genomics by assisting CSHL researcher David Jackson with the cellular analysis of *Arabidopsis* genes of unknown function. An Internet "super site" will support the laboratories with online protocols, custom analysis tools, shared databases, and collaborative bulletin boards.

The project will be kicked off in June 2003 with a focus workshop of faculty advisors drawn from two- and four-year colleges representing six regions of the United States. During the dissemination phase, in 2004–2005, faculty advisors will organize week-long training workshops to reach 144 instructors.

Student Instruction

In 2002, we brought our newly enlarged facility into full operation—including three teaching labs and a computer lab. With an expanded menu of lab field trips, we provided lab experiences for more than 14,000 middle- and high-school students—an increase of 30% over 2001. We also marked an exciting milestone, with 100,000 students having participated in lab experiments at the DNALC since the opening of our first laboratory in 1988. Although our programs are targeted primarily at school groups, we also welcomed 6000 members of the general public, who combine a visit to *The Genes We Share* exhibit with *Long Island Discovery*, Cablevision's multimedia production that chronicles the colorful history of Long Island.

Genetics as a Model for Whole Learning (GMWL), our program of in-school instruction and lab field trips for 5–7th graders, reached over 17,000 students in 40 school districts and private schools on Long Island. Field trips to the DNALC combine lab work—such as extracting DNA or transforming DNA into bacteria—with a tour of our new museum exhibit, *The Genes We Share*. The multimedia exploration *Anastasia: Dead or Alive?* continues to be popular for field trips, but faculty are also using the WWW version in their own classrooms.

During the year, we renewed a contract with the New York City Board of Education to provide the *GMWL* program to more than 1800 minority students in Community School District #29 in Queens. We continued our ongoing partnership with CUNY's *Gateway Institute for Pre-College Education*, which

provides enrichment to prepare minority high school students for success in higher education. We provided *gratis* lab field trips for 450 *Gateway* students and minority students from Central Islip High School, Elmont Memorial High School, Theodore Roosevelt High School, Martin Van Buren High School, and Midwood High School.

Founded in 1985, the DNALC's *Curriculum Study* program remains the nation's oldest and largest coordinated effort in the country to bring molecular biology and recombinant DNA technology into the science classroom. Our 38 member districts include both public and private schools in Suffolk, Nassau, Queens, the Bronx, and Manhattan. In 2002, we welcomed three new members to the *Curriculum Study* Program: Kings Park Central School District, Fordham Preparatory School, and North Shore Hebrew Academy High School.

Great Moments in DNA Science, the *Curriculum Study* Honors Student Seminars, attracted 467 area high school students during three evenings in April. The speakers, and their topics of discussion, were:

- Peter Mombaerts, The Rockefeller University: Cloning and Embryonic Stem Cells
- Vivek Mittal, Cold Spring Harbor Laboratory: Exploring Cancer with DNA Microarrays
- Maureen O'Leary, Stony Brook University: The Origins of Whales: Discovering an Evolutionary Transition from Land to Sea Using Molecules and Bones

The summer proved to be our busiest and most productive workshop season to date. We conducted 29 student workshops at the DNALC and DNALC *West*, reaching a total of 676 students—a 34% increase over 2001 attendance. Off-site, we supported *gratis* workshops for 72 minority students at Central Islip High School (Suffolk), Wyandanch High School (Suffolk), Brooklyn Technical High School (Brooklyn), and John F. Kennedy High School (Bronx).

Staff and Interns

The DNALC bid farewell to several staff members in 2002. Maureen Cowan left her position as a middle school educator to pursue a career as a chemistry teacher at St. Mary's School in Manhasset. Our talented multimedia designer Wen-Bin Wu returned to Hong Kong to run his family's import-export business. Hong Zhou departed as manager of our *DNA Sequencing Service*.

The ranks of the instructional group were swelled by the arrival of Erin Maroney, Kimberly Kessler, and Michael O'Brien. Erin has a degree in plant and soil science from the University of Vermont (2001). She teaches and helps administer the middle school program. With a degree in biology from Boston College (2002), Kimberly manages our after-school intern program, in addition to teaching middle and high school students. Michael graduated from SUNY at Albany in May 2002 with a degree in Biochemistry and Molecular Biology. In addition to teaching high school labs, he manages the *DNA Sequencing Service*. Kimberly and Michael are both "alumni," having visited the DNALC as high school students. Kimberly performed labs here as part of SUNY Stony Brook's *Women in Science and Engineering*. Michael was a student of Fred Gillam at Sachem High School, who was among the first high school faculty to implement the *DNA Science* curriculum.

The *BioMedia* Group continues to thrive, and has nearly doubled in size in response to recent ambitious projects, including *DNA Interactive* and *Inside Cancer*. Thus, we were delighted to welcome three new members to our design staff: Eun-Sook Jeong, Darius Farraye, and Karwai Pun. With Master of Art degrees from Long Island University at C.W. Post and Honglk University in Seoul, Eun-Sook has crossed several continents in her career as an interior designer and multimedia artist. A native of Korea, she began her career at the DNALC as a summer intern while finishing work toward a master's degree. Darius has a strong background in multimedia web design and received a degree in Interactive Design from The Pratt Institute in 2002. Karwai joined us after having received degrees in art history and multimedia design, and working for an Internet design firm in Norway. A native of Long Island, she also visited the DNALC with her Northport High School biology class.

Fund-raising at the DNALC got a boost in 2002 with the addition of Erin Wahlgren as Senior Development Officer. Erin graduated from Simmons College in Boston with a double major in English and

Art History, and went on to hold development positions at the New York Public Library and Old Westbury Gardens. Although Erin reports to the Development Department, she is an integral part of the DNALC team.

Our high school intern program continues to offer students the opportunity to conduct independent research projects under the direction of Scott Bronson and Jennie Aizenman. Michelle Louie and Dan DeRoulet researched techniques for tagging *Arabidopsis* genes with the jellyfish gene encoding green fluorescent protein. Saroja Bangaru spent the summer with CSHL scientist David Jackson and studied the control of morphogenesis in plants, using maize as a model system. Caroline Lau was selected as an Intel finalist (international) for her work on striped bass population genetics. Jared Winoker and Kunal Kudakia researched human diversity using a combination of computer tools and techniques developed at the DNALC.

In addition to preparing reagents used in our teaching labs, interns play a critical role in our free *DNA Sequencing Service*. Lara Abramowitz, Sirish Kondabolu, and Jonathan Mogen (Half Hollow Hills High School), Eric Paniagua (Long Island School of the Gifted), Alex Hogg (Friend's Academy), Michelle Louie (King's Park High School), and senior intern Alina Duval (Hofstra University) worked together to meet the increasing demand for the DNALC's on-site sequencing service. Joining the intern program in 2002 were Pushpa Abraham (Kings Park High School), Lara Abraham (Half Hollow Hills High School), Jennifer Aiello (Kings Park High School), Christina Bezas (Huntington High School), Michael Casimir (Ward Melville High School), Daisy Choi (Cold Spring Harbor High School), Kimberly Izzo (Kings Park High School), David Wagman (Syosset High School), and Phillip Witkin (Syosset High School).

Bringing DNALC *West* online required recruiting and training a new group of high school interns: Robert Weintraub (Walt Whitman High School); Alinea Noronha (Herricks North High School); and Devin Chu (Archbishop Molloy High School).

The *BioMedia* Group was delighted to welcome Felix Hu back for the summer and Christmas breaks. Felix started his first term at Georgia Tech last year. Tracy Mak, a veteran *BioMedia* intern, also started her first college term last year at Cornell. Felix's sister Regina Hu (Northport High School) and Watson School graduate student Elizabeth Thomas continue to work for us during the year. Ariel Gitlin (Cold Spring Harbor High School) and Ryan Chiu (Horace Mann High School) started as new *BioMedia* interns. Ray Zhang (Elwood John Glenn High School) worked with us for the summer. Amelia Dorrer (C.W. Post) joined the team as a college intern. *BioMedia* interns help with a number of different tasks having to do with web site development, such as writing and editing scripts, animations, and web pages; designing new material; beta-testing web sites; and reviewing video clips.

Several art students took short-term positions at the DNALC to work on production of *The Genes We Share* exhibit, including Dana Liebowitz (Bennington College), Matt Mottola (New York Institute of Technology), Kerry Janney (C.W. Post), and Greg Furjanic (C.W. Post). Exhibit interns performed a range of tasks, such as drafting plans, sculpting and painting the human origins "cave," assembling display cases, filling blood bags with theatrical blood, and hand lettering signage.

The following interns left to pursue their scientific interests in college and/or research. We congratulate Marie Mizuno and Jonathan Mogen for their acceptance into the "Partners for the Future" program. Marie is currently working in the lab of Yuri Lazebnik researching apoptosis while Jonathan is working with Yi Zhong investigating neurofibromatosis 1 (*NF1*) and *presenilin* genes. Eric Paniagua (Long Island School for the Gifted) joined the lab of Jerry Yin to research molecular mechanisms involved in long-term memory. In August, we bid farewell to the following interns as they began their freshman year at the following institutions: Yan Liang Huang (Harborfields High School) University of Notre Dame, Caroline Lau (Syosset High School) Princeton University, Janice Lee (Oyster Bay High School) Boston University, Jarrett Linder (Half Hollow Hills High School) Cornell University, and Alex Witkowski (Cold Spring Harbor High School) SUNY Albany. Daniel DeRoulet (Columbia University) and Rebecca Yee (Wellesley College) used their summer breaks to assist with summer workshops and advanced research projects. Additionally, Andrew Diller left the DNALC to pursue a career in the fine arts and Wayne Chiang (Cold Spring Harbor High School) moved back to Taiwan with his family.

