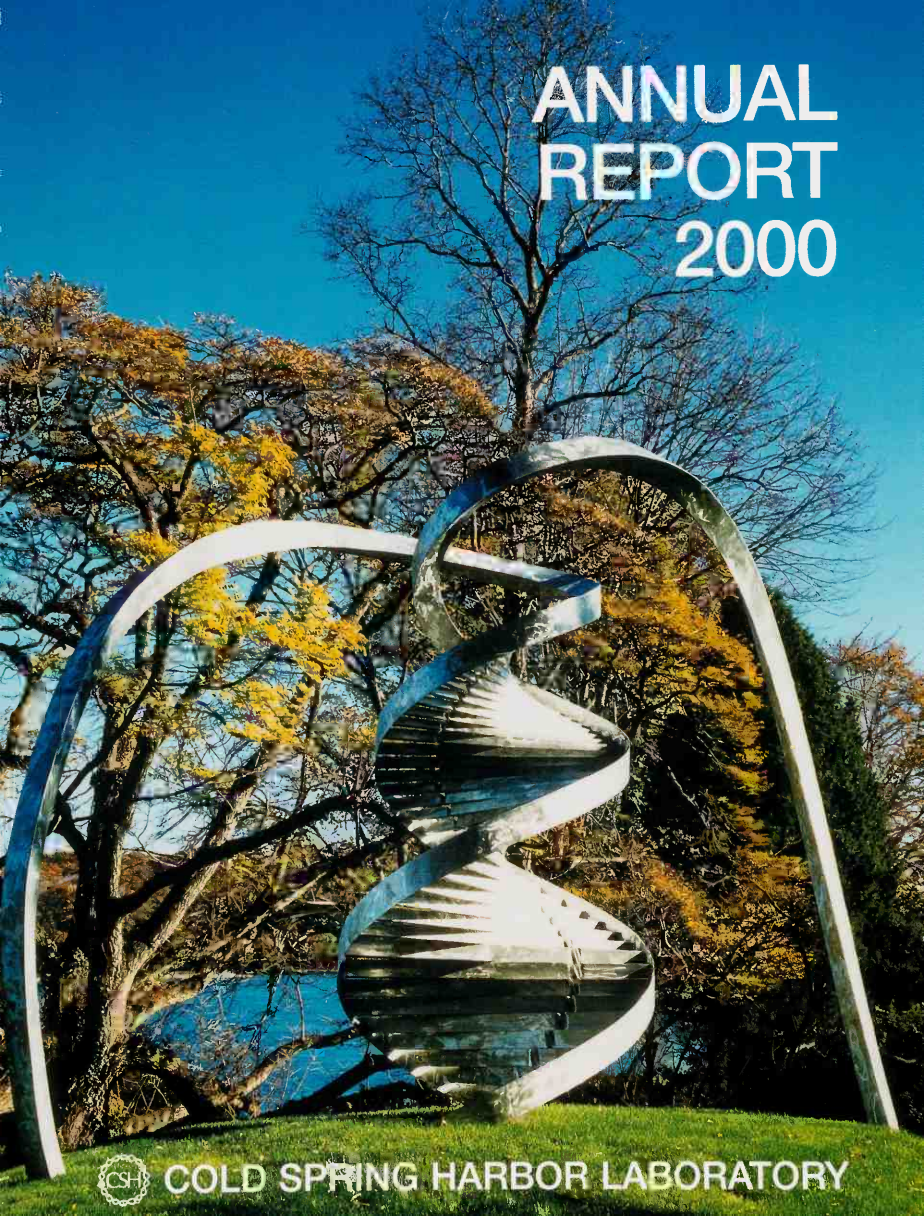
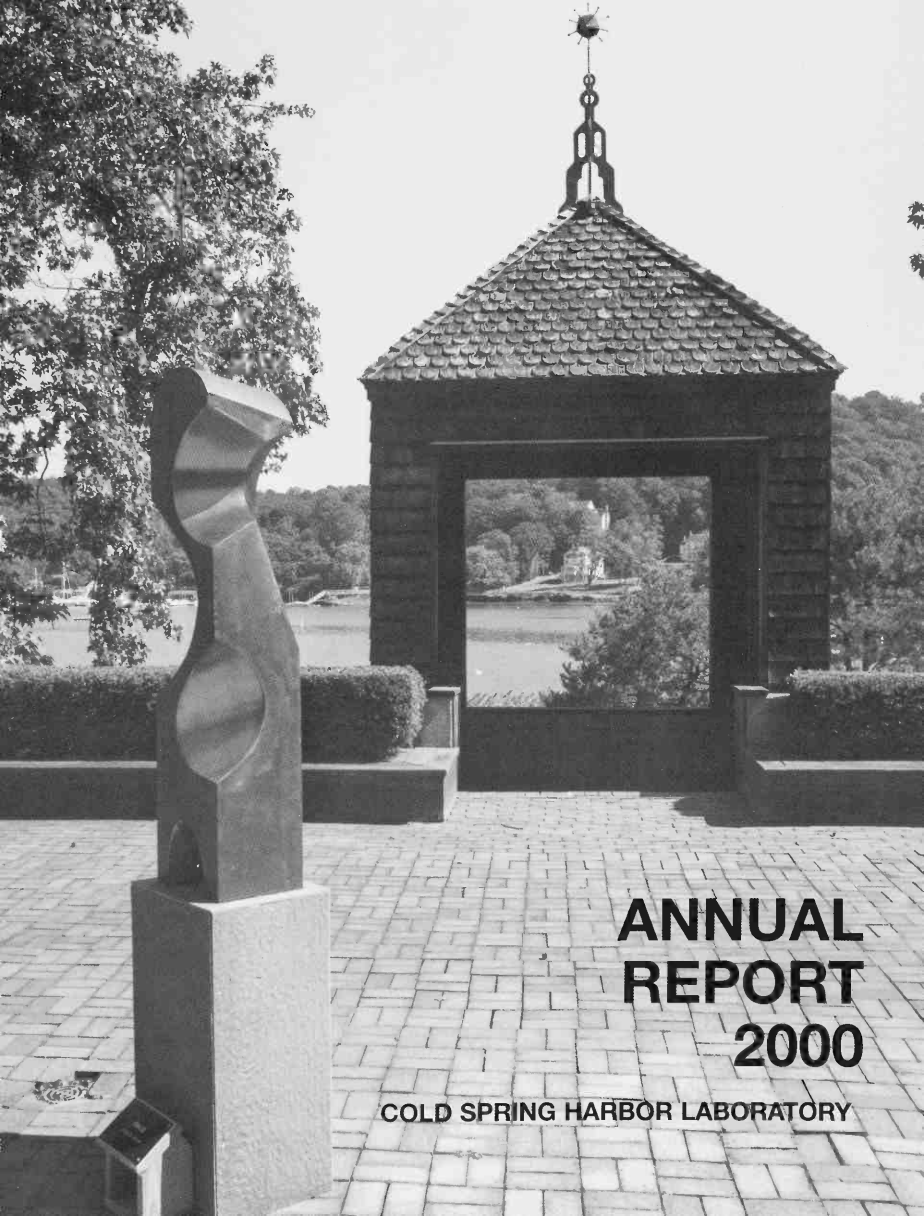
ANNUAL REPORT 2000



COLD SPRING HARBOR LABORATORY

Correction

On page 385, in the Financial Statements section of the Annual Report, Comparative Operating History 1996–2000, the Net Operating Excess for the year 2000 was erroneously printed as $1,224 (Dollars in Thousands). The correct Net Operating Excess is breakeven and should have been indicated, as in other columns, by "–". All other numbers are correct as printed.



ANNUAL REPORT 2000

COLD SPRING HARBOR LABORATORY

ANNUAL REPORT 2000

Cold Spring Harbor Laboratory
P.O. Box 100
1 Bungtown Road
Cold Spring Harbor, New York 11724

Website: http://www.cshl.org

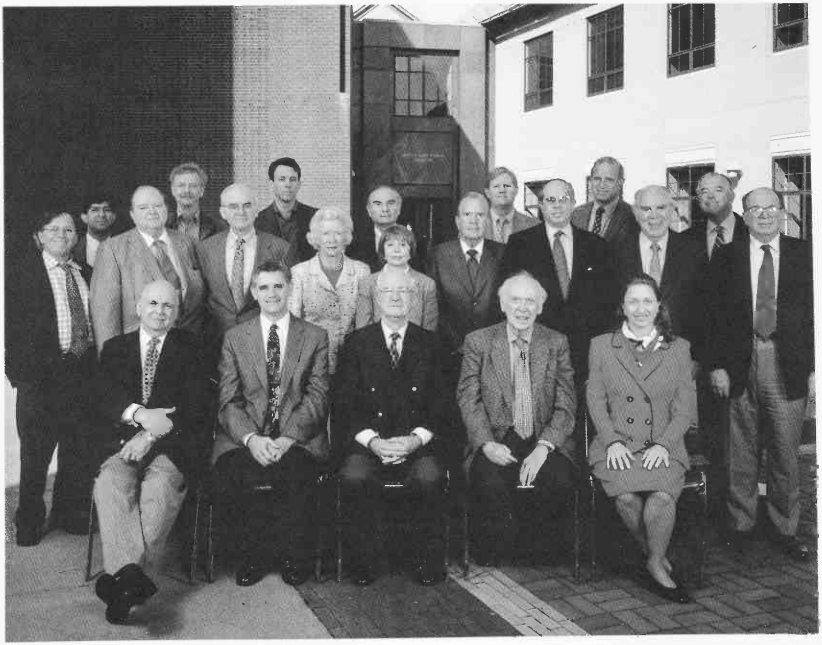
Managing Editor Jeff Picarello
Editorial Staff Dorothy Brown, Denise Roberts
Project Coordinators Inez Sialiano, Denise Weiss, Denise Roberts
Nonscientific Photography Miriam Chua
Desktop Editor Susan Schaefer
Cover Designer Tony Urgo
Book Designer Emily Harste

Front cover: *Spirals Time–Time Spirals*, a sculpture by Charles A. Jencks, installed at CSHL on August 31, 2000.

Back cover: The Samuel Freeman Building, part of the Neuroscience Center at CSHL. Dedicated: May 3, 2000. (Photo by Jeff Goldberg, Esto Photographics, Inc.)

Section title pages: Miriam Chua

Contents



CSHL Board of Trustees (November 4, 2000)

Front row: E.S. Marks, B. Stillman, W.R. Miller, J.D. Watson, L.N. Grace

Middle row: M. Ptashne, W.L. Matheson, C.E. Harris, M.F. Gerry, H.A. Dolan, A.M. Spiro, J.L. Spingarn, W.E. Murray, L.B. Polsky

Back row: J. Kuriyan, R. Jaenisch, J. Goldfield, J.J. Phelan, R.D. Lindsay, W.D. Pidot, L.C. Quick, Jr.

Not shown: E.E. Harlow, S. Hockfield, L. Mendell, C.J. Sherr, E.M. Skolnick, W.S. Robertson, H. Solomon

Officers of the Corporation

William R. Miller, *Chairman*
Edwin S. Marks, *Vice Chairman*
Lola N. Grace, *Secretary/Treasurer*
Whitney D. Pidot, *Asst. Secretary*

Bruce W. Stillman, *Director and Chief Executive Officer*
James D. Watson, *President*
W. Dillaway Ayres, Jr., *Chief Operating Officer**
G. Morgan Browne, *Chief Financial Officer**

Board of Trustees

Scientific Trustees

Edward E. Harlow, Ph.D.
Massachusetts General Hospital

Jeff Hawkins
Handspring, Inc.

Susan Hockfield, Ph.D.
Yale University

Rudolf Jaenisch, M.D.
Massachusetts Institute of Technology

John Kuriyan, Ph.D.
HHMI, Rockefeller University

Lorne Mendell, Ph.D.
State University of New York, Stony Brook

Mark Ptashne, Ph.D.
Memorial Sloan-Kettering Cancer Center

Edward M. Scolnick, M.D
Merck & Co.

Charles J. Sherr, M.D., Ph.D.
HHMI, St. Jude Children's Research Hospital

Bruce Stillman, Ph.D.
Cold Spring Harbor Laboratory

Individual Trustees

David H. Deming
Oyster Bay, New York

Helen Dolan
Oyster Bay, New York

Jacob Goldfield
New York, New York

Lola N. Grace
Glen Head, New York

Charles E. Harris
New York, New York

Robert D. Lindsay
Locust Valley, New York

Edwin S. Marks
Kings Point, New York

William L. Matheson
Mill Neck, New York

William R. Miller
New York, New York

Douglas P. Morris
Laurel Hollow, New York

William E. Murray
New York, New York

Whitney D. Pidot
Locust Valley, New York

Leon B. Polsky
New York, New York

Leslie C. Quick, Jr.*
Laurel Hollow, New York

William S. Robertson
Huntington, New York

Howard Solomon
New York, New York

Arthur M. Spiro
Kings Point, New York

James D. Watson
Cold Spring Harbor, New York

Honorary Trustees

Bayard Clarkson, M.D.
Memorial Sloan-Kettering Cancer Center, New York, New York

John Cleary, Esq.
Oyster Bay, New York

Martha Farish Gerry
Mill Neck, New York

H. Bentley Glass
Boulder, Colorado

Townsend J. Knight
New York, New York

Mary D. Lindsay
New York, New York

David L. Luke III
New York, New York

Wendy Vander Poel Russell
Oyster Bay, New York

*Appointed November, 2000, and effective January, 2001.
*Deceased

Governance

The Laboratory is governed by a 30-member Board of Trustees which meets three or four times a year. Authority to act for the Board of Trustees between meetings is vested in the Executive Committee of the Board of Trustees. The Executive Committee is composed of the Officers of the Board and any other members who may be elected to the Executive Committee by the Board of Trustees. Additional standing and *ad hoc* committees are appointed by the Board of Trustees to provide guidance and advice in specific areas of the Laboratory's operations.

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

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

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

Committees

Audit

Whitney D. Pidot, Chairman
William L. Matheson
William R. Miller
Arthur M. Spiro

Building

Helen A. Dolan, Chairman
Arthur Brings
John P. Cleary, Esq.
Mary D. Lindsay
Nancy Marks
Wendy Vander Poel Russell
Elizabeth Watson

Commercial Relations

William R. Miller, Chairman
W. Dillaway Ayres, Jr.
G. Morgan Browne
John P. Cleary, Esq.
John P. Maroney
Bruce Stillman, Ph.D.

Compensation

William R. Miller, Chairman
Lola N. Grace
Whitney D. Pidot
Leslie C. Quick, Jr.+

Development

Leslie C. Quick, Jr., Chairman+
W. Dillaway Ayres, Jr.
Richard Cosnotti
Martha F. Gerry
Laurie J. Landeau, V.M.D.
Mary D. Lindsay
David L. Luke III
William R. Miller
Wendy Vander Poel Russell
James L. Spingarn
James D. Watson, Ph.D.

DNA Learning Center

Arthur M. Spiro, Chairman
W. Dillaway Ayres, Jr.
Helen A. Dolan
Jack Leahy
David Micklos
Bruce Stillman, Ph.D.
Vincent Torti

Executive Committee

William R. Miller, Chairman
W. Dillaway Ayres, Jr.
G. Morgan Browne
Lola N. Grace
Edwin S. Marks
Leslie C. Quick, Jr.+
James L. Spingarn
Arthur M. Spiro
Bruce Stillman, Ph.D.

Finance and Investment

Lola N. Grace, Chairman
W. Dillaway Ayres, Jr.
G. Morgan Browne
Charles E. Harris
William D. Keen
Robert D. Lindsay
Edwin S. Marks
William R. Miller
William E. Murray
Leon B. Polsky
William S. Robertson
Lari C. Russo
Howard Solomon

Nominating

Robert D. Lindsay, Chairman
W. Dillaway Ayres, Jr.
William R. Miller
Whitney D. Pidot
Leslie C. Quick, Jr.+
Bruce Stillman, Ph.D.

Banbury Center, Robertson/Meier Houses

William S. Robertson, Chairman
W. Dillaway Ayres, Jr.
Jan Witkowski, Ph.D.
James D. Watson, Ph.D.

Robertson Research Fund

Carl Schafer, Chairman
W. Dillaway Ayres, Jr.
G. Morgan Browne
Katherine Ernst
Winship Herr, Ph.D.
Walter Meier, M.D.
William R. Miller
William S. Robertson
Bruce Stillman, Ph.D.

Tenure and Appointments

Edward Harlow, Ph.D.
Susan Hockfield, Ph.D.
Rudolf Jaenish, Ph.D.
John Kuriyan, Ph.D.
Lorne Mendell, Ph.D.
Mark Ptashne, Ph.D.
Edward M. Scolnick, M.D.
Charles J. Sherr, M.D., Ph.D.
Bruce Stillman, Ph.D.

+Deceased

Leslie C. Quick, Jr.

Leslie C. Quick, Jr. (1926–2001)

Leslie Quick, who long lived near our Lab in Laurel Hollow, was a happy, generous philanthropist who took much pleasure in using his seaplane to commute to and from New York City. The main office of Quick & Reilly, his extraordinarily successful discount stock brokerage firm, was in the Wall Street area. His formative school years were on Long Island, where he attended Andrew Jackson High School in Queens and there met his future wife, Regina Clarkson. Both of his parents were New Yorkers. His father was of Episcopalian background and was a manager at Allied Chemical, a position that early in Les's life necessitated frequent changes of job location before his parents settled in Queens Village. On his mother's side, he was descended from the Galway man, Henry Gratin Kilroy, who after coming to the United States became a policeman assigned to harbor patrol. This twist of fate allowed him to open a speakeasy on Staten Island with contraband collected in exercise of his official duties. Some 30 years later, his equally entrepreneurial grandson pursued a business degree at Pennsylvania Military College (now Widener University) after his wartime service in the Army Air Force in the Pacific. Ten days after his graduation in June 1950, he married Regina and took a position with the Addressograph Company as a Wall Street representative.