Dave Micklos

2002 Workshops, Meetings, and Collaborations

January 14–15	Site visit by Lisa Darmo, Elizabeth Paine, and Lawrence Wallace, Carolina Biological Supply Company, Burlington, North Carolina
January 17–19	*DNA Interactive* Advisory Board Meeting, Banbury Center and DNALC, CSHL
January 22	Site visit by Philip Batterhan, International Congress of Genetics, Melbourne, Australia
January 28	*New York City Genes* teacher training, York College, Queens, New York
January 28–31	The O'Reilly Bioinformatics Technology Conference, Tucson, Arizona
January 30	National Institute of Social Sciences Issues Discussion Group, Colony Club, New York, New York
February 1	Inter-School Exchange Reception
February 8	Site visit by Richard Riley, Clemson University, Clemson, South Carolina
February 19	Site visit by James Ravanis and Steven Spofford, Rindge School of Technical Arts, Cambridge, Massachusetts
February 19–22	Site visit by Phoon Lee Chaeng, Goh-Goh Poh Gek, Cheong Kim Fatt, and Lee Seng Hai, Singapore Ministry of Education; Anne Dhanaraj, Singapore Science Centre; and Lee Sing Kong, Singapore National Institute of Education
February 25	Site visit by Gail Carmack, UTeach Program, University of Texas-Austin
February 25–27	National Institutes of Health *Science Education Partnership* Award Meeting, Houston, Texas
February 26	Site visit by Regan Huff, McWane Center, Birmingham, Alabama
February 28	Site visit to Biogen, Cambridge, Massachusetts
March 6–8	National Institute of Standards and Technology, *Best Practices* Meeting, Gaithersburg, Maryland
March 7	Site visit by Bonnie Kaiser and Robert Schill, The Rockefeller University, New York, New York; and Catherine Rubin, EduChange
March 8	Site visit by Jennifer Chidsey and Paul Flagg, Ross School, East Hampton, New York
March 11	Exhibit development meetings with Mark Holterman, University of Illinois at Chicago; and David Teplica
March 11–13	Howard Hughes Medical Institute Undergraduate Program Review, Chevy Chase, Maryland
March 15–20	*DNA Interactive* editorial meeting, London and Cambridge, England
March 20–24	Howard Hughes Medical Institute, *Vector Bioinformatics* Workshop, Contra Costa County Office of Education, Pleasant Hill, California
March 27–30	National Science Teachers Association Annual Meeting, San Diego, California
April 3	*Your Genes, Your Health* interview, Dominick Sabatino, Nassau University Medical Center, East Meadow, New York
April 4	Site visit by Carson Powers, David Tesseo, Jean Caron, Phil Maniscalo, Martha Grossel, and Richard Hinman, Science Center of Eastern Connecticut, New London
April 9	*Your Genes, Your Health* interview, Anthony Cervo
April 14–16	National Institutes of Health ELSI conference, *American Eugenics and the New Biology: Perspectives and Parallels*, Banbury Center, CSHL
April 15	*Great Moments in DNA Science* Honors Students Seminar, CSHL
April 20–21	Presentation for *Genomic Revolution* exhibit opening, North Carolina Museum of Natural Sciences, Raleigh
April 23	*Great Moments in DNA Science* Honors Students Seminar, CSHL
	Your Genes, Your Health interview, Robert Desnick, Mount Sinai School of Medicine, New York, New York
April 24	National Institute of Social Sciences Board Meeting, Harvard Club, New York, New York
April 25	Presentation for Sayville High School Career Café Day
April 27	Presentation for Arbor Day Festival, Planting Fields Arboretum, Oyster Bay, New York
April 29	*Great Moments in DNA Science* Honors Students Seminar, CSHL
May 7–9	*DNA Interactive* filming, San Francisco, California
May 10	Site visit by Huanming Yang, Beijing Genomics Institute, China
May 13–15	National Human Genome Research Institute ELSI Project, *Eugenics Image Archive*, Editorial Advisory Panel Meeting, Banbury Center, CSHL
May 17	Site visit by Eugene Mitacek and Suzanne Ettinger, New York Institute of Technology, Old Westbury
May 21	*DNA Interactive* editorial meeting, DNALC
	Your Genes, Your Health interview, Edwin Kolodny, New York University School of Medicine, New York
May 22	Site visit by Don Colbert, SUNY Binghamton
May 28–June 1	Teacher training workshop, National Institute of Education, Singapore
May 29–30	Site visit by Janna Greenhalth and Pat Quinn, Science Center of Eastern Connecticut,

	New London
June 3–14	Teacher training workshops, National Institute of Education, Singapore
June 11	*Your Genes, Your Health* interview, Susan Fishbein, Late Onset Tay-Sachs Foundation, Glenside, Pennsylvania
June 13	Site visit by Kevin Seeley, Pall Corporation, Port Washington, New York
June 17–21	Howard Hughes Medical Institute, *Vector Bioinformatics* Workshop, Southwest Foundation for Biomedical Research, San Antonio, Texas
June 19	Josiah Macy, Jr., Foundation Meeting, DNALC
June 20	*Your Genes, Your Health* interview, Judith Willner and Randi Zinberg, Mount Sinai School of Medicine, New York, New York
June 24	DNA Learning Center *West* opening and reception, Lake Success, New York
June 24–28	Howard Hughes Medical Institute, *Vector Bioinformatics* Workshop, Stowers Institute for Medical Research, Kansas City, Missouri
June 25	Site visit by Frederick Seitz and Florence Arwade, Lounsbery Foundation
June 27–July 3	*Fun With DNA* Workshop, DNALC
	Fun With DNA Workshop, DNALC *West*
	World of Enzymes Workshop, DNALC
	DNA Science Workshop, DNALC
July 8–12	*Fun With DNA* Workshop, DNALC
	Fun With DNA Workshop, DNALC *West*
	World of Enzymes Workshop, DNALC
	DNA Science Minority Workshop, Central Islip High School, New York
July 8–26	Pfizer *Leadership Institute in Human and Molecular Genetics*, DNALC
July 9	*Your Genes, Your Health* interview, the McHale family
July 12	Site visit by Woodrow Wilson Biology Institute participants
July 15	Site visit by *Gateway Institute for Pre-College Education* administrators and teachers
July 15–19	*Green Genes* Workshop, DNALC
	DNA Science Workshop, DNALC
	DNA Science Minority Workshop, Wyandanch High School, New York
July 16	*Your Genes, Your Health* interviews, Michael Shelanski, Columbia University, New York, New York; and Thomas Winiewski, New York University School of Medicine, New York
July 19	Site visit by Mary Miller and Rob Semper, Exploratorium Origins Project, San Francisco, California
July 20–23	*DNA Interactive* editorial meeting, DNALC
July 22–26	*Fun With DNA* Workshop, DNALC
	DNA Science Minority Workshop, John F. Kennedy High School, Bronx, New York
	Genomic Biology & PCR Workshop, DNALC
July 26	Site visit by Matt Ridley, author and chairman of the International Centre for Life, Newcastle upon Tyne, United Kingdom
July 29–August 2	*World of Enzymes* Workshop, DNALC
	Green Genes Workshop, DNALC
	Genetic Horizons Workshop, DNALC
	DNA Science Workshop, DNALC *West*
	DNA Science Minority Workshop, Brooklyn Technical High School, New York
August 5–9	*Fun With DNA* Workshop, DNALC
	Fun With DNA Workshop, DNALC *West*
	World of Enzymes Workshop, DNALC
	Genomic Biology & PCR Workshop, DNALC
	Howard Hughes Medical Institute, *Vector Bioinformatics* Workshop, National Center for Biotechnology Information, Bethesda, Maryland
August 12	Site visit by Lawrence Scherr, North Shore–Long Island Jewish Health System
August 12–16	*Fun With DNA* Workshop, DNALC
	Green Genes Workshop, DNALC
	DNA Science Workshop, DNALC
	Howard Hughes Medical Institute, *Vector Bioinformatics* Workshop, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts
August 15	*Your Genes, Your Health* interview, Julie Zale
August 19–23	*Fun With DNA* Workshop, DNALC
	World of Enzymes Workshop, DNALC
	DNA Science Workshop, DNALC
	DNA Science Workshop, Cambridge, Massachusetts
	Howard Hughes Medical Institute, *Vector Bioinformatics* Workshop, Foundation for Blood Research, Scarborough, Maine

August 26–30	*Fun With DNA* Workshop, DNALC
	World of Enzymes Workshop, DNALC *West*
	DNA Science Workshop, DNALC
September 6–9	*DNA Interactive* editorial meeting, DNALC
September 18	*The Genes We Share* exhibit opening
	Site visit by Lisa Darmo, Charles Matthews, George Ross, and Lawrence Wallace, Carolina Biological Supply Company, Burlington, North Carolina
September 25	Site visit by Prince Andrew, Duke of York
September 26–29	*Museums, Media and the Public Understanding of Research* International Conference, Science Museum of Minnesota, St. Paul
September 27	Presentation for Science Center of Eastern Connecticut, New London
September 27–30	Multimedia Educational Resource for Learning and Online Teaching (MERLOT) International Conference, Atlanta, Georgia
October 3	Site visit by Tim Herman and Michael Patrick, Center for BioMolecular Modeling, Milwaukee School of Engineering, Wisconsin
October 6–8	*DNA Interactive* Advisory Panel Meeting, Banbury Center, CSHL
October 7	Site visit by Barbara Delatiner, New York Times
October 9	Site visit to the Carolina Biological Supply Company, Burlington, North Carolina
October 15	National Institute of Social Sciences Board Meeting, New York, New York
October 19–25	Planning and web site development meeting, National Institute of Education, Singapore
October 20	*Your Genes, Your Health* interview, Melissa Goldman
October 25	Site visit by Judith Kirk, Westmead Hospital, Sydney, Australia; and Joe Sambrook, Peter MacCallum Cancer Institute, Melbourne, Australia
October 30–November 2	National Association of Biology Teachers Annual Convention, Cincinnati, Ohio
November 5	*DNA Interactive* filming, Charlottesville, North Carolina
November 7–8	Site visit by Connie Barnes, Elizabeth Button, Diane Carpenter-Crews, Jim Moody, and Mary Sokolowski, SUNY Binghamton
November 13	Site visit by Kathleen Belton and Drew Bogner, Molloy College, Rockville Center, New York
November 14	Site visit by Colin Goddard, OSI Pharmaceuticals, and William Hurt, actor
November 18	Site visit by Daniela Gieseler, German public television
November 22	Site visit by Mary Miller, Exploratorium Origins Project, San Francisco, California
November 25	Site visit by Russ Hodge, European Molecular Biology Laboratory, Heidelberg, Germany
December 2–6	*DNA Interactive* editorial meeting, London, England
	Site visits to Max Planck Institute, Berlin and Leipzig, Germany
December 19	Site visit by Julie Clayton, *Nature* Magazine

Sites of Major Faculty Workshops 1985–2002

Key: High School **College** *Middle School*

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	**Foothill College, Los Altos Hills**	**1997**
	University of California, Davis	1986
	San Francisco State University	**1991**
	University of California, Northridge	**1993**
	Canada College, Redwood City	1997
	Pierce College, Los Angeles	**1998**
	California Lutheran University, Thousand Oaks	1999
	Laney College, Oakland	1999
	California State University, Fullerton	**2000**
	Salk Institute for Biological Studies, La Jolla	2001
	Contra Costa County Office of Education, Pleasant Hill	2002
COLORADO	Colorado College, Colorado Springs	1994
	United States Air Force Academy, Colorado Springs	**1995**
	University of Colorado, Denver	1998
CONNECTICUT	Choate Rosemary Hall, Wallingford	1987
DISTRICT OF COLUMBIA	**Howard University**	**1992,1996**
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
	CityLab, Boston University School of Medicine	1997
	Dover-Sherborn High School, Dover	1989
	Randolph High School	1988
	Winsor School, Boston	1987
	Boston University	**1994,1996**
	Whitehead Institute for Biomedical Research, Cambridge	2002
	Biogen, Cambridge	2002
MICHIGAN	Athens High School, Troy	1989
MISSISSIPPI	Mississippi School for Math & Science, Columbus	1990,1991
MISSOURI	Washington University, St. Louis	1989
	Washington University, St. Louis	**1997**
	Stowers Institute for Medical Research, Kansas City	2002
NEW HAMPSHIRE	St. Paul's School, Concord	1986,1987
	New Hampshire Community Technical College, Portsmouth	**1999**
NEVADA	University of Nevada, Reno	1992
NEW YORK	Albany High School	1987