Les stayed with Addressograph for five years before taking a $6000 pay cut to move into publishing consultantcy. This step soon led to his 13 years at Forbes Magazine, where he rose to become a Vice President as its business manager. The prospect of being himself in finance, as opposed to being part of writing about it, led him, in 1969, to join Wallace Forbes in launching a money management company. Three years later, Les bought the company, and during the course of the next two years partnered with Kevin Reilly to form the brokerage firm Quick & Reilly. The days were not easy, with disagreements between the partners leading to Reilly's departure within two years. By then, the course of Quick & Reilly was irrevocably set by Leslie's decision to become a discount broker. This was the first time any member of the New York Exchange had ever offered discounted commissions of 50% or more to all comers. By then the firm had acquired a Palm Beach office, and the phones there as well as in the New York City office were ringing off the hook. Through all the subsequent ups and downs of the market, Quick & Reilly never made less than 30% pretax profits. Much of the success came from Les's uncanny genius for reading market trends as well as his ability to build long-term friendships through fair dealing and unbending integrity.

His four sons, Leslie III, Tom, Peter, and Christopher, were brought into the family business even before they had finished college. With them, he spent one frantic Sunday poring over the finances of a NYSE specialist firm entering bankruptcy. The next morning, Les offered to buy the firm for 4 million dollars and so beat a very annoyed Carl Icahn. Now led by his son Christopher, the specialist firm makes markets in 430 companies that comprise 17% of total big board volume. Earlier, Les founded a clearing firm after rivals, upset by his successful discounting, had coerced their previous clearing firm to stop handling Quick & Reilly trades. Within a year, his new business turned a profit. Now it successfully operates under the leadership of his oldest son, Leslie III. At all times, Les showed real flair for sensing out companies whose stock prices would soon move upward. Upon visiting a drill bit firm in Texas where he learned that their rival, Hughes Tools, would soon have a superior product, he rushed home to tell his brokers to load up on Hughes Tools. That move covered half the costs of his sons' college tuition.

Quick & Reilly went public in 1983, giving it capital that let it expand faster, eventually to comprise some 120 investor centers serving more than 1.3 million clients. Three years ago,

in 1998, Quick & Reilly was sold to the Fleet Financial Group in Boston under an arrangement that would let Leslie and his family continue to control its operations for the five succeeding years. Although Les had by then passed 70, his was still a vigorous presence, both physically and intellectually, and he will never be remembered for ever having voluntarily slowed down. At the age of 60, he acquired the pilot's license to let him, not his pilot, be in command of his seaplane and no one was surprised when he piloted it under the Brooklyn Bridge. And at 65, he acquired his very own ocean-going 126-foot yacht that he brought several times into Kinsdale on trips to Ireland where he hoped to pinpoint the exact birthplace of his grandfather so as to qualify for dual Irish citizenship.

On Long Island, Les and Jean presided over three successively larger homes. The first was in Levittown, where mass-assembled homes let denizens of Brooklyn and Queens move into clean air and safer streets. From there they moved to a Woodbury house that gave them the space for their ever-growing family, and where their sons used their bikes to deliver the morning papers before they went off to school. By 1978, Les had the means to move into one of the large comfortable brick homes built on the site of the former Rutherford estate just to the north of the Laurel Hollow Police Station. Their Florida houses in the Palm Beach region were likewise increasingly commodious, with the current family home on the beach near Lost Tree Golf Club. It was formerly Greg Norman's residence. Liz and I were invited there two winters ago, arriving to find that Les, just six months recovered from a successful heart by-pass operation, proposed piloting us in his Cessna on a joy ride to and from Vero Beach. Without hesitation, we accepted, knowing that Les in no way would take a risk that would cut down a life that gave him so much joy.

To his last days, he was as much a happy kid as he was a caring, honest leader of business and a devoted family man. He bought his first Rolls Royce the day Roy Kroc died. He didn't want to mistakenly not enjoy his new wealth before it was too late. At the same time, he was increasingly active in supporting Catholic causes, being a key founding Trustee of the Inner City Scholarship fund set up by Cardinal Terence Cook, as well as playing a major role in the transformation of St. Francis Hospital in Port Washington into one of our nation's leading centers for heart surgery. In addition, he was a prime patron of the Alfred E. Smith Foundation and served on the board of Fairfield University. For all his many services to the Catholic Church, he became a Knight of Malta and a Knight of the Equestrian Order of the Holy Sepulchre of Jerusalem. He was also long a Trustee of his alma mater Widener University.

Once he came on our Board just three years ago, he quickly saw our challenges ahead and, never being a passive member of any Board, soon was chairing our Board's Development Committee. Then the chief item on its agenda was funds to support our soon to be launched School of Biological Sciences. Immediately he stepped up to bat with a million-dollar gift to endow a student fellowship, as well as an equally large sum to endow a fund for our Core Course in Scientific Reasoning and Logic. Soon to follow were 300,000 more dollars to endow one of our faculty lectureships. More recently, Les was excited by our plans to expand our cancer research efforts in our new Woodbury Genome Center as well as new facilities on our main campus. But by then he had all too suddenly learned that the summer pain in his legs that he thought was sciatica had a much more ominous origin—a malignant chondrosarcoma that had already spread to his lungs. This awful news came only several days after he had been honored as Irishman of the Year at the Waldorf Astoria by the Irish Historical Society. It was a proud occasion for him and his family and all those countless individuals blessed to have felt his warmth and vigor on behalf of the public good.

Although his prognosis was inherently grim, the thought of Les dying did not want to enter our minds. He was irreplaceable and much too nice to go this way of pain. Just before Christmas, Liz and I brought him and Jean a Guiness-filled cake to share with his children. Although his cancer was filling up his lungs, he had just gone with Tom to buy a new motorcycle and already been taken for a ride. Later, he had Chris sneak him out of his house to let him one last time see our Lab buildings and beautiful snow-covered grounds. Later in Palm Beach, when he knew the end was near, he banished temporarily the thought by ordering a red Bentley convertible. Learning that one of this color was not available, he settled for a second Rolls Royce.

His last days were spent in Boston at the Dana Farber Cancer Center, whose anti-cancer drugs five years before had cured a son of his daughter Nancy of leukemia. But even their newest weapons were not equal to his relentless cancer, and he died with Jean and his children about him on Thursday, March 8. On Saturday, a private funeral Mass was said at St. Dominic's in Oyster Bay, with burial in the beautifully sylvan Locust Valley Cemetery. The following Monday, Cardinal Egan celebrated a Mass for him in a filled St. Patrick's Cathedral on Fifth Avenue.

At six in the evening, the day after Les's death, the Lab held a long-scheduled, pre-St. Patrick's Day concert in Grace Auditorium by the Irish-American ballad singer, Cathie Ryan. Her haunting songs four months before had highlighted the Waldorf banquet honoring Les. Singing again *Danny Boy*, the tears it so easily generates flowed even more copiously.

Our good must stop dying of cancer.

16 April 2001 **James D. Watson**

DIRECTOR'S REPORT

There are times when it is exhilarating, but also humbling, to be a member of a privileged fellowship who collectively call themselves scientists. The year 2000 was such a moment, for we saw extraordinary progress in understanding the fabric of life, the sequence of bases in DNA that encode our genetic information. Following past successes in the sequencing of the genomes of many microbes—the yeast *Saccharomyces cerevisiae* and the small worm *Caenorhabditis elegans*—a flurry of important genome sequences emerged during 2000, all with great fanfare. Early in the year, we saw the publication of the sequence of the genome of the fruit fly *Drosophila melanogaster*, which was a result of a successful collaboration between academic scientists and the private sector, a harbinger of future genome projects. In the middle of the year came the highly coordinated, multinational announcement that the drafts of the human genome were complete, following intervention and direction from President Clinton in this country and Prime Minister Blair in the U.K. The publication of the draft human sequences appeared in early 2001, again with much public attention. With lesser blowing of the trumpet, the last month of the year saw the publication of the complete sequence of the first plant genome from *Arabidopsis thaliana*. Thus, for the first time, it is possible to gaze into the intimate genetic details of organisms from all kingdoms of life. As a consequence, public interest in biology and medicine is at an all-time high and probably rivals public interest in science following other monumental scientific and engineering feats, such as landing a man on the moon and the splitting of the atom. We have been fortunate to witness and participate in these dramatic advances. A new era of biology has blossomed that will long have profound consequences for science and indeed for society as a whole.