	Bronx High School of Science	1987
	Columbia University, New York	1993
	Cold Spring Harbor High School	1985,1987
	DeWitt Middle School, Ithaca	*1991,1993*
	DNA Learning Center	1988–1995, 2001–2002
	DNA Learning Center	**1990,1992,1995,2000**
	DNA Learning Center	*1990–1992*
	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
	Orchard Park Junior High School	*1991*
	Plainview-Old Bethpage Middle School	*1991*
	State University of New York, Purchase	1989
	State University of New York, Stony Brook	1987–1990
	Titusville Middle School, Poughkeepsie	*1991,1993*
	Wheatley School, Old Westbury	1985
	US Military Academy, West Point	**1996**
	Stuyvesant High School, New York	1998–1999
	Trudeau Institute, Lake Saranac	2001
NORTH CAROLINA	North Carolina School of Science, Durham	1987
OHIO	Case Western Reserve University, Cleveland	1990
	Cleveland Clinic	1987
	North Westerville High School	1990
OKLAHOMA	School of Science and Mathematics, Oklahoma City	1994
	Oklahoma City Community College	**2000**
	Oklahoma Medical Research Foundation, Oklahoma City	2001
PENNSYLVANIA	Duquesne University, Pittsburgh	1988
	Germantown Academy	1988
SOUTH CAROLINA	Medical University of South Carolina, Charleston	1988
	University of South Carolina, Columbia	1988
TEXAS	J.J. Pearce High School, Richardson	1990
	Langham Creek High School, Houston	1991
	Taft High School, San Antonio	1991
	Trinity University, San Antonio	**1994**
	University of Texas, Austin	**1999**
	Austin Community College-Rio Grande Campus	2000
	Southwest Foundation for Biomedical Research, San Antonio	2002
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
WASHINGTON	**University of Washington, Seattle**	**1993,1998**
	Fred Hutchinson Cancer Research Center, Seattle	1999,2001
WEST VIRGINIA	Bethany College	1989
WISCONSIN	Marquette University, Milwaukee	1986,1987
	University of Wisconsin, Madison	1988,1989
	Madison Area Technical College	**1999**
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	2001–2002
SWEDEN	Kristineberg Marine Research Station, Fiskebackskil	1995

COLD SPRING HARBOR
LABORATORY PRESS

2002 PUBLICATIONS

SERIALS

Genes and Development, Volume 16, 1–3320

Genome Research, Volume 12, 1–2003

Learning and Memory, Volume 9, 1–442

Protein Science, Volume 11, 1–2980

Cold Spring Harbor Symposia on Quantitative Biology: Volume 66: *The Ribosome*

LABORATORY MANUALS AND HANDBOOKS

Protein-Protein Interactions: A Molecular Cloning Manual, Erica Golemis (ed.)

DNA Microarrays: A Molecular Cloning Manual, David Bowtell and Joe Sambrook (eds.)

Proteins and Proteomics: A Laboratory Manual, Richard Simpson

Manipulating the Mouse Embryo: A Laboratory Manual (3rd ed.), Andras Nagy, Marina Gertsenstein, Kristina Vinterstein, and Richard Behringer

Lab Ref: A Handbook of Recipes, Reagents, and Other Reference Tools Required at the Bench, Jane Roskams and Linda Rodgers (eds.)

TEXTBOOKS

Discovering Genomics, Proteomics, & Bioinformatics, A. Malcolm Campbell and Laurie J. Heyer

GENERAL INTEREST AND CHILDREN'S BOOKS

Ageless Quest: One Scientists Search for Genes That Prolong Life, Lenny Guarente

I Wish I'd Made You Angry Earlier: Essays on Science, Scientists, and Humanity, Max Perutz

Have a Nice DNA and *Gene Machines* Fran Balkwill and Mic Rolph (*Enjoy Your Cells* series)

Staying Alive: Fighting HIV/AIDS, Fran Balkwill and Mic Rolph

OTHER

Retroviruses CD, John M. Coffin, Stephen H. Hughes, and Harold E. Varmus

Essentials from Cells: A Laboratory Manual CD, David L. Spector and Robert D. Goldman

CSHL Annual Report 2001

Banbury Center Annual Report 2001

Watson School of Biological Sciences Annual Report 2001

Administrative and Financial Annual Report 2001

The journal publishing program

A selection of recently published books

COLD SPRING HARBOR LABORATORY PRESS

After a promising start, 2002 became a financially challenging year. A substantial decline in journal advertising sales, increased pressure for discounts from book resellers, and grant pressures on authors that delayed completion of important books all combined to produce financial results that were below expectation. Total income from our publishing program decreased by 7% to $9,050,700, and the net operating excess declined by more than 30%.

Nevertheless, Cold Spring Harbor Laboratory Press continues to make an important contribution to the Laboratory's educational mission, with an international publishing program that addresses three distinct audiences. Through research journals, scientific monographs, and laboratory manuals, the program assists the continuing professional education of working scientists and the training of graduate students for advanced degrees. In addition, textbooks are published for undergraduates. And a growing number of books are published for readers who are not technically trained but interested in biological research and its impact on medical practice and social policy. In 2002, each of these three audiences found something new to interest them from Cold Spring Harbor.

Books

A complete list of the 2002 publications appears opposite. Ten new books, one expanded paperback edition, and two CDs were published.

Professional scientists worldwide rely on the technical manuals published by the Laboratory, many of which have originated in practical courses taught here. More than 20 manuals are now available, including the 2001 edition of *Molecular Cloning* by Joe Sambrook and David Russell, the world's most widely used manual of DNA techniques, which continued to sell strongly throughout the year. New titles added in 2002 included the first editions of three manuals for important, emerging sets of technologies. *Proteins and Proteomics* by Richard Simpson provides an authoritative, protocol-based approach to proteomics. *Protein-Protein Interactions*, edited by Erica Golemis, presents methods for dissecting intracellular machinery. And in *DNA Microarrays*, the expertise of many scientists in this rapidly evolving field was welded into a coherent whole by the creative editing of David Bowtell and Joe Sambrook. Another new and important release was the third edition of the classic developmental biology manual *Manipulating the Mouse Embryo*, first published in 1982 and entirely revised and rewritten for this edition by Andras Nagy, Marina Gertsenstein, Kristina Vinterstein, and Richard Behringer.

Academic books in electronic form are slowly gaining popularity, and several new approaches to this medium were made during the year. The proteomics and microarrays manuals were published in association with dedicated web sites containing supplementary material and links to on-line resources such as reference databases. Similar sites were constructed in association with popular textbooks (e.g., *Bioinformatics* and *Genes & Signals*), offering resources for teaching and downloadable figures that can be turned easily into slide presentations. In addition, for the first time, two established Cold Spring Harbor books, *Retroviruses* and *Cells: A Laboratory Manual*, were republished on compact disk, ensuring that although not necessarily available in print, their contents would remain accessible to the scientific community in an easily searchable form. Improved accessibility also prompted our decision to make available on-line, free of charge, the entire text of several specialized monographs through the BookShelf project at the National Center for Biotechnology Information. We also licensed 13 books in electronic form to the companies Netlibrary and Ebrary, both of which provide academic and institutional business information specialists with consolidated on-line reference material. These initiatives are at early stages, but it is important that we explore emerging business models as scientists' reliance on Internet-delivered information continues to grow.

Some kinds of books, however, seem destined to remain most popular in print form. These include a new kind of manual we have pioneered in partnership with author Kathy Barker. Focused more on the human aspects of science than the technical, her books *At The Bench*, advising novices on how

to prosper in a laboratory environment, and *At The Helm*, on succeeding as a newly appointed principal investigator, were high on our list of best-sellers this year. Academic institutions are beginning to pay close attention to the professional career development of their scientific staff. The print handbook *Lab Ref*, by Jane Roskams and Linda Rodgers, also did well as an easy-to-use bench resource.

There were two releases in our general interest category. The 1998 collection of essays by Max Perutz *I Wish I'd Made You Angry Earlier* was much admired and sold very well. Although in his 80s, Max was prolific, and so many of his warm, insightful commentaries and book reviews appeared in subsequent years that we began a dialog about the publication of a paperback edition containing some of the newer essays. Sadly, Max died in February 2002 as the project began to take shape. So the expanded paperback edition became his memorial, assembled with the help of his children Vivienne and Robin, and with the addition of a wonderful tribute to Max by his friend and colleague John Meurig Thomas.

The second title in this category was *Ageless Quest*, by MIT's Lenny Guarente, a book on a theme perfectly summarized by its subtitle *One Scientist's Search for Genes That Prolong Youth*. The author made appearances at bookstores on both coasts to talk about his research and sign books, and he gave interviews to radio, television, and print journalists. His book was warmly received and was particularly appreciated by fellow scientists for its honest description of a life in a hot area of research and the clarity of his explanations of complex technicalities. Its particularly striking cover, a young woman wistfully contemplating her image in a mirror, typified what has become a much commented on feature of all our books, both popular and professional—their attractiveness as objects, in both design and production. These features, and the accuracy of typesetting, reflect the skills of our very able Production Department.

The success of a book like *Ageless Quest* in reaching its intended popular audience is critically dependent on the willingness of bookstores (including on-line booksellers) and library suppliers to stock it. After two years of relentless persistence by our Sales Department, relationships with the major players in the commercial marketplace have now been cemented. As a result, popular science books from Cold Spring Harbor can now be found reliably in bookstores at Harvard, Boston, Stanford, and other prominent universities, as well as in retail stores in major cities and the most dominant on-line bookshops. A particularly satisfying consequence of these new business relationships was the decision in Ann Arbor, Michigan, to base a community reading project around Philip Reilly's 2002 paperback *Abraham Lincoln's DNA and Other Adventures in Genetics*. With the assurance that the book would be readily available in local stores, the committee of "Ann Arbor Reads" arranged a three-month program of events surrounding the book, beginning January 2003, that includes talks by the author, lectures in schools and colleges on themes from the book, library reading groups, and other community-oriented activities.

Cordial relationships with retailers and wholesalers are also critical to the further development of our textbook program, since professors recommending books to students expect them to be immediately available nearby. The textbook list expanded in 2002 with the release of an innovative book for advanced genetics courses entitled *Discovering Genomics, Bioinformatics, and Proteomics*, by Malcolm Campbell and Laurie Heyer of Davidson College. Copublished with the notable textbook publishing house Benjamin Cummings, it is an excellent introductory book that offers a less mathematically challenging approach than our market-leading textbook, David Mount's *Bioinformatics: Genome and Sequence Analysis*, which continued to be widely adopted in many American universities. Another gratifying commercial development was the designation of six of our books as Special Selections by the Library of Science Book of the Month Club.

The marketing of future textbooks will be assisted by the staff of the Customer Service Department, who, in addition to their regular duties of fulfilling book orders and journal subscriptions, adopted telemarketing to inform prospective buyers about the availability of newly published books. The Press web site and the ability to broadcast e-mail messages are also invaluable additions to the techniques available for book promotion. Many thousands of scientists worldwide have elected to receive announcements of new books and special offers, and the web site contains the definitive catalog of all books currently available from the Press. Nevertheless, traditional promotional activities continued vigorously, with attractively redesigned newsletters and catalogs, a range of book-specific brochures and advertisements, and exhibits at more than a dozen major scientific conferences.

There was further consolidation of Cold Spring Harbor Laboratory Press Europe in 2002, with distribution in Oxford and active sales representation in several European countries. The sales goal for Europe was met, encouraging further improvement and expansion in 2003. New book distributors were appointed in Latin America, Southeast Asia, Egypt, Turkey, and Slovakia, widening an established distribution network in Japan, Korea, and India. International links were forged and maintained through attendance at the London and Frankfurt Book Fairs. Negotiations for translation rights have now resulted in the publication of Cold Spring Harbor books in more than 10 languages.