The revolution of recombinant DNA that emerged in the early 1970s made it possible to obtain unlimited amounts of virtually any DNA. Imaginations ran wild, but like so many endeavors in science, technical limitations still kept biologists at bay. Perhaps the clearest and most difficult technical challenge was to figure out how to read the sequence of individual genes. By the mid 1970s, just as I was entering graduate school in Australia, two vastly different approaches to DNA sequencing emerged. One from Wally Gilbert's laboratory in Cambridge in the U.S. used a chemical sequencing approach, whereas that from Fred Sanger's laboratory in Cambridge in the U.K. employed enzymatic methods. The two different technologies from the two Cambridges were complementary, but early on, each had its advocates as to which one was best. It was rather amusing to listen to young Australian scientists returning from studies in either the U.S. or the U.K. tout the virtues of *their* favorite approach to gene sequencing, depending on the country of origin. In those days, both methods were important, and it was necessary to learn both to be a successful graduate student. In retrospect, these developments proved, unwittingly, to be the first multinational collaboration in the DNA sequencing era, for the sequencing problem was essentially solved and now could be scaled up.

Progress was rapid, and within a year, the genome of a bacteriophage (a virus of bacteria) called ϕX174, which contains approximately 5375 nucleotides, was reported from Sanger's group. It was equally exciting to learn in 1978, during my first visit to Cold Spring Harbor for the annual Symposium, of Greg Sutcliffe's determination of the complete sequence of the then-favorite bacterial cloning plasmid, pBR322, using the Maxam and Gilbert technique. It was only

a matter of time before the concept of sequencing the whole human genome began to be discussed, notably at a meeting organized by Robert Sinsheimer in 1985 at the University of California, Santa Cruz. But the idea of determining the complete sequence of the human genome was controversial, as many biologists saw the cost as being too high. This was a time when grants were particularly difficult to obtain because of limited funding, and there was considerable concern that such an ambitious project could not be completed for technical reasons, even if sufficient funds could be found. During the Cold Spring Harbor Symposium in 1986, Jim Watson brought together some of the leading biologists to discuss the genome sequencing proposal: Was it feasible and, of particular importance, who should fund the considerable cost? Those days now seem far in the distant past, but it was only 14 years ago, a short time in the history of molecular biology. Wally Gilbert's prediction at that meeting that we would all carry our genetic sequence on a credit card then seemed far too fanciful, but it is now well within the realm of possibility.

Shortly after the 1986 meeting, spurred by the start of a human genome project by the Department of Energy and helped by discussions organized by the National Academy of Sciences, the National Institutes of Health began the project with considerable enthusiasm. Much of the early success of the project in the U.S. was due to the outstanding leadership of Jim Watson in establishing in this country the NIH Human Genome Research Center (later an Institute at the NIH). All biology benefited by his promoting the sequencing of model organisms, such as yeast, *Drosophila*, and *C. elegans*, and by focusing early money on ethical issues. The introduction of new techniques was of great importance to the success of the project, particularly the contribution by Lee Hood and Mike Hunkapiller in developing the automated DNA sequencing machines that eventually got the job done. Francis Collins, the second and current director of the U.S. effort, skillfully directed the difficult stage of scaling up the human genome sequencing project and shepherding the public effort to near completion. In the U.K., John Sulston and the Wellcome Trust, together with Bob Waterson in this country, played an essential role early on by committing considerable effort and resources (and their scientific reputations) to model organism genome sequencing and later, at a critical time to the human genome project, when it needed to scale up. Later, the introduction of a private effort by Craig Venter and his colleagues at Celera dramatically accelerated the progress, although the introduction of the private sector into genome sequencing has created the new problem that not all of the sequences are available to all those who want to use them.

Congratulations are due to the community of genome sequencers, particularly those supported by public funds, from the heads of the various genome centers down to the technicians who did much of the work. They have shown that it is possible to undertake large, internationally based science projects and get the job done with a remarkable degree of cooperation. But more importantly, they have shown that it is possible for very talented scientists to participate in large multi-investigator science projects that benefit all of science, even though individuals might not have received the recognition that might have come had they continued to run a conventional, small laboratory. I have observed at the annual Cold Spring Harbor genome meeting a real sense of purpose and camaraderie among the scientists who cooperated in this project. Unfortunately, this message has been lost to the public, who have instead been inundated with the public-private competition issue by the popular press. One hope is that history will focus on the truly remarkable collegiality that was evident during this time.

The genome projects have also benefited us all in another direct way. Now that many genomes are complete, funding has picked up because we have captured the imagination of the public and Congress and because there is a more realistic hope of successfully tackling human diseases, particularly cancer.

The first eukaryotic genome to be sequenced was that of the baker's yeast, *S. cerevisiae*, completed in 1996. Done in cottage industry style by investigators from many countries, the yeast genome became the model for how complete genome sequences might be used. My own work on the replication of chromosomes, in part using yeast as a model organism, has greatly benefited from the freely available genome sequence. Now that we have the human genome sequence almost complete, it is perhaps worth reflecting on how the yeast genome has helped progress in yeast biology and biology in general.

Knowing the entire genome sequence immediately establishes a closed system for understanding information flow within the organism. Since all of the genes are known, when a new activity or function is discovered, it is possible to search the entire genome to discover whether there are other similar proteins that might perform related functions. In this manner, families of proteins are rapidly discovered. Because biology reuses protein domains and activities for multiple purposes, progress in understanding one pathway will often lead to insight into other biochemical processes. This iterative accumulation of biological knowledge will not only help us to understand the biochemical processes in yeast, but, because most yeast genes have functional or sequence-related homologs in the human genome, also make it possible to predict with high accuracy protein function in human cells. For this to occur efficiently, it is necessary to know the entire human genome sequence, and as more genomes are sequenced, including those of other model organisms, the information flow from one system to another will increase rapidly. This is, perhaps, the strongest argument for sequencing the genomes of all of the important organisms used in modern biological and biomedical research.

Developments in the methodology to rapidly analyze proteins by mass spectrometry have also dovetailed with whole genome sequencing in a most productive and informative way. Very small amounts of proteins can be fragmented and the mass of the protein fragments determined by mass spectrometry. These masses can then be compared to a computer-generated database of all predicted fragments from all predicted proteins encoded in a genome. If sufficient fragments are determined experimentally, then the proteins can be uniquely identified. So it is now possible, and indeed routine in the yeast community, to use antibodies to isolate a protein present in a complex mixture of proteins from cells and then rapidly identify all of the proteins with which it interacts. Since most biological functions involve proteins working together in biochemical pathways, we will move toward the possibility of knowing many of the protein networks in cells, and even the protein modifications that occur in response to signals that the cell receives. Such an analysis and the insight it provides were not possible without knowing the entire genome sequence.

Another important technical advance from studies on yeast that has been effectively integrated with whole genome sequencing is the development of DNA microarrays. The concept of arraying small amounts of DNA for biological analysis emerged from the pioneering studies of Ed Southern in the 1970s, when he showed that it was possible to transfer DNA to fixed membrane supports and hybridize DNA or RNA to the arrayed DNA. Once the entire genome of yeast was known, it became practical to array all the predicted genes on glass slides so that all of the genes of yeast could be analyzed simultaneously, instead of one gene at a time as was done previously. In this way, the dynamics of gene expression patterns could be followed as cells responded to experimentally induced signals such as nutrient starvation, changing of carbon sources, and commitment to divide or exit from the cell division cycle. The method has become one of the most powerful tools to study the reaction of an organism to biological perturbations. It has only been possible by having the complete genome sequence available.

Coupled with the protein analysis described above, DNA microarray experiments allow a very intimate look at the molecular physiology of a cell and how it functions. We can see, on a whole genome scale, how cells work and how they adapt to their changing environment. From what we

have learned from studies on yeast biology during the last few years, it is probable that cell, tissue, and organism physiology will return as a dominant area of investigation but studied now at the molecular level. When this type of analysis is applied to animal studies, it will be possible to see how an organism responds to all sorts of perturbations, leading to an unprecedented understanding of biology and physiology. This is happening at a rapid pace. Already, DNA arrays are being used effectively to study the response of animal cells to extracellular signaling and to drugs used in the clinic. A particularly innovative analysis is under way at Cold Spring Harbor Laboratory by Tim Tully and Josh Dubnau who have identified fruit fly genes whose expression is altered during the process of learning a task or consolidating memories of the learned task. It is almost like watching the brain think!

With so much data, the field of bioinformatics, or computer-assisted analysis of biological information, has rocketed to become one of the most exciting fields of biology. Judging by the number of applications we get for a limited number of places available, the two bioinformatics courses taught each year at Cold Spring Harbor are now the most popular of all our advanced courses. Again, the yeast community has been a pioneer in the field of bioinformatics because of the availability of the entire genome sequence during the last six years and because of the need to deal with the vast amounts of data that derive from whole genome experiments. But bioinformatics includes much more than the analysis of DNA sequences. Efforts within the yeast community have developed databases that link the scientific literature to the genome. Genes and their protein products, and even the pathways in which they function, have become a foundation for computationally organizing biological information. Searching these databases has become as routine as searching the printed literature. This is perhaps the best argument for supporting a single electronic database of all research papers so that the biological literature can be fully integrated with the large number of databases that are being developed by computer scientists. Publishers of the scientific literature must strive to make science as easy as possible by ensuring that the on-line literature is linked in a seamless and accessible way.

Because biomedical and biological research has flourished, the literature is growing at a pace that far surpasses even the most avid reader. And yet the biological community has only just begun to use computational approaches to analyzing the literature. Information science will become a dominant field of biology and computer scientists will need to be integrated as much as possible with biologists. At Cold Spring Harbor, we are strengthening our already broad bioinformatics research by adding computer scientists to our faculty so that they can develop new technologies in consort with biologists.

Within the yeast community, there have been a number of initiatives aimed at providing whole genome resources, such as creating a complete set of yeast strains in which all genes have been deleted; or tagging all proteins with various sequence tags, marking each gene with a unique bar code; or attempting to identify all possible protein-protein interactions using genetic methods. In retrospect, some of these initiatives have not been as productive as anticipated in the beginning. For example, the time it takes to delete a gene is very short compared to the time it takes to know the consequences of doing such a deletion. Therefore, the availability of a complete set of gene disruptions has not yet hastened progress. Thus, we should think carefully before whole genome approaches for other organisms are attempted.

So what of the human genome? Because it is our genome, there have been sophistic writings in both the scientific and public literature that the gene sequences will reveal what it is to be human, reveal the nature of the soul, and even explain human behavior. Most of this has been overly excessive piffle put forth by those who can only be excused for getting too caught up in the momentous occasion. Many discussions about the implications of the genome sequence have also been flavored by the advent of cloning animals from single cells and the consequent,

way too premature talk of human cloning. Knowing our gene sequence, or even a mouse genome sequence, is not going to help overcome all the very considerable technical obstacles that still exist in cloning other mammals. We simply do not understand enough about the methods for producing animals from individual cells. We do not know much about how gene expression programs are reset before development can occur. Clearly, research on cloning should proceed, but knowing the full human genome sequence will only marginally help solve the significant hurdles that exist, and the two areas of science must not get confused when there is discussion about future possibilities.

The achievement of obtaining the sequences of many genomes, including the human genome, is a major milestone in science. Certainly, when the double helix was revealed, it was unimaginable that the entire nucleotide sequence of a genome could be obtained. I still find it humbling to realize that we are in a golden age of biology that will have far-reaching consequences not only for our own science, but also for humankind. At the same time, we should have realistic expectations of what will emerge from these spectacular developments.

On a practical level for most scientists, research has been made much easier because of the reagents that have derived from the genomics age. Clones of genes and fragments of genomes are readily available, as are the predicted sequences of most proteins (we do not yet have the computer tools to predict all protein sequences accurately). These resources have been put to great use, speeding up the pace of biological discovery manyfold. This progress in itself has been a silent revolution, perhaps only appreciated by the scientists actually doing the work. Many of the advantages which have become available to the yeast community during the past six years are now available to those working on human biology, including arrays of human genes, protein analysis by mass spectrometry, and comparisons to the predicted "proteome"—the set of all proteins. It is now possible to analyze the changes in gene expression of the entire set of known human genes in response to physiological changes in cells and tissues. Although it is early in this analysis, new and exciting findings have been reported in the literature. Such experiments have already led to new methods for diagnosing human disease and to the discovery of new targets for therapy. In some cases, the cause of disease has been discovered by the power of being able to compare gene sequences between diverse species, such as those of *Drosophila* and human, or even yeast and human.

One of the most interesting aspects of whole genome sequencing in humans is the diversity of sequences that are being uncovered. It is estimated that there is one difference between individuals for each 1300 bases in the human genetic code of 3,000,000,000 bases. This means that we are all about 99.9% identical, something that itself is quite remarkable. But if turned around the other way, then it means that there are about 3 million differences at the primary DNA level between individuals. Most of this variation will not be expressed, but some of it will. This means that individuals will not only have different shapes and sizes—something that we all know about—but also have different probabilities of being afflicted by disease and, when treated, different responses to drugs and other therapy. Such variation will become valuable for predicting how patients might respond to certain drugs, allowing treatments to be targeted to individuals who will benefit from the drugs while avoiding adverse effects of the same drugs in others.

Knowing more about human genomic variation also has the potential to change how we view ourselves as a species. By knowing more about human DNA variation, we will realize that traditional ethnic and cultural boundaries will not be reflected in our DNA, but rather will be purely a human invention with no genetic (and maybe even no biological) basis. If this turns out to be true, and it is really understood by the lay public, then cultural and ethnic differences may not be as dominant in future human endeavor. But, change will occur only very slowly, and this may be an unattainable utopian goal.

Clearly, whole genomic approaches to biology are changing the way we think about dealing with human disease, and perhaps this is what the public finds the most appealing. One example at Cold Spring Harbor Laboratory is the whole genome analysis of human cancer by Michael Wigler, Robert Lucito, Masaaki Hamaguchi, Vivek Mittal, and their colleagues. By combining a method called RDA (representational difference analysis) developed in Wigler's laboratory about eight years ago with high-density arrays of human DNA placed on glass slides, it is now possible to analyze small biopsies from human cancers and compare DNAs from the normal and cancer cells. Initially focusing on breast cancer, this research is already leading to exciting new results. There are new possibilities for diagnosing the disease and for identification of new anti-cancer targets. From such genomic analyses, cancers that look alike to a pathologist can be molecularly identified and classified, and understood as being separate diseases with separate outcomes.

Such analysis of breast cancer has also led to the discovery of new gene mutations in human cancers. It is already clear from the analysis of breast cancer samples that many altered regions of the genome have not been characterized, particularly in cancers derived from patients who have no known family history of the disease, which represents the vast majority of breast cancer cases. By correlating the alterations with the type and severity of the cancer, and with the response of the cancer to existing treatments, such knowledge will lead to better decision-making by oncologists. But the more important goal is to identify those genome alterations that suggest new, cancer-specific targets for therapy. Already the identification of one genetic lesion in a subset of human breast cancers has led to a novel therapy. In about 25% of breast cancers, a gene called *Erb2* is overexpressed. This knowledge led to the development by Genentech of an antibody-based drug called Herceptin which has proven effective in treating a subset of breast cancer patients. Our goal for the cancer gene discovery at Cold Spring Harbor Laboratory is to identify the key therapy targets in all classes of breast cancer. Furthermore, the methods that have been developed and applied for breast cancer research can easily be applied for other cancers, given sufficient funding.

Such scenarios will play out for many human diseases. But a caution should be noted as we all celebrate the fantastic achievements of the recent past and speculate on how quickly genomics will change our real understanding of biology. Genes make proteins, and proteins, not genes, determine how we look, how we behave, what diseases we get and how they are treated, and even how we respond to our environment. Because of gene splicing, gene rearrangements, and developmental diversity in gene expression patterns between individuals, biology is much more complex than the genomic view would suggest. Proteins can be modified in many different ways, thereby changing their function. It is possible for a single gene to encode many proteins with diverse, and sometimes even opposite, functions. This is not always apparent from gazing at the gene sequence, even with powerful computers. Our present methods for discovering the functions of proteins and the pathways in which they operate are not yet rapid enough, particularly when applied to mammalian biology. Although integrative approaches, such as comparing gene functions across diverse species, are clearly paying dividends, biology is much more complex than the set of genes that make up a genome. We need to think about greatly improving computational approaches to biology, finding better experimental ways to characterize protein diversity, and most importantly, speeding up discovery of protein function. Once this can be achieved, and we learn how to integrate this knowledge into a molecular understanding of cell and organism physiology, the true power of the genome will be unleashed.

HIGHLIGHTS OF THE YEAR

The research and education programs at the Laboratory continue their strong momentum. The Watson School of Biological Sciences recruited its second class of students this year, and the DNA Learning Center underwent extensive renovations that will further its educational objectives. The Meetings and Courses program and Banbury Center continue to be invaluable resources for scientific information, and the Cold Spring Harbor Laboratory Press added new projects and properties to its long list of titles. In this, the year of the Human Genome, Cold Spring Harbor Laboratory was a bustling center of scientific activity.

Research

Cancer

Scott Lowe

Malignant melanoma is an aggressive, deadly cancer that does not respond to conventional chemotherapy. Other aggressive, chemoresistant cancers—and approximately half of all cancers—are characterized by mutations in the *p53* tumor suppressor gene. Malignant melanomas, however, do not typically display mutations in the *p53* gene.