Two more books were added to the popular *Enjoy Your Cells* series for children. The author, Fran Balkwill, is now Professor of Cancer Biology at St. Bartholomew's Hospital, London, but despite the demands of a busy research career, she is increasingly involved in science education. *Gene Machines* and *Have A Nice DNA* once again show how good she is at describing scientific concepts in clear, uncluttered language, and her text is perfectly complemented by Mic Rolph's colorful and entertaining artwork.

Ten years after we began distributing their first children's books, Fran and Mic's work has become familiar to scientists all over the world. One of them is Siamon Gordon, Professor of Experimental Pathology at Oxford, who was born and medically trained in South Africa. At his urging, Fran, Mic, and Cold Spring Harbor Laboratory Press began the development of a book in the characteristic style of the *Enjoy Your Cells* series but dedicated to the goal of informing South African teenagers about the biology of HIV/AIDS, their risk of infection, and ways of preventing it. The authors researched this project during a 2001 visit to South Africa, and the book, entitled *Staying Alive: Fighting HIV/AIDS*, was written with much advice from the teachers, students, and community workers they met on their travels. With the help of start-up grants, we were able to print 20,000 copies of the book. In July, the authors, Siamon Gordon and his wife Lyndall, social scientist Linzi Rabinowitz, my son Tony, and I spent three weeks travelling in three provinces of South Africa, visiting schools, squatter camps, and orphanages in urban and rural areas, to distribute these copies free of charge. In each province, we publicized the location of distribution sites for the book. We were also able to develop a network of educators willing to use the book in a variety of settings and tell us about their experience. During our visit, we were privileged to meet teachers, social workers, doctors, politicians, patient activists, and others struggling with the tragedy of AIDS in South Africa. They included Education Minister Dr. Kadar Asmal who, in endorsing the book, gave us our mission statement: "Since AIDS has no cure or vaccine, education must be the social vaccine." As the year ended, we were excited and honored by the news that our application to the Bill and Melinda Gates Foundation for funds to further the work with *Staying Alive* in 2003 had been approved.

Journals

Genes & Development's circulation in 2002 reflected its continued strength in institutional subscriptions. All research journals that formerly enjoyed high levels of individual subscriptions are seeing those subscriptions decline as ever-larger numbers of institutions network the electronic edition of the journal to their entire staff. *Genes & Development* is no exception. Nevertheless, manuscript submission continued to increase, up by 8%, and the journal's impact factor rose to 20.88, maintaining its position among the top ten elite journals in experimental biology. Rudi Grosschedl (University of Munich) completed his first year as European Editor.

The circulation of *Genome Research* showed a 10% growth in institutional subscriptions. Manuscript submission increased by 70%, assisted by implementation of an entirely electronic manuscript submission and review system, and the journal's impact factor rose to 8.56.

The circulation of *Learning & Memory* improved by 3%. The manuscript submission rate increased by 62% and the journal's impact factor was 3.52.

The contract to publish the Protein Society's journal *Protein Science* began in 2001 and for the second year in a row, institutional subscriptions rose strongly, by 40%. Its impact factor was 3.47. Our success in increasing the journal's visibility, the attractive redesign, and reliable production schedules were key assets in our winning the RNA Society's competition to publish its journal *RNA*, starting in January 2003.

The journal program's expansion and its tight editorial focus on high-quality gene and protein science offer the opportunity to develop the sale of site licenses to companies and packages of on-line-only journals to library consortia, particularly abroad. This new income stream supplements declining revenue from traditional subscription sales. This program was very successful, resulting in ten new contracts during the year with associated revenue of more than $200,000. Representation at the London Online Conference resulted in several new leads for consortia and corporate clients abroad.

Total advertising sales in journals fell in 2002 by 30% compared with the previous year. *Genome Research* was particularly affected by the economic downturn that hit genomics and bioinformatics companies, many of which closed their doors for good; however, the gap was partly offset by improved revenue from *Protein Science*, which rode a rising tide of commercial interest in proteomics. RNA science is also in a growth phase, driven by the recent solution of the structure of the ribosome and the discovery of RNA interference as a means of silencing genes, so there are encouraging revenue prospects for the journal *RNA* in the coming year.

There is much current debate about the responsibility of publishers to make scientific information more readily available. Centralized, free databases of published papers are important resources in this respect. Cold Spring Harbor journal papers older than one year are already freely available at the journals' web sites, and in 2002, our Journal Production Department embarked on the unexpectedly lengthy and technically complex process of depositing these papers in the PubMedCentral database at the National Library of Medicine. This task is now expected to be completed early in 2003. The Department survived an abrupt and complete change in personnel early in the year and is now managed and staffed by experienced publishing professionals who have not only maintained impressive on-time publishing schedules, but also successfully dealt with a number of innovations such as the handling of digital artwork from authors, on-line manuscript submission and review, and the ability to publish papers on-line ahead of print.

Staff

The staff members of the Press (as of December 2002) are listed elsewhere in this volume. As always, I am enormously grateful to all of them for the care, pride, and professionalism they bring to the work they do. Publishing is the work of teams, and the achievements listed in this report are the product of talented individuals willing to pool their skills for the benefit of the program as a whole.

Our spacious and comfortable offices on the Woodbury Campus, in which we celebrated our first anniversary, house 40 of our staff members, with one other in our valuable office on the main campus and another in our much used writing center, the Meier House on the Banbury Estate. Another three staff members are in offices in San Diego, New Mexico, and Connecticut, and a further three in our warehouse at Hauppauge.

In 2002, we welcomed seven new staff members: Debbie Banninger, Cindy Blaut, Melissa Frey, Pauline Henick, Mary Mulligan, Linda Sussman, and Rita Wallace. We also bade farewell to Alison Herlihy, Nora Ruth, Laurie Saukas, Michele Schoudel, and Peri Zeenkov.

Finally, I would like to emphasize the vital role that the senior staff of the Press play in this expanding enterprise. Jan Argentine, Editorial Development Manager; Ingrid Benirschke, Marketing Manager; Kathy Cirone, Circulation Manager; Kathryn Fitzpatrick, Marketing Manager; Nancy Hodson, Operations Manager; Geraldine Jaitin, Customer Service Manager; Bill Keen, Finance Director; Guy Keyes, Sales Manager; Marcie Siconolfi, Advertising Manager; Linda Sussman, Journal Production Manager; Denise Weiss, Book Production Manager; the editors of our journals, Terri Grodzicker at *Genes & Development* and Laurie Goodman at *Genome Research*; and Elizabeth Powers, Executive Assistant. The Press and the Laboratory are fortunate to have these talented and dedicated individuals in key positions as a foundation for continued growth.

John R. Inglis

FINANCE

FINANCIAL STATEMENTS

CONSOLIDATED BALANCE SHEET
December 31, 2002
With comparative financial information for the year ended December 31, 2001

Assets:	*2002*	*2001*
Cash and cash equivalents	$ 15,998,950	25,917,330
Accounts receivable:		
Publications	1,507,910	2,070,714
Other	621,788	1,396,569
Grants receivable	5,155,499	5,194,624
Contributions receivable	4,485,178	6,681,094
Publications inventory	2,214,499	2,027,130
Prepaid expenses and other assets	1,630,366	2,436,437
Investments	174,146,179	185,947,777
Investment in employee residences	4,347,627	4,495,774
Restricted use asset	2,240,000	1,400,000
Land, buildings and equipment, net	108,416,078	108,104,940
Total assets	$ 320,764,074	345,672,389
Liabilities and Net Assets:		
Liabilities:		
Accounts payable and accrued expenses	$ 4,347,089	5,928,294
Notes payable	170,119	202,154
Bonds payable	45,200,000	45,200,000
Deferred revenue	3,648,453	2,615,372
Total liabilities	53,365,661	53,945,820
Net assets:		
Unrestricted	154,939,682	170,584,179
Temporarily restricted	10,132,940	13,179,303
Permanently restricted	102,325,791	107,963,087
Total net assets	267,398,413	291,726,569
Total liabilities and net assets	$ 320,764,074	345,672,389

CONSOLIDATED STATEMENT OF ACTIVITIES
Year ended December 31, 2002
With comparative totals for the year ended December 31, 2001

	Unrestricted	*Temporarily Restricted*	*Permanently Restricted*	*2002 Total*	*2001 Total*
Revenue and other support:					
Public support (contributions and non-government grant awards)	$ 11,374,015	10,077,203	3,605,790	25,057,008	22,251,408
Government grant awards	22,680,109	–	–	22,680,109	20,728,880
Indirect cost allowances	15,073,780	–	–	15,073,780	14,274,887
Program fees	2,910,787	–	–	2,910,787	2,677,232
Publications sales	9,050,738	–	–	9,050,738	9,940,567
Dining services	2,850,201	–	–	2,850,201	2,628,965
Rooms and apartments	2,222,483	–	–	2,222,483	1,890,226
Royalty and licensing fees	1,173,659	–	–	1,173,659	2,234,554
Investment income (interest and dividends)	5,515,942	–	–	5,515,942	8,285,120
Miscellaneous	583,033	–	–	583,033	541,080
Total revenue	73,434,747	10,077,203	3,605,790	87,117,740	85,452,919
Net assets released from restrictions	13,123,566	(13,123,566)	–	–	–
Total revenue and other support	86,558,313	(3,046,363)	3,605,790	87,117,740	85,452,919
Expenses:					
Research	42,240,020	–	–	42,240,020	39,464,156
Educational programs	12,105,086	–	–	12,105,086	11,490,571
Publications	9,345,581	–	–	9,345,581	9,800,811
Banbury Center conferences	1,141,308	–	–	1,141,308	1,026,037
Dolan DNA Learning Center programs	2,368,850	–	–	2,368,850	1,468,244
Watson School of Biological Sciences programs	1,648,168	–	–	1,648,168	1,104,729
General and administrative	10,562,264	–	–	10,562,264	9,739,917
Dining services	4,146,716	–	–	4,146,716	3,551,730
Total expenses	83,557,993	–	–	83,557,993	77,646,195
Excess of revenue and other support over expenses	3,000,320	(3,046,363)	3,605,790	3,559,747	7,806,724
Other changes in net assets:					
Net (depreciation) appreciation in fair value of investments	(18,644,817)	–	(9,243,086)	(27,887,903)	(16,949,723)
(Decrease) increase in net assets	(15,644,497)	(3,046,363)	(5,637,296)	(24,328,156)	(9,142,999)
Net assets at beginning of year	170,584,179	13,179,303	107,963,087	291,726,569	300,869,568
Net assets at end of year	$ 154,939,682	10,132,940	102,325,791	267,398,413	291,726,569

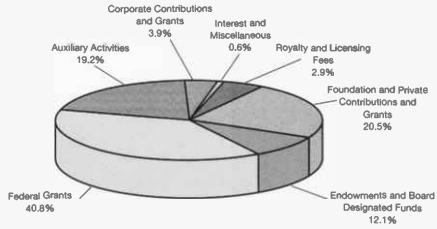
CONSOLIDATED STATEMENTS OF CASH FLOWS
Years ended December 31, 2002 and 2001
With comparative financial information for the year ended December 31, 2001