To explore alternative explanations for the origins and properties of malignant melanoma, and to identify potential targets and strategies for therapy, Scott Lowe and his colleagues have examined the status of other genes known to function downstream from p53 in a pathway leading to "apoptosis" or "programmed cell death." When intact, this pathway rids the body of abnormal, precancerous cells by triggering a cellular self-destruct mechanism. When this pathway is disrupted (by the loss of p53 function, for example), precancerous cells survive and proliferate, resulting in cancer.

This year, Scott and postdoctoral fellow Marisol Soengas found that malignant melanomas often lose a key trigger of programmed cell death, a protein called Apaf-1 (*apo*ptosis *a*ctivation *f*actor-1). They also discovered that the loss of Apaf-1 in melanoma cells is associated with resistance to the chemotherapy drug adriamycin. Most significantly, Scott and Marisol showed that restoring Apaf-1 in melanoma cells rescues the ability of these tumor cells to kill themselves in response to adriamycin. Thus, accurate diagnosis and treatment of malignant melanoma, and perhaps other cancers, should include an assessment of the status of Apaf-1. Scott and Marisol were joined in this work by Yuri Lazebnik, who is examining how Apaf-1 acts in concert with an enzyme called caspase-9 to trigger the cell death machinery.

RNA Splicing and Breast Cancer

In 1977, CSHL scientist Richard Roberts and his colleagues published a paper whose title began "An amazing sequence arrangement..." Rich's use of the adjective "amazing" in a serious scientific article was highly unusual and strangely prescient, because the paper reported a discovery for which he would later win the Nobel prize. A number of papers from Cold Spring Harbor Laboratory and MIT published the discovery that genes are not usually contiguous, but rather occur as blocks of relevant coding sequences (later called exons) that are interspersed with blocks of intervening, noncoding sequences (introns).

Introns are removed at the level of RNA by a splicing mechanism. This year, Adrian Krainer and his colleagues discovered why particular mutations in the *BRCA1* gene alter premessenger RNA splicing and thereby predispose individuals to breast and ovarian cancer.

Adrian Krainer

Although complex in detail, the basic splicing mechanism involves cutting a premessenger RNA molecule at two sites (the splice junctions), discarding the intron thus released, and joining the exons together. This basic splicing mechanism is mediated by the binding of splicing factors to the splice junctions and the action (e.g., cutting and joining) of splicing factors at these sites. Recently, however, additional sites that stimulate splicing have been identified within exons. These sequences are called exonic splicing enhancers or ESEs. Adrian and his CSHL bioinformatics colleague Michael Zhang have developed rules for predicting the presence of ESEs within genes of particular interest. They discovered that a mutation in the *BRCA1* gene—known to alter splicing and predispose individuals to breast and ovarian cancer—disrupts an ESE. In addition, Adrian found that disruption of ESE function may contribute to many other diseases, including cystic fibrosis, neurofibromatosis, Wiskott-Aldrich syndrome, and Menkes disease.

Papillomavirus DNA Replication and Cervical Cancer

DNA contains specialized regions—called origins of replication—that enable the stable "seal" between the two strands of the double helix to be broken. This breaking of the seal between the strands of DNA is the first step in DNA replication, the process that copies DNA. It results from the action of enzymes called DNA helicases that bind to origins of replication and unwind the DNA. Arne Stenlund is investigating how the DNA helicase of papillomavirus binds to the origin of replication in the viral genome and initiates the unwinding of viral DNA prior to DNA replication, a process necessary for the virus to multiply and spread. Papillomaviruses are a family of DNA tumor viruses that infect mammals and cause both benign and malignant epithelial tumors such as polyps and cancer of the cervix. Virtually all human cervical cancer can be attributed to papillomavirus infection. Therefore, the papillomavirus DNA helicase is an attractive target for antiviral drugs and other treatment strategies.

Arne Stenlund

Arne, together with Grace Chen (now at the University of Michigan Medical School), found that a complex mechanism governs how the papillomavirus DNA helicase works. The enzyme, made up of a protein called E1, binds to the viral origin of replication by nestling up to another papillomavirus protein, E2. Together, these proteins bind to adjacent sites at the origin, where E1 then becomes an active DNA helicase and begins to unwind the double helix. Arne and Grace have discovered that E2 acts as a loading factor that deposits E1 on a specific sequence of DNA, the papillomavirus origin of replication.

Leemor Joshua-Tor

Using X-ray crystallography, Leemor Joshua-Tor and postdoctoral fellow Eric Enemark have determined the three-dimensional structure of a part of E1 that binds to DNA and to E2. By obtain-

ing a detailed view of E1's biochemical properties and structure, Arne and Leemor have uncovered interesting clues about how the papillomavirus DNA helicase is put together, how it functions, and what steps might be taken to block the function of this enzyme as a way to prevent cervical cancer.

Neuroscience

Brain Development: How does a single cell—a fertilized egg—become a complex organism composed of billions of cells of thousands of different kinds? How do cells arise at the correct time and place during the development of an organism and adopt the specialized characteristics that make them bone, muscle, or nerve? How is cell number controlled so that tissues, organs, and body parts adopt the correct size? Regulated transitions from cell proliferation to cell differentiation—during which cells stop growing and adopt specialized characteristics—are the key to these phenomena.

Gregori Enikolopov

Since 1992, Grigori (Grisha) Enikolopov has made a series of important discoveries concerning how the biochemical messenger nitric oxide (NO) instructs the cells in a developing organism to stop dividing, enabling them to differentiate into specialized cell types. Building on that research, Natalia (Natasha) Peunova, Hollis Cline, and Grisha found that experimentally altering NO levels in the developing brain of tadpoles significantly affects brain volume and the number of brain cells. When they artificially decreased NO levels in the brains of developing tadpoles, brain cell proliferation increased, leading to larger than normal brains (too little NO, too many brain cells). Conversely, when they artificially increased NO levels, brain cell proliferation decreased, and the resulting brains were smaller than normal (too much NO, too few brain cells).

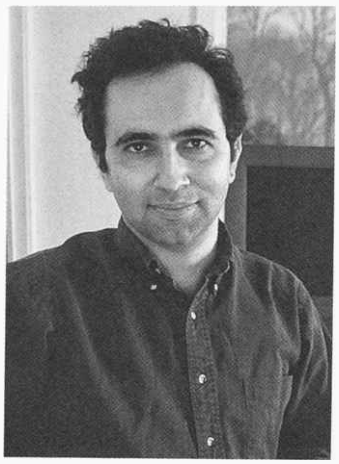
This experiment enabled Natasha, Grisha, and Holly to provide the first definitive demonstration of the essential role of NO as an inhibitor of nerve cell proliferation during vertebrate brain development. Fruit flies, frogs, mice, and humans all have similar sets of enzymes that produce NO. Thus, Grisha and his colleagues are uncovering universal principles concerning the antiproliferative power of NO and how this power is harnessed to create everything from diverse blood cell types, to correctly proportioned body parts, to the complex anatomy of the brain.

Hollis Cline

Brain Imaging: Forget gigabytes. Even the most powerful computers available today are no match for the complexity, efficiency, and information-processing capacity of the human brain. Underlying the brain's far superior design are the billion-million or so connections between brain cells—called synapses—that form vast neural networks in which brain cells, or neurons, are connected to thousands of other neurons. These networks—and their ability to be shaped by experience—enable us to receive, process, store, and retrieve all manner of information about our world. Unfortunately, the extremely tiny size of synapses and the limitations of conventional experimental techniques have hampered detailed studies of these essential structures. (One trillion synaptic compartments, or "dendritic spines," could fit into a thimble.) This year, Karel Svoboda overcame these technical obstacles to gain an extremely close look at the properties of dendritic spines and synapses that govern brain function.

Using "two-photon" microscopy (a high-resolution imaging technique whose application to neuroscience Svoboda pioneered), Karel and postdoctoral fellow Bernardo Sabatini watched calcium flow into single dendritic spines of electrically stimulated brain neurons. These measurements enabled Karel and Bernardo to determine the number and type of "calcium channels" present at synapses in a region of the brain important for learning and memory, the hippocampus. Calcium channels are molecular gates that open in response to electrical stimulation and allow calcium to flow into dendritic spines. Once inside, calcium triggers biochemical events in the spine that modify synaptic strength.

The strengthening of synapses between neurons in response to experience is believed to underlie complex brain functions such learning and memory. Karel, Robert Malinow, and several other CSHL neuroscientists have made a series of significant discoveries about this process. Visualizing how neurons communicate with each other on the most basic level, as Karel has done by imaging calcium, provides important clues for understanding how our brains outperform the most sophisticated computers and, in so doing, enable us to grasp the human experience.