	2002	2001
Cash flows from operating activities:		
Decrease in net assets	$ (24,328,156)	(9,142,999)
Adjustments to reconcile (decrease) increase in net assets to net cash provided by operating activities:		
Depreciation and amortization	5,165,482	4,620,198
Net depreciation (appreciation) in fair value of investments	27,594,426	16,949,723
Contributions restricted for long-term investment	(5,020,794)	(7,504,820)
Restricted use asset	(840,000)	(1,400,000)
Changes in assets and liabilities:		
Decrease (increase) in accounts receivable	1,337,585	(341,935)
Decrease (increase) in grants receivable	39,125	(1,542,261)
Decrease in contributions receivable	1,754,273	1,425,568
(Increase) decrease in publications inventory	(187,369)	300,183
Decrease (increase) in prepaid expenses and other assets	806,071	(744,895)
Decrease in accounts payable and accrued expenses	(1,581,205)	(2,000,575)
Increase (decrease) in deferred revenue	1,033,081	(79,689)
Net cash provided by operating activities	5,772,519	538,498
Cash flows from investing activities:		
Capital expenditures	(5,476,620)	(13,401,394)
Proceeds from sales and maturities of investments	185,039,464	58,387,861
Purchases of investments	(200,832,291)	(53,119,270)
Net change in investment in employee residences	148,147	(1,448,345)
Net cash used in investing activities	(21,121,300)	(9,581,148)
Cash flows from financing activities:		
Permanently restricted contributions	3,605,790	1,421,839
Contributions restricted for investment in land, buildings, and equipment	1,415,003	6,082,981
Decrease in contributions receivable	441,643	23,948
Repayment of notes payable	(32,035)	(25,778)
Net cash provided by financing activities	5,430,401	7,502,990
Net decrease in cash and cash equivalents	(9,918,380)	(1,539,660)
Cash and cash equivalents at beginning of year	25,917,330	27,456,990
Cash and cash equivalents at end of year	15,998,950	25,917,330
Supplemental disclosures		
Interest paid	$ 1,132,279	1,447,427
Noncash investing and financing activities:		
Contributed property	$ 840,000	1,400,000

COMPARATIVE OPERATING HISTORY 1998–2002
(Dollars in Thousands)

	1998	1999	2000	2001	2002
Revenue:					
Main Lab:					
Grants and contracts	$ 24,025	27,179	30,345	34,716	37,872
Indirect cost allowances	11,054	11,207	12,718	14,134	14,987
Other	9,441	9,426	10,618	12,528	10,918
CSHL Press	6,341	6,400	8,684	9,941	9,051
Banbury Center	1,444	1,848	1,856	1,666	1,763
Dolan DNA Learning Center	1,334	1,392	1,471	1,878	2,978
Watson School of Biological Sciences	–	218	682	927	1,496
Total revenue	53,639	57,670	66,374	75,790	79,065
Expenses:					
Main Lab:					
Research and training	24,025	27,179	30,345	34,716	37,872
Operation and maintenance of plant	5,549	5,765	6,589	7,027	8,661
General and administrative	3,378	3,844	6,162	6,492	6,395
Other	7,328	7,863	7,075	9,505	8,550
CSHL Press	6,141	6,077	8,186	9,515	8,962
Banbury Center	1,321	1,614	1,702	1,536	1,597
Dolan DNA Learning Center	1,228	1,280	1,362	1,801	2,780
Watson School of Biological Sciences	–	218	682	927	1,496
Total expenses, excluding depreciation and amortization	48,970	53,840	62,103	71,519	76,313
Excess before depreciation, amortization and designation of funds	4,669	3,830	4,271	4,271	2,752
Depreciation and amortization	(3,443)	(3,526)	(3,974)	(4,620)	(5,165)
(Designation) release of funds (2)	(750)	–	(297)	349	1,848
Net operating excess (deficit)	$ 476	304	–	–	(565)

(1)The above amounts are presented on a combined basis for all funds for which Cold Spring Harbor Laboratory prepares operating budgets.
(2) Funds designated to underwrite future direct and indirect expenses of new research programs.



COLD SPRING HARBOR LABORATORY
SOURCES OF REVENUE
DECEMBER 31, 2002

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 2002.

GRANTS January 1, 2002–December 31, 2002

COLD SPRING HARBOR LABORATORY

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*2002 Funding†*	
FEDERAL GRANTS				
NATIONAL INSTITUTES OF HEALTH				
Equipment	Dr. Lowe	04/01/02 03/31/03	393,302	*
Program Projects	Dr. Herr	01/01/77 12/31/06	4,712,097	*
	Dr. Stillman	08/01/87 07/31/05	3,633,903	
Research Support	Dr. Cline	12/06/95 11/30/04	745,769	
	Dr. Cline	03/01/98 03/31/06	509,098	
	Dr. Enikolopov	08/01/99 05/31/04	344,836	
	Dr. Enikolopov	09/12/94 11/30/03	409,907	
	Dr. Enikolopov	03/11/02 12/31/03	207,500	*
	Dr. Grewal	03/01/00 02/28/05	268,800	
	Dr. Hannon	09/01/00 08/31/05	289,743	
	Dr. Hannon	03/01/01 02/28/03	166,000	
	Dr. Helfman	09/01/99 06/30/04	397,701	
	Dr. Hernandez	07/01/99 06/30/04	237,827	
	Dr. Herr	03/01/02 02/28/06	298,800	*
	Dr. Hirano	07/01/01 06/30/05	351,623	
	Dr. Hirano	05/01/96 04/30/04	353,055	
	Dr. Huang	08/01/01 06/30/06	425,391	
	Dr. Joshua-Tor	05/01/01 03/31/06	443,500	
	Dr. Joshua-Tor	02/15/02 01/31/07	398,500	*
	Dr. Joshua-Tor	08/01/96 11/30/04	249,216	
	Dr. Krainer	06/01/01 05/31/06	534,241	
	Dr. Lowe	07/01/99 06/30/04	334,902	
	Dr. Malinow	05/01/92 04/30/05	565,432	
	Dr. Malinow	04/01/95 02/28/03	419,634	
	Drs. McCombie/Wigler/Zhang	01/01/99 12/31/03	484,705	
	Dr. Neuwald	09/30/98 08/31/06	373,500	
	Dr. Skowronski	04/01/98 03/31/03	502,367	
	Dr. Spector	04/01/90 03/31/03	721,989	
	Dr. Stein	09/01/02 06/30/05	613,047	*
	Dr. Stillman	07/01/91 05/31/04	536,168	
	Dr. Svoboda	12/01/98 11/30/03	406,883	
	Dr. Svoboda	12/01/02 11/30/07	326,523	*
	Dr. Tonks	08/01/91 03/31/06	609,418	
	Dr. Tonks	05/01/97 06/30/05	348,600	
	Drs. Tully/Zhong	09/28/00 06/30/03	274,509	

*New Grants Awarded in 2002.
†Includes Direct & Indirect Cost.

Grantor	Program/Principal Investigator	Duration of Grant	2002 Funding*	
	Dr. Wigler	09/30/02 07/31/04	166,000	*
	Dr. Wigler	05/01/99 07/31/03	996,358	
	Dr. Xu	08/01/01 07/31/05	303,219	
	Dr. Xu	01/01/98 12/31/02	311,754	
	Dr. Yin	09/30/02 07/31/04	166,000	*
	Dr. Zhang	08/01/00 07/31/03	403,616	
	Dr. Zhang	09/30/97 08/31/03	641,728	
	Dr. Zhong	02/01/96 06/30/03	292,073	
Fellowships	E. Govek	05/01/01 04/30/03	24,766	
	Dr. Kuhlman	12/01/02 11/30/05	38,320	*
	Dr. Lucito	06/01/02 05/31/07	114,280	*
	Dr. Ronemus	12/01/01 11/30/04	48,148	
Training Support	Dr. Helfman	09/01/84 12/31/03	303,114	
Course Support	Macromolecular Crystallography	09/01/00 08/31/05	51,223	
	C. elegans	08/01/98 07/31/06	63,937	
	Neurobiology Short-term Training	07/01/01 06/30/06	160,527	
	Cell and Developmental Biology of *Xenopus*	04/01/93 03/31/04	18,974	
	Cancer Center Research Workshops	01/01/83 03/31/05	308,013	
	Acquiring and Analyzing Genomic Sequence Data	04/01/95 03/31/04	89,045	
	In Situ Immunochemistry, In Situ Hybridization, and Live Cell Imaging	07/01/98 06/30/03	73,128	
	Bioinformatics: Writing Software for Genome Research	07/01/00 06/30/05	73,094	
	Making and Using DNA Microarrays	09/30/99 06/30/04	65,652	
Meeting Support	Zebrafish Development and Genetics	04/18/00 03/31/03	18,000	
	Axion Guidance and Neural Plasticity	06/01/00 05/30/05	26,468	
	Molecular Chaperones and the Heat Shock Response	04/01/02 03/31/03	13,000	*
	Evolution of Developmental Diversity	04/17/02 03/31/07	13,000	*
	Molecular Genetics of Aging	06/01/02 05/31/03	23,241	*
	Germ Cells	07/01/02 06/30/03	6,000	*
	Mouse Molecular Genetics	07/12/02 06/30/07	17,438	*
	Translational Control	07/05/02 06/30/07	8,000	*
	Gene Expression and Signaling in the Immune System	04/19/02 03/31/07	2,000	*
	Cancer Genetics and Tumor Suppressor Genes	08/07/00 07/31/05	15,000	
	The 67th Symposium: The Cardiovascular System	05/15/01 04/30/06	5,000	
NATIONAL SCIENCE FOUNDATION				
Research Support	Dr. Cline	09/01/99 08/31/04	119,499	
	Dr. Helfman	02/01/99 01/31/04	115,202	
	Dr. Jackson	08/01/02 07/31/05	110,000	*
	Dr. Jackson	10/01/01 09/30/06	131,263	
	Drs. Martienssen/McCombie/Stein/Lucito	09/01/01 08/31/05	490,077	
	Dr. Martienssen	10/01/01 09/30/06	136,489	
	Dr. Stein	09/01/02 08/31/04	297,412	*
	Dr. Timmermans	07/01/02 06/30/05	116,471	*

*New Grants Awarded in 2002.
*Includes Direct & Indirect Cost.

Grantor	Program/Principal Investigator	Duration of Grant	2002 Funding*	
Training Support	Undergraduate Research Program	06/01/00 05/31/03	65,204	
Course Support	Advanced Bacterial Genetics	05/01/99 04/30/04	72,785	
	Arabidopsis Molecular Genetics	04/01/00 03/31/03	66,500	
	Cell and Development Biology of *Xenopus*	09/01/02 08/31/06	21,520	*
Meeting Support	Evolution of Developmental Diversity	03/15/02 02/28/03	10,000	*
	Molecular Genetics of Bacteria and Phages	07/01/02 06/30/03	8,000	*
UNITED STATES DEPARTMENT OF AGRICULTURE				
Research Support	Dr. Jackson	09/01/01 08/31/03	98,250	
	Drs. Martienssen/McCombie	09/01/01 09/30/03	250,000	
	Dr. Martienssen	12/01/00 11/30/03	85,000	
	Dr. Stein	09/01/00 08/31/04	849,094	
	Dr. Stein	09/15/00 09/14/04	255,264	
	Dr. Stein	10/01/02 09/30/04	220,000	*
	Dr. Timmermans	09/01/01 08/31/03	65,960	
UNITED STATES DEPARTMENT OF THE ARMY				
Research Support	Dr. Conklin	09/30/02 09/29/05	161,216	*
	Dr. Hannon	06/01/00 07/01/04	126,000	
	Dr. Hannon	04/01/02 03/31/06	801,448	*
	Drs. Hannon/Lowe	09/01/02 08/31/05	166,000	*
	Dr. Lazebnik	06/01/01 05/31/04	203,637	
	Dr. Van Aelst	09/01/01 08/31/03	167,000	
	Dr. Zhong	04/01/02 03/31/04	166,000	*
Fellowship Support	M. Carmell	05/01/02 04/30/05	22,000	*
	Dr. Du	06/01/02 05/31/05	50,000	*
	Dr. Hamaguchi	04/15/00 05/15/04	65,203	
	Dr. Hannon	06/01/00 05/31/04	80,858	
	Dr. Kannanganattu	01/01/01 12/31/03	44,622	
	Z. Nahle	07/01/02 06/30/05	22,000	*
	Dr. Narita	07/01/01 06/30/04	51,247	
	Y. Seger	07/01/01 06/30/04	22,000	
	Dr. Silva	11/01/01 10/31/03	49,000	
DEPARTMENT OF ENERGY				
Meeting Support	The 68th Symposium	09/01/02 08/31/03	15,000	*
MISCELLANEOUS GRANTS				
Research Support				
American Cancer Society	Dr. Wigler	01/01/96 12/31/03	10,000	
American Cancer Society	Dr. Wigler	01/01/01 12/31/05	70,000	
AKC Canine Health Foundation	Drs. McCombie/Hannon	07/20/02 06/30/03	33,000	*
Babylon Breast Cancer Coalition	Dr. Wigler	12/01/02 12/31/03	10,000	*

*New Grants Awarded in 2002.
*Includes Direct & Indirect Cost.