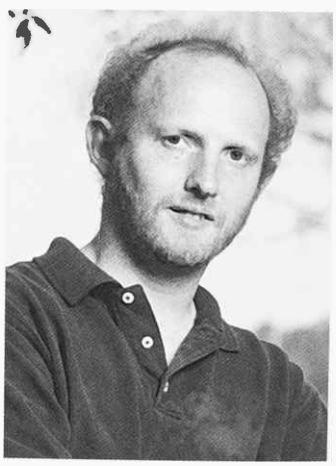
Dmitri Chklovskii

Theoretical and Computational Neuroscience

The discoveries made by traditional neuroscientists result from experiments with living tissue. In contrast, theoretical and computational neuroscientists use mathematics to describe how the brain works. Much of Dmitri (Mitya) Chklovskii's theoretical neuroscience research is based on the idea that biology and evolution impose several design constraints on the structure of the nervous system. For example, scientists have long believed that neurons are positioned and connected in the brain in such a way as to minimize the length—and hence the volume—of wiring.

Mitya has developed a mathematical formulation of this "wiring economy" principle that enables several predictions to be made about how neurons are connected to each other in the brain. Mitya's "wire length minimization" model corresponds closely with the observed properties of the mammalian visual cortex, a brain region that processes visual information. The human brain contains approximately 300 million feet of wiring packed into a 1.5-quart volume, but the brain would be much larger if it did not employ the strategy Mitya has uncovered for fitting the necessary wiring into the available, limited space.

Recently, Mitya has turned his efforts to understanding the neurological basis of optical illusions. By determining what happens when the brain is tricked, e.g., by an optical illusion, Mitya aims to uncover new principles of brain function.



Robert Martienssen

Plant Molecular Biology and Genetics

Owing to its small size and short generation time (~6 weeks from seed to adult), the mustard relative *Arabidopsis thaliana* has

W. Richard McCombie

become a workhorse of plant molecular biology and genetics. In addition to pursuing their specific research interests, W. Richard McCombie and Robert Martienssen helped organize the *Arabidopsis* Genome Initiative, a large-scale international effort established in 1996 to sequence the entire genome of this plant species.

This year, and well-ahead of schedule, the *Arabidopsis* Genome Initiative reached its goal of sequencing the entire *Arabidopsis* genome and thus obtained the first-ever complete genome sequence from a plant. This achievement has profound implications for biology, medicine, agriculture, and the environment because it will enable detailed studies of the entire genetic structure of plants to be carried out. Such studies will yield a great deal of new information about plant growth; crop yields; disease and drought resistance; ripening and spoilage; pharmaceutical, vitamin, and "edible vaccine" production; and many other aspects of plant biology.

Symposium LXV

From May 31 to June 5, biologists from a number of research fields gathered for the 65th annual CSHL Symposium, this year titled "Biological Responses to DNA Damage." On Sunday, June 4, meeting attendee Jan H.J. Hoeijmakers of Erasmus University in Rotterdam delivered the annual Dorcas Cummings Memorial Lecture to a scientific and public audience, a very successful annual event hosted by the CSHL Association. Hoeijmakers' talk, "Maintaining Nature's Perfection: Cancer and Aging and the Condition of Our Genes," was timely because of its relevance to cancer, but also fascinating because we must inherit and maintain our DNA in as best shape as we can to avoid acquired genetic diseases. The lecture, Symposium, and program were an unparalleled success.

Jan H.J. Hoeijmakers

Cancer Center Designation Renewed

Cold Spring Harbor Laboratory has been a National Cancer Institute (NCI)-designated basic cancer center since 1987. After a recent review and site visit, CSHL's cancer center status was renewed by the NCI, which rated the Laboratory's overall program in cancer research as "outstanding." CSHL was one of only a handful of NCI designees to receive this distinction. Designated cancer centers receive a Cancer Center Support Grant from the NCI, which helps fund the scientific infrastructure of the cancer research program. Funds from the NCI enable cancer centers to take advantage of new research opportunities, state-of-the-art technologies, and other NCI-supported resources to advance cancer research.

Scott Lowe, a CSHL Professor since 1995, has been named Deputy Director of the CSHL Cancer Center. Scott has made leading contributions to the identification and characterization of genetic pathways that suppress tumor development and, when disrupted, make tumors resistant to cancer therapy. As Deputy Director, Scott will help me lead the Cancer Center in new directions and ensure that its resources continue to flourish. Scott's

appointment follows Winship Herr's seven-year role as an energetic and very effective Deputy Director. Winship has stepped down to focus on the development of the Watson School (for which he serves as Dean) and his other duties as Assistant Director of the Laboratory.

Denise Roberts, the Laboratory's new Research Administrator, has also assumed the administrative duties for the Cancer Center.

Watson School of Biological Sciences

CSHL's graduate school—the Watson School of Biological Sciences—welcomed its second class of students this fall. On August 28, nine new students joined the Watson School's ranks, the second class to enter the program. Hailing from across the country and around the world, the students dove quickly into their rigorous programs, enrolling in the core courses and beginning the laboratory rotations that mark their first year of study.

Honorary Board of Trustees member David L. Luke III continues to lead the fund-raising efforts to fully fund the Watson School programs. This year, nearly $7 million was raised to support the Watson School, its graduate program, and its students.

Presidential Events

James D. Watson, president of CSHL, has had another busy year, most recently promoting his newest book, *A Passion for DNA: Genes, Genomes, and Society*. The collection of essays, published by the CSHL Press, was nationally publicized through an exhausting 4-month book tour. Locally, Jim was a big hit at Huntington's Book Revue, where hordes of fans lined up for the Nobel Laureate's autograph. Jim was also honored on July 4 with the prestigious Philadelphia Liberty Medal. Jim received the award, together with Francis H.C. Crick, for the "tremendous impact of their science on our lives and public policies."

Lewis Miller, an Australian artist and winner of the prestigious Archibald Prize—Australia's premier award for portraiture—returned to CSHL for a month this summer. Miller, who visited the Laboratory during the 1998 Symposium to sketch attendees and Laboratory personnel, has many works exhibited throughout Blackford Hall. His mission this summer was more specific: I commissioned Miller to paint a full-sized portrait of James D. Watson.

James Watson meets young fans while promoting *A Passion for DNA*.

Miller's efforts were a success: A seven-foot representation of CSHL's President now hangs in a place of honor inside Grace Auditorium, where Jim has spent countless hours lecturing to public and scientific audiences. While at the Laboratory, Miller also completed several other portraits, including sketches of Charles and Helen Dolan, long-time supporters of the Laboratory. Mrs. Dolan, who currently serves on the Laboratory's Board of Trustees, was particularly pleased with the charcoal drawings Miller presented to the couple. In all, Miller completed more than 15 portraits while at the Laboratory, and each was framed for display around the Laboratory campuses.

Helen Dolan

Charles Dolan

30th Anniversary Reunion of Yeast Course

Since the inception of CSHL's Yeast Genetics Course 30 years ago, nearly 500 scientists from around the world have traveled to Cold Spring Harbor to learn more about this relatively simple organism. Although simple in structure, yeast undergoes many of the complex biological processes of higher-order organisms, making it a model system for the study of molecular biology. CSHL's Yeast Genetics Course has helped to elevate yeast to its present "stardom," and most of the outstanding yeast geneticists/molecular biologists of the past half-century have either taken the course, taught the course, or both.

To celebrate the great science and lifelong friendships that have resulted from the Yeast Genetics Course, the Laboratory held a reunion for course participants on August 11. The event brought together students and instructors of the course—both past and present—for 2 days of reminiscing and sharing of current research. The reunion illustrated the influence that the course has had on its participants, as well as the impact that these investigators have had on science. "It's hard to imagine where we'd be today without yeast," said Jim Watson, who kicked off the reunion celebration. More than 35 alumni of the Yeast Genetics Course attended the reunion, taking part in the 2-day mélange of lectures, reflections, and activities, including the now-famous "plate race."

Jim Hicks, Ira Herskowitz, and Chris Kaiser at the reunion.

Banbury Center

One of the highlights of the Banbury Center year is the annual Executives' Conference. Begun in 1986 to bring top executives in the pharmaceutical and biotechnology industries together to learn about the newest concepts and technologies, the meetings attracted both speakers and invited guests of the highest quality. The 2000 meeting, entitled "Human Consciousness and Intelligence," lived up to this tradition. The keynote speaker was Oliver Sacks, the eminent psychologist and best-selling author. Oliver is the first Banbury Center

visitor to have been immortalized by Hollywood, when he was played by Robin Williams in the film *Awakenings*, based on his book. Howard Gardner spoke on his theory of multiple intelligences, and there was a spirited discussion of human intelligence following a presentation by Charles Murray, coauthor of *The Bell Curve*. Participants were treated to appearances by Alex the African Gray Parrot (by Irene Pepperberg) and, at the other extreme, robots (by Howard Gardner). In between, Stephen Pinker discussed his ideas on language, while Vilayanur Ramachandran reviewed the neuropsychological basis of sensory perception. We are grateful to David Deming of J.P. Morgan for helping us to present such an extraordinary meeting.