Grantor	Program/Principal Investigator	Duration of Grant	2002 Funding*	
Michael Scott Barish Human Cancer Grant sponsored by the 1 in 9: The Long Island Breast Cancer Action Coalition	Dr. Wigler	01/01/02 12/31/02	66,010	*
Breast Cancer Research Foundation	Dr. Wigler	10/01/00 09/30/03	1,000,000	
The Charles Dana Foundation	Siegel Fund for Cancer Pharmacogenomics	01/01/02 12/31/02	100,000	*
DARPA/NYU Consortium Agreement	Drs. Wigler/Mittal	09/18/01 09/17/04	226,127	
DOE/University of Arizona Consortium Agreement	Drs. McCombie/Martienssen/Stein	09/15/02 09/14/03	36,742	*
Ellison Medical Foundation	Dr. Grewal	09/01/99 08/31/03	50,000	
Forbeck Research Foundation	Dr. Lowe	11/09/02 10/31/03	1,000	*
Fraxa Research Foundation	Dr. Yin	05/01/02 04/30/03	50,000	*
Glen Cove C.A.R.E.S.	Dr. Hamaguchi	04/01/02 03/31/03	25,000	*
Irving Hansen Foundation	Dr. Tansey	08/01/02 07/31/03	20,000	*
Anya Hulbert/Matt Ridley	Watson Archives	09/01/02 08/31/03	10,000	*
Helen Hoffritz Foundation	Dr. Cline	12/01/96 01/31/03	30,000	
Helicon	Dr. Tully	07/01/02 06/30/03	168,000	*
Immuno-RX	Dr. Mittal	08/01/02 07/31/04	205,903	*
Lehrman Trust	Library Support	04/01/00 03/31/03	140,108	
L.I.A.B.C. Long Islanders Against Breast Cancer	Dr. Wigler	01/01/02 12/31/02	106,846	*
Long Beach Cancer Coalition	Dr. Muthuswamy	09/01/02 08/31/03	2,500	*
Elizabeth McFarland Breast Cancer Research	Dr. Wigler	09/01/00 12/31/03	31,946	
McKnight Endowment	Dr. Svoboda	08/01/02 07/31/04	100,000	*
Millennium Pharmaceuticals	Dr. Lowe	10/02/02 10/01/03	20,000	*
Miracle Foundation	Dr. Wigler	04/01/02 03/31/03	100,000	*
Louis Morin Charitable Trust	Drs. Joshua-Tor/Dubnau	01/01/00 11/30/03	151,917	*
The News Corporation Foundation	Siegel Fund for Cancer Pharmacogenomics	01/01/02 12/31/02	100,000	*
NIH/Baylor College of Medicine Consortium Agreement	Dr. Mills	09/01/01 08/31/06	208,113	
NIH/Cal Tech Consortium Agreement	Dr. Stein	09/15/00 06/30/03	277,399	
NIH/Cal Tech Consortium Agreement	Dr. Stein	07/01/02 06/30/04	274,780	*
NIH/Cal Tech Consortium Agreement	Dr. Stein	07/01/02 06/30/04	181,399	*
NIH/Columbia University Consortium Agreement	Dr. Lowe	09/30/00 07/31/05	494,898	
NIH/Jackson Laboratory Consortium Agreement	Dr. Stein	01/01/02 12/31/02	50,000	*
NIH/Nanaprobes, Inc. Consortium Agreement	Dr. Spector	09/01/02 02/28/03	15,770	*
NIH/Northwestern University Consortium Agreement	Dr. Spector	07/01/01 12/31/02	66,400	
NIH/SUNY-Downstate Medical School Consortium Agreement	Dr. Yin	09/01/01 06/30/06	90,000	
NIH/Washington University Consortium Agreement	Dr. Stein	03/01/99 10/31/03	45,348	
NIH/Washington University Consortium Agreement	Dr. Stein	09/30/99 10/31/03	17,174	
NIH/UCSD Consortium Agreement	Dr. Zhang	09/01/01 08/31/04	144,316	
NSF/Rutgers University Consortium Agreement	Drs. Spector/Martienssen	10/01/00 09/30/05	443,408	
NSF/University of Arizona Consortium Agreement	Drs. McCombie/Martienssen/Stein	09/15/02 09/14/03	171,835	*
NSF/SUNY-StonyBrook Consortium Agreement	Dr. Jackson	09/01/02 08/31/04	190,004	*
NSF/Yale University Consortium Agreement	Drs. McCombie/Martienssen	09/15/01 09/14/03	49,690	

*New Grants Awarded in 2002.
*Includes Direct & Indirect Cost.

Grantor	Program/Principal Investigator	Duration of Grant	2002 Funding*	
NSF/University of Wisconsin Consortium Agreement	Dr. Martienssen	09/01/00 08/31/05	239,131	
New York State Funds	Library Support	07/01/02 06/30/03	4,409	*
NYSTAR/Mt. Sinai School of Medicine Consortium Agreement	Dr. Svoboda	10/01/02 09/30/03	175,000	*
NY Botanic Garden Plant Consortium	Dr. McCombie	03/01/02 12/31/03	81,442	*
Mr. and Mrs. Edmond Nouri	Dr. Tully	04/01/00 03/31/03	122,869	
OSI Pharmaceuticals	Dr. Hannon	10/21/02 10/20/03	1,000,000	*
OSI Pharmaceuticals	Drs. Hannon/Mittal	10/21/02 10/20/05	450,000	*
The David and Lucille Packard Foundation	Drs. Chklovskii/Mainen/Zador	07/01/01 06/30/04	421,496	
The Perkin Fund	Women's Partnership For Science	06/01/02 05/31/04	37,500	*
Plainview/Oyster Bay Cancer Coalition	Dr. Muthuswamy	09/01/02 08/31/03	7,000	*
Rockefeller University Agreement	Dr. Mills	09/01/02 08/31/03	67,413	*
SAIC-NCI	Dr. Hannon	10/21/02 10/21/03	500,000	*
Ann and Herb Siegel Philanthropic Foundation	Siegel Fund for Cancer Pharmacogenomics	01/01/00 11/30/03	300,000	
Starr Foundation	Drs. Stillman/Hatchwell	06/01/02 05/31/03	316,393	*
Strauss Hawkins Foundation	Drs. Brody/Zador	01/30/02 01/29/03	100,000	*
Sungene Plant Consortium	Dr. Martienssen	07/01/00 06/30/05	135,000	
Seraph Foundation	Dr. Enikolopov	10/01/01 09/30/03	29,000	
Tularik, Inc.	Dr. Wigler	11/01/97 10/31/03	660,000	
Tularik, Inc.	Dr. Wigler	01/01/02 12/31/03	500,000	*
USDA/University of Arizona Consortium Agreement	Drs. McCombie/Martienssen/Stein	09/15/02 09/14/03	171,830	*
The V Foundation	Dr. Muthuswamy	05/01/02 04/30/04	50,000	*
Westvaco Plant Consortium	Dr. Martienssen	01/01/98 12/31/02	135,343	
Whitehall Foundation, Inc.	Dr. Huang	08/16/01 08/15/04	75,000	
Zeneca Plant Consortium	Dr. Martienssen	07/20/99 07/19/04	135,000	
Fellowships				
AACH Amgen Fellowship	Dr. Wendel	07/01/02 06/30/03	30,000	*
American Cancer Society	Dr. Hastings	01/01/00 12/31/02	34,000	
Burroughs Wellcome	Dr. Zito	09/01/02 08/31/03	58,000	*
CaP CURE Prostate Cancer	Dr. Lucito	01/01/02 12/31/03	100,000	*
CSHL Association Funds	Labwide Support	04/01/02 03/31/03	224,535	*
Cure Autism Now	Dr. Takahashi	06/01/02 05/31/04	40,000	*
Deutsche Forschungsgemeinschaft (DFG)	Dr. Rumpel	11/01/02 10/31/04	28,176	*
Dorcas Cummings Memorial Fund	Undergraduate Research Program	01/01/02 12/31/02	9,198	*
Epilepsy Foundation	Dr. Haas	07/01/02 06/30/03	40,000	*
Eppley Foundation	Dr. Huang	01/01/02 12/31/02	22,000	*
Goldring Fellowship	Dr. Stillman	09/01/00 08/31/03	30,000	
Helen Hay Whitney Foundation	Dr. Zito	07/01/00 06/30/03	45,000	
	Dr. Karpova	07/01/02 06/30/03	41,000	*
	Dr. Sheu	04/01/01 03/31/04	42,500	
Howard Hughes Medical Institute	Graduate Student Support	09/01/94 08/31/03	60,850	
Human Frontier Science Program Organization (HFSPO)	Dr. Ango	04/01/02 03/31/05	42,000	*
	Dr. Bureau	10/01/01 09/30/03	42,000	
	Dr. Iijima	04/26/02 04/25/04	42,000	*
	Dr. Scheuss	09/01/02 08/31/05	42,000	*
Jane Coffin Childs	Dr. Gillespie	07/01/02 06/30/03	40,500	*
Jephson Educational Trust	Undergraduate Research Program	01/01/02 12/31/02	10,000	*
Johns Hopkins/Ellison Foundation Consortium Agreement	Dr. Kass-Eisler	01/01/02 03/31/03	110,984	*

*New Grants Awarded in 2002.
*Includes Direct & Indirect Cost.

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*2002 Funding**	
Ambassador Felix Schnyder Memorial Fund, administered by The Lauri Strauss Leukemia Foundation	Dr. Van Aelst	01/01/02 12/31/02	15,000	*
The Leukemia & Lymphoma Society	Dr. Duelli	12/31/00 12/30/03	35,000	
	Dr. Losada	07/01/01 06/30/04	50,000	
	Dr. Noma	07/01/02 06/30/05	40,000	*
	Dr. Speck	07/01/01 06/30/04	40,000	
The Leukemia & Lymphoma Society Special Fellow Award	Dr. Tansey	07/01/01 06/30/06	100,000	
Life Science Research Foundation	Dr. Samuel	01/01/02 05/31/03	58,142	*
Maxfield Foundation	Dr. Lazebnik	12/01/00 11/30/03	5,000	
NARSAD	Dr. Haas	07/01/02 06/30/04	30,000	*
	Dr. Barria-Roman	07/01/02 06/30/04	30,000	*
Pew Charitable Trust	Dr. Huang	07/01/02 06/30/06	60,000	*
Rita Allen Foundation	Dr. Hannon	10/01/00 09/30/03	50,000	
Searle Scholars Program	Dr. Mainen	07/01/01 06/30/04	80,000	
SUNY-Stony Brook Training Grant	Dr. L. Zhang	2001 2002	38,154	
Wellcome Trust	Dr. Akerman	10/01/01 09/30/03	29,513	
Wellcome Trust	Dr. Newey	10/01/01 09/30/03	26,306	
Course Support				
Eppley Foundation	Stem Cell	04/01/02 12/31/02	34,870	*
Eppley Foundation	Human Neurological Diseases	06/01/02 12/31/02	23,750	*
Esther A. & Joseph Klingenstein Fund, Inc.	Neuro Course Support	03/01/02 02/28/05	50,000	*
Merck & Co, Inc.	Developmental Disabilities in Children	04/01/01 03/31/03	46,192	
Howard Hughes Medical Institute	Advanced Neurobiology Courses	01/01/91 12/31/03	330,000	
Meeting Support				
Alfred P. Sloan	DNA Jubilee	06/01/02 03/31/03	25,000	*
Dana Foundation	DNA Jubilee	10/01/02 09/30/03	100,000	*
March of Dimes	Germ Cells	04/01/02 03/31/03	5,000	*
Alzheimer's Association	Heat Shock	01/01/02 12/31/02	2,000	*
Merck & Co, Inc.	Heat Shock	01/01/02 12/31/02	1,000	*
Invitrogen Life Technologies	Heat Shock	01/01/02 12/31/02	2,000	*
Medical & Biological Laboratories, Inc. Ltd.	Heat Shock	01/01/02 12/31/02	1,000	*
Lalor Foundation	Germ Cells	09/01/00 08/31/03	7,250	

*New Grants Awarded in 2002.
*Includes Direct & Indirect Cost.

BANBURY CENTER

Grantor	Program/Principal Investigator	Duration of Grant	2002 Funding*
FEDERAL SUPPORT			
NIH–National Human Genome Research Institute	American Eugenics and the New Biology: Perspectives and Parallels	2002	$33,099
National Institute of Justice of the U.S. Department of Justice	Microbial Forensics	2002	30,000 *
NIH–National Institute of Mental Health and National Institute of Child Health and Human Development (through a grant to the University of Illinois, Urbana)	RNA Metabolism and the Fragile X Syndrome	2002	32,752 *
National Science Foundation (through a grant to New York University)	Designer Molecules for Biosensor Applications	2002	19,208 *
U.S. Department of Energy (NNSA, CNBP)	Microbial Forensics	2002	10,000 *
NONFEDERAL SUPPORT			
Meeting Support			
Amyotrophic Lateral Disease Association	Neurodegenerative Disease Models: From Pathogenesis to Therapeutics	2002	19,894 *
Bill and Melinda Gates Foundation (through a grant to Albert B. Sabin Vaccine Institute, Inc.)	Global Vaccine Shortage: The Threat to Children and What to do About It	2002	28,224 *
Howard Hughes Medical Institute	DNA Interactive Advisory Panel	2002	20,921
Howard Hughes Medical Institute	DNA Interactive Advisory Panel, Second Meeting	2002	21,381
Huntington's Disease Society of America	Neurodegenerative Disease Models: From Pathogenesis to Therapeutics	2002	19,894 *
ICF Ventures	Phage Therapy—Potential and Challenges	2002	50,000 *
Herbert J. Siegel Fund for Cancer Pharmacogenomics	A Critical Review of Melanoma—Biology and Therapy	2002	34,607
WADA Health, Medical and Research Committee	Genetic Enhancement of Athletic Performance	2002	39,619 *
Yamanouchi USA Foundation	Glucocorticoid Regulatory Mechanisms and Pathophysiology	2002	41,825

*New Grants Awarded in 2002.

DOLAN DNA LEARNING CENTER

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*2002 Funding**
FEDERAL GRANTS			
National Institutes of Health ELSI Research Program	Creation of a *Digital Image Archive on the American Eugenics Movement*	03/98–03/03	$ 183,660
National Institutes of Health	Creation of *Inside Cancer*	01/01–12/03	274,051
NONFEDERAL GRANTS			
Howard Hughes Medical Institute	Precollege Science Education Initiative for Biomedical Research Institutions	09/99–08/03	149,664
Howard Hughes Medical Institute	DNA Interactive Education Program	01/02–12/02	951,932
Josiah Macy, Jr. Foundation	*DNA from the Beginning*	10/97–09/02	215,251
Pfizer Foundation	*Leadership Institute in Human and Molecular Genetics*	01/02–12/02	69,618

The following schools each awarded a grant for the *Genetics as a Model for Whole Learning* Program:

Bay Shore Union Free School District	$1,000	Massapequa Union Free School District	1,650
Bellmore–Merrick Central High School District	5,300	North Bellmore Union Free School District	1,000
Brooklyn District #17	1,400	Northport–East Northport Union Free School District	6,160
Buckley Country Day School	365	North Shore Hebrew Academy	700
Commack Union Free School District	425	Old Westbury School of the Holy Child	2,115
East Meadow Union Free School District	4,780	Oyster Bay–East Norwich Central School District	2,592
East Williston Union Free School District	1,680	Port Washington Union Free School District	10,275
Elmont Union Free School District	500	Progressive School of Long Island	300
Elwood Union Free School District	3,235	Queens Community School District #29	33,375
Farmingdale Union Free School District	980	Rockville Centre Union Free School District	5,210
Garden City Union Free School District	6,075	Saint Aidan School	250
Great Neck Union Free School District	7,225	Saint Anne School	350
Green Vale School	1,180	Saint Dominic Elementary School	3,500
Half Hollow Hills Central School District	4,900	Saint Edward School	2,025
Harborfields Central School District	9,595	Saint Savior School	350
Herricks Union Free School District	1,125	South Huntington Union Free School District	12,650
Holy Family Regional School	250	Syosset Central School District	21,075
Jericho Union Free School District	5,800	Three Village Central School District	750
Lawrence Union Free School District	6,480	United Nations School	500
Locust Valley Central School District	16,125	Whitby School	250
Lynbrook Union Free School District	500	Yeshiva Darchei Torah	250
Mamaroneck Central School District	2,800		

The following schools each awarded a grant for *Curriculum Study*:

Bellmore–Merrick Central High School District	1,100	Long Beach City School District	1,100
Bethpage Union Free School District	1,100	Massapequa Union Free School District	2,200
Commack Union Free School District	1,100	North Shore Central School District	2,200
Elwood Union Free School District	1,100	North Shore Hebrew Academy High School	2,000
Fordham Preparatory School	2,000	Oceanside Union Free School District	1,100
Great Neck Union Free School District	1,100	Oyster Bay–East Norwich Central School District	2,200
Green Vale School	1,100	Plainedge Union Free School District	1,100
Harborfields Central School District	2,200	Plainview–Old Bethpage Central School District	2,200
Island Trees Union Free School District	1,100	Portledge School	1,100
Jericho Union Free School District	1,100	Port Washington Union Free School District	1,100
Kings Park Central School District	2,000	Roslyn Union Free School District	1,100
Lawrence Union Free School District	2,200	Sachem Central School District	1,100
Levittown Union Free School District	1,100	South Huntington Union Free School District	1,100
Locust Valley Central School District	1,100		

INSTITUTIONAL ADVANCEMENT

It is a pleasure to acknowledge the philanthropy of the many thoughtful people who are increasing the pace of biomedical discovery at Cold Spring Harbor Laboratory. With the fruits of the human genome project in hand, the challenge to control cancer is no longer theoretical or technical, but financial. It is private funding that makes the difference and stimulates breakthrough science.

The gifts reported here will help to control cancer, increase our understanding of neurological disorders, and assure the education of the next generation of scientific leaders. Private gifts fund the innovation in molecular biology that characterizes the advanced education and research at Cold Spring Harbor Laboratory.

We deeply appreciate such commitment to the best science for a better life.

Rod Miller, Vice President for Institutional Advancement

Cold Spring Harbor Laboratory is a nonprofit research and educational institution, chartered by the State of New York. Less than half of the Laboratory's annual revenues are derived from Federal grants and contracts, and thus, we rely heavily on support from the private sector: foundations, corporations, and individuals. Contributions from the private sector are tax-exempt under the provisions of Section 501(c)(3) of the Internal Revenue Code. In addition, the Laboratory is designated a "public charity" and, therefore, is enabled to receive funds resulting from the termination of "private foundations."

Foundations, corporations, and individuals can give to Cold Spring Harbor through a variety of methods:

Gifts of Money can be made directly to Cold Spring Harbor Laboratory.

Securities: Stock certificates may be reassigned directly or transferred through your broker. Appreciated securities should be given outright, which will avoid capital gains taxes on the appreciated value. Securities that have decreased in value should be sold, and the proceeds donated. In this way, a donor will receive a deduction for both the loss and the charitable contribution.

Life Insurance: You may designate the Cold Spring Harbor Laboratory as the beneficiary of an existing or new policy, or irrevocably assign ownership of the policy. There are estate tax benefits in either case. If ownership is assigned, there is an immediate tax deduction.

Pooled Income Funds: Combine gifts from a number of donors in a pool for attractive investment and tax purposes.

Appreciated Real Estate or Personal Property: Sizable tax benefits can result from such donations; the Laboratory can use some in its program and can sell others.

Charitable Remainder Trusts can be structured to suit the donor's specific desires as to extent, timing, and tax needs.

Bequests: Designating Cold Spring Harbor Laboratory as beneficiary ensures that a bequest will be utilized as specified.

Conversion of Private Foundation to "Public" Status on Termination: This may be done by creating a separate fund within Cold Spring Harbor Laboratory whereby the assets of the private foundation are accounted for as a separate fund and used for the purposes specified by the donor. Alternatively, the private foundation can be established as a supporting organization of Cold Spring Harbor Laboratory.

Matching Gifts: Many employers will match gifts to Cold Spring Harbor Laboratory and/or the Watson School of Biological Sciences. Please check with your employer to augment your gift.

For additional information, please contact the Office of the Vice President for Institutional Advancement, Cold Spring Harbor Laboratory, One Bungtown Road, Cold Spring Harbor, New York 11724. Phone number: 516-367-6858.

CAPITAL AND PROGRAM CONTRIBUTIONS

January 1, 2002–December 31, 2002

Contributions of $5,000 and above, exclusive of Annual Fund

In 2002, Cold Spring Harbor Laboratory received significant support in the form of capital, program, and gifts-in-kind contributions from individuals, foundations, and corporations.

Rita Allen Foundation, Inc.
Arrow Electronics
Babylon Breast Cancer Coalition
The Michael Scott Barish Human Cancer Grant sponsored by 1 in 9: The Long Island Breast Cancer Action Coalition
Benjamin Development Co.
Mr. William Braden
The Breast Cancer Research Foundation*
Gladys Brooks Foundation
Cambrex Corporation
CaP CURE
The Charles A. Dana Foundation
Mr. and Mrs. Charles F. Dolan
Ellison Medical Foundation
The Eppley Foundation for Research
Forest Laboratories, Inc.
Mr. Robert B. Gardner, Jr.
Genzyme Corporation
Glen Cove C.A.R.E.S.
The Lillian Goldman Charitable Trust
Goldring Family Foundation
Mr. and Mrs. John S. Grace
Irving A. Hansen Memorial Foundation
The William A. Haseltine Foundation
Jeff Hawkins and Janet L. Strauss
Human Genome Sciences, Inc.
Dr. Anya Hurlburt and Dr. Matt Ridley
Mary Woodard Lasker Charitable Trust
The Lehrman Institute
Estate of Elisabeth Sloan Livingston
Long Islanders Against Breast Cancer (L.I.A.B.C.)
Richard Lounsbery Foundation
Marks Family Foundation
G. Harold and Leila Y. Mathers Charitable Foundation
The Maxfield Foundation
Breast Cancer Awareness Day in memory of Elizabeth McFarland
The Miracle Foundation
Louis Morin Charitable Trust
The News Corporation Foundation
Mr. and Mrs. Edmond J. Nouri
David and Lucile Packard Foundation
Peninsula Community Foundation
The Perkin Fund*
Pew Charitable Trust
Plainview-Old Bethpage Breast Cancer Coalition
William Townsend Porter Foundation
William E. and Maude S. Pritchard Charitable Trust
Mr. and Mrs. John R. Reese
The Seraph Foundation
Ann L. and Herbert J. Siegel Philanthropic Fund
Alfred P. Sloan Foundation
The Starr Foundation
Lauri Strauss Leukemia Foundation
The Times Mirror Foundation
Tularik, Inc.
The V Foundation*
Dr. and Mrs. James D. Watson
The Whitehall Foundation

Total	**$8,407,076.94**

* New Pledges Awarded in 2002.

WATSON SCHOOL OF BIOLOGICAL SCIENCES CAPITAL CAMPAIGN

January 1, 2002–December 31, 2002

Contributions and pledges, exclusive of Annual Fund

The Watson School of Biological Sciences at Cold Spring Harbor Laboratory was established in 1998 to provide graduate education in the biological sciences at the Ph.D. level. The mission of the doctoral program is for students to gain extensive knowledge in biology through a combined research and education experience, with strong guidance from the faculty, and to complete the program in approximately four years. This accelerated program is designed to increase the number of highly skilled biologists available to apply the wealth of data from the human genome.

In its fourth year, the Watson School matriculated 6 additional students, bringing the total number to 26. In 2002, the Watson School received significant support from individuals, foundations, trusts, and corporations.

Purpose	Donor
Arnold and Mabel Beckman Graduate Studentship	Arnold and Mabel Beckman Foundation
Core Course	Arnold and Mabel Beckman Foundation
Dean's Chair	Annenberg Foundation Lita Annenberg Hazen Foundation
Fellowships	Bristol-Myers Squibb Company The Charles A. Dana Foundation Mr. and Mrs. Alan E. Goldberg William Randolph Hearst Foundation Mr. and Mrs. David H. Koch Mr. and Mrs. Robert D. Lindsay and Family Estate of Elisabeth Sloan Livingston The Miller Family Foundation
Lectureships	Mr. and Mrs. George Cutting The William Stamps Farish Fund* Florence Gould Foundation Joy and George Rathmann/The Rathmann Family Foundation Ziering Family Foundation
Visiting Lectureship	Dr. and Mrs. Mark Ptashne
The Watson Fund for Innovative Graduate Education	Anonymous The William H. Donner Foundation The David and Lucile Packard Foundation The Seraph Foundation The G. Unger Vetelsen Foundation Mark and Karen Zoller

Total	**$5,766,654**

*New Pledges Awarded in 2002.

ANNUAL CONTRIBUTIONS

Corporate Sponsor Program

In 2001, a significant change was made to the structure of the Corporate Sponsor Program with the introduction of a new category, Corporate Benefactor. This category is intended for the very large pharmaceutical companies that have, over the years, been formed by the amalgamation of companies which were each a Corporate Sponsor. Pfizer Inc., which was about to merge with Warner Lambert, was the founding member of this group. We are delighted that no fewer than five companies followed Pfizer's example in 2002. These companies are Aventis Pharma AG; Bristol-Myers Squibb Company; GlaxoSmithKline; Eli Lilly and Company; Novartis Pharma AG; and Pfizer Inc. This support from some of the largest pharmaceutical companies made a most significant contribution to the Program.

The members of the Program receive special privileges in acknowledgment of their contributions. We waive all on-site fees for 16 representatives of the Corporate Benefactors and 8 representatives of the Corporate Sponsors at our meetings. In 2002, Corporate Sponsors could select from 20 meetings in Grace Auditorium. Of these scientists, Benefactors and Sponsors may send six and three, respectively, to meetings at Banbury Center, where attendance is otherwise only by invitation. Member companies receive gratis copies of Cold Spring Harbor Laboratory Press publications including the journals *Genes & Development, Learning & Memory, Protein Science*, and *Genome Research*. Our Sponsors are acknowledged in all relevant publications, including the books of abstracts given to each of the 7000 participants who come to the meetings each year. The names of the sponsoring companies are listed on the poster describing the meetings, and this is mailed to approximately 20,000 scientists throughout the world. In addition, the companies are listed on Cold Spring Harbor Laboratory Web Sites—those of the Meetings Office and Banbury Center.

The Corporate Sponsor Program continues to be critical to the vigorous meetings program held at Cold Spring Harbor Laboratory, whether at Grace Auditorium on the main Laboratory campus or at the Banbury Center. Only with the strong foundation provided by the program can we plan with confidence for the year's meetings and introduce new and unusual topics. We are, then, very grateful to all of the companies that joined us in 2002, and they are listed below.

CORPORATE BENEFACTORS
Aventis Pharma AG
Bristol-Myers Squibb Company
GlaxoSmithKline
Eli Lilly and Company
Novartis Pharma AG
Pfizer Inc.

CORPORATE SPONSORS
Abbott Laboratories
Amersham Biosciences, Inc.
Amgen Inc.
Applied Biosystems
AstraZeneca
BioVentures, Inc.
Chugai Pharmaceutical Co., Ltd.
Cogene BioTech Ventures, Ltd.
Diagnostic Products Corporation
Forest Laboratories, Inc.
Genentech, Inc.
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Association President's Report

2002 was another active year for our Association. Community outreach went particularly well, with close to 100 new members joining. Unrestricted funding for our most creative young researchers remained strong despite the lackluster economy.

Thanks to our generous supporters, the Laboratory continues to provide some of the top postdoctoral researchers in the world with opportunities to pursue highly unique avenues of investigation, rather than serving as assistants in established laboratories. This year, our Association members raised $790,729.51 for our Annual Fund, ensuring that this important Cold Spring Harbor Laboratory tradition endures.

Leadership is key to the Association. We welcomed David Banker, Lori Garofalo, and Richard Torrenzano as new Directors. Our most sincere thanks for exceptional outreach and genuine enthusiasm for the Laboratory during their tenures went to retiring Directors Nicholas Bartilucci, Robin Hadley, and Eileen Pulling.

Our February 4 Annual Meeting was followed by a delicious, heart-healthy dinner based on recipes from our engaging featured lecturer, Dr. Walter C. Willett, Professor of Epidemiology and Nutrition and Chairman of the Department of Nutrition at Harvard School of Public Health, and Professor of Medicine at Harvard Medical School. Much of Dr. Willett's work focuses on dietary determinants of heart disease and cancer, the subject of his recent book, *Eat, Drink and Be Healthy: The Harvard Medical School Guide to Healthy Eating*.

The annual *Jazz at the Lab* fundraiser on April 13 was an even greater financial success than last year. Featuring the classic jazz greats—Joe Bushkin on piano, Harry Allen on tenor sax, John Allred on trombone, Bob Merrill on trumpet, and Bucky Pizzarelli on guitar—the evening will long be remembered by guests who enjoyed a night of world-class jazz, preserved, as in 2001, on a CD. The concert was

Mary Beth Donohue, Bill Sheeline, Mary Lindsay, Cora Michalis at *Jazz at the Lab.*

followed by a delicious buffet dinner and a vibrant late-set performance. Special thanks for a beautifully organized and creative evening go to Committee co-chairs Mary Beth and Joe Donohue and Lisa and Gil Ott, and the Underwriting co-chairs, Susan and Mark Hollo, Susan and Bill Sheeline, Ginny and David Knott, and Cathy and Doug Soref. The evening's financial success was due primarily to the generosity of our Jazz 2002 Underwriters, listed below:

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Special events expanded this year to include a new and very successful annual science fair for local first graders from the Goose Hill School. Lots of input and organization from our own science teacher Director, Mary Alice Kolodner, made for a day that was a real hit with the children. Our young scientists, many of whom are supported by the annual fund, used the opportunity to hone their "explanation" skills to show our young neighbors exactly what it is that scientists do.

The Dorcas Cummings Lecture was held on June 2, 2002. Our guest speaker, Dr. Richard Lifton of the Yale University School of Medicine, Department of Genetics, fascinated the audience with his discussion on "Salt and Blood Pressure: New Insights from Human Genetic Studies." Thanks to the yeoman outreach efforts and organizational saavy of co-chairs, Lynn Gray and Cynthia Stebbins, 27 terrific dinner parties followed the lecture. A very special thank you to the friends in our community who generously hosted these dinners, welcoming into their homes visiting scientists from around the country world, our own Lab scientists, and friends.

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My wife Jamie and I enjoyed hosting the Partnership Picnic for Lab scientists and their neighborhood "partners" on Sunday September 22. The balmy weather and blue skies set the tone for a relaxed and sociable afternoon. Mary Beth and Joe Donohue graciously offered to host our Major Donor party on October 26. We were intrigued to learn from Senthil Muthuswamy, our featured scientific speaker, about his latest breast cancer research findings. There could be no better setting than the elegant comfort of the Donohue's living room to learn how our Association annual fund supports research that seeks to understand the causes of breast cancer.

The third annual Biotechnology Luncheon on November 21 was sponsored once again by the U.S. Trust Company at their attractive 47th Street offices. Guest speakers Dr. Emilio Emini, Senior Vice President of Vaccine and Biologics Research, Merck Laboratories, and Dr. John Inglis, Executive Director of the Cold Spring Harbor Laboratory Press addressed the topic, "Staying Alive: Fighting HIV/AIDS." Dr. Emini shed some light on a Merck AIDS vaccine currently in clinical trials, and Dr. Inglis discussed his fascinating trip to South Africa this summer where he and collaborators distributed nearly 20,000 copies the CSHL Press's children's book about AIDS prevention. Association Director Larry Remmel is largely responsible for filling every seat in the house for this enlightening, information-packed luncheon.

David H. Deming
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In 2002, the Laboratory recorded with deep appreciation generous support from the Estate of Elisabeth Sloan Livingston, a charitable remainder trust from Mrs. Samuel D. Parkinson, the commitment of a trust by Florence Strelkowski, and from another close supporter, a bequest in a will for the benefit of the Laboratory.

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The Undergraduate Research Program introduces college students to the skills they need to become research scientists. Twenty-five participants, 11 men and 14 women, from eight countries conducted research under the guidance of staff scientists for ten weeks this summer on projects of mutual interest.

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