Some years ago, the world was astonished and shocked by the cloning of Dolly, the sheep. It seemed to be an extraordinary step in the manipulation of living organisms. However, Dolly herself was the only success of many attempts, and subsequent studies have only achieved limited success. Cloning is very difficult. The Banbury Center meeting "Mammalian Cloning: Biology and Practice" tried to identify those elements of the biology underlying cloning that might be responsible for the low success rate. Almost all of the leading scientists cloning a wide variety of animals participated, including Ian Wilmut, leader of the team that cloned Dolly.

A special feature of the 2000 program at Banbury Center was the large number of meetings on neuroscience, reflecting the increasing research effort in neuroscience at the Laboratory. The topics ranged from the experimental ("Structure, Mechanism, and Function of CaMKII") to the theoretical ("Natural Stimulus Statistics"). Neural Networks and Cognition was particularly noteworthy because neural networks underlie the Palm operating system. The meeting was funded by Jeffrey Hawkins, the developer of the Palm OS and cofounder of Handspring, Inc., who is now a Trustee of the Laboratory. The Swartz Foundation, established by another good friend of the Laboratory, Jerry Swartz, funded a meeting called "Toward Animal Models of Attention and Consciousness," discussing how the brain is able to focus selectively on items of interest in the world around us.

Robertson Research Fund

Since 1973, the Robertson Research Fund has been the primary in-house support for scientists at Cold Spring Harbor Laboratory. The fund has grown from its original 1973 total of $8 million to more than $95 million for the year 2000. This year, Robertson funds supported cancer research in the labs of David Helfman, Michael Hengartner, Tatsuya Hirano, Ryuji Kobayashi, Yuri Lazebnik, Greg Hannon, and Dick McCombie; neurobiology research in the labs of Grigori Enikolopov, Roberto Malinow, Tim Tully, Jerry Yin, and Yi Zhong; and plant research in the lab of Robert Martienssen. Robertson funds also supported several new investigators, including Andrew Reiner, Shivinder Grewal, David Jackson, Zachary Mainen, and Anthony Zador.

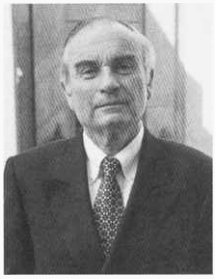
The Marie H. Robertson Memorial Fund, devoted to neuroscience, provided support to Grigori Enikolopov and Zachary Mainen and provided funds for a neurobiology seminar program and a Banbury Center meeting on neuroscience.

Cold Spring Harbor Laboratory Board of Trustees

Cold Spring Harbor Laboratory's Board of Trustees has led the Laboratory through another year. At its two annual meetings, the Board members continued to help shape the vision and

drive of this institution. This year, the Board welcomed the addition of Jacob Goldfield, a private investor.

The Board of Trustees bid a fond farewell to two long-standing Board members, Martha Farish Gerry and John J. Phelan. Mrs. Gerry has served on the Board for more than 10 years, serving on the Banbury Program, Building, DNA Learning Center, and Development Committees; she was also Secretary of the Board from 1997 to 2000. Mr. Phelan, who led the Board's Commercial Relations Committee since 1996, served the Laboratory for more than 8 years and was also represented on the Board's Audit and Executive Committees. Both Trustees were honored at the Board's November 4 meeting for their outstanding service and unwavering loyalty to Cold Spring Harbor Laboratory.



John J. Phelan

CSHL Association

The CSHL Association (CSHLA) held its annual meeting on February 5, 2000. The membership honored its retiring directors, including Mary Ann Charlston and Mary D. Lindsay (who was simultaneously named an Honorary Director). The Association also elected several new directors, including William F. Gerry, Lynn M. Gray, Susan Hollo, and Allen Dulles Jebsen. George W. Cutting, Jr., a former president of the CSHLA and a former honorary director, was reelected and returned as an active director this year.

The annual meeting also featured a lecture by Dr. Steven Pinker, Professor of Psychology in the Department of Brain and Cognitive Sciences at the Massachusetts Institute of Technology. His lecture, titled "Words and Rules: The Ingredients of Language," discussed the components and importance of human communication.

Martha Farish Gerry

On April 15, 2000, the Association hosted *Jazz at the Lab 2000*, its second jazz benefit. The performance featured The Harold Betters Quartet, with Harold Betters on trombone, Kevin Moore on piano, Charles Ramsey on bass, and Cecil Brooks III on drums. Kenny Blake, a saxophonist, and Eric Johnson, a guitarist, were also featured in the performance.

The annual major donors' cocktail party was held in the Lloyd Harbor home of Mrs. John W.B. (Robin) Hadley. The event, held November 11, 2000, provided an elegant and enjoyable setting in which the Laboratory's scientists were able to personally thank the generous individuals who help to support their work.

DNA Learning Center

During the year, the DNA Learning Center (DNALC) multimedia group opened a major new Internet site, *Image Archive on the American Eugenics Movement*. The site provides students, teachers, scholars, and the interested public with an extraordinary window into a "hidden" chapter of history and a cautionary tale for our current rush to exploit the human genome. Through more than 1500 images and documents, most never publicly released before, the archive traces the rise and fall of this ill-guided experiment to protect the "American germ plasm from pollution by 'bad genes.'" With the support of many prominent geneticists and public citizens, eugenicists successfully lobbied for coercive social legislation to restrict European immi-

DNALC Internet site, *Image Archive on the American Eugenics Movement.*

gration, to prevent race mixing, and to sterilize "hereditary defectives." The *Eugenics Archive* makes no attempt to lead users to a "correct" interpretation of the materials; however, contextual narratives remind users that the vast majority of scientific "facts" presented by eugenicists were fundamentally flawed and have been discredited by modern research standards. It is especially appropriate that the Laboratory should sponsor this effort, because from 1910 to 1940, the Eugenics Record Office at Cold Spring Harbor was an epicenter of the American movement.

By far, the most exciting event at the DNALC was the completion of the BioMedia Addition. Construction began on April 18, 2000, and included the addition of a suite of administrative offices, an additional teaching lab, a computer lab, a lunchroom, and additional exhibition space. Through the generosity of The Dolan Family Foundation, established by CSHL Trustees Charles and Helen Dolan, the DNALC also gained a state-of-the-art facility for multimedia production, now called the "multitorium." The facility includes high-tech equipment and the latest technology, which will enhance the DNALC's mission to educate the students of Long Island. In June, 2001, the site will be dedicated and renamed *The Dolan DNA Learning Center*.

CSHL Press

The Cold Spring Harbor Laboratory Press had a very successful year. One highlight was the eagerly anticipated December appearance of the celebrated laboratory manual *Molecular Cloning*, in a new edition by Joe Sambrook and David Russell that is already a best-seller. Eleven other new titles were published, including three manuals, a monograph on translational control, the annual symposium volume, an elegant book on embryonic development, and a compendium of classic papers in cell biology. A memorial volume for Alfred Hershey was also released. Several of the new titles were aimed at nonspecialist readers, most notably Jim Watson's essay collection *A Passion for DNA*, which was particularly popular. Altogether, 220 books are in print and are available at the attractively redesigned Web Site CSHLPress.com. The book program's sales support was strengthened through contracts with major booksellers and distributors in the U.S. and the creation of a European sales team centered in Oxford.

In the textbook division, four major projects were initiated and discussion of co-publishing contracts initiated with three major textbook marketing firms. Particularly satisfying was the agreement reached with Benjamin Cummings for a fifth edition of Jim Watson's classic text *Molecular Biology of the Gene*.

The journal program continued to advance. Subscriptions increased, advertising sales rose, and the measurable impact of all three journals was either maintained or strengthened. The on-line editions were enhanced with new features such as freely accessible back issue archives. The Protein Society's journal *Protein Science*, acquired from another publisher, was redesigned both in print and on-line and relaunched under the CSH banner in December.

Major Gifts

2000 was an exciting year for fund-raising. Several major campaigns continue to be successful: the campaign to fund the new Genome Research Center; the BioMedia Addition to the DNA Learning Center; and a continuing campaign for the Watson School of Biological Sciences. Results for all were astounding, and we are very fortunate to have such generous and supportive friends at Cold Spring Harbor Laboratory.

The Genome Research Center

As the Genome Research Center in nearby Woodbury nears completion, the fund-raising effort to support this new facility is already under way. A pledge of support was made by Mrs. Joy Levy, wife of the late Senator Norman J. Levy, who has offered to make the Genome Research Center the beneficiary of the efforts of the Norman J. Levy Foundation. To start the process, Mrs. Levy has pledged $250,000; her brother and sister-in-law, Jerry and Linda Saslow, have pledged $50,000 as well. The William & Maude Pritchard Charitable Trust has made a gift of $280,500 to support the Genome Research Center, and Arrow Internet Business Group and the Irving A. Hansen Memorial Foundation pledged $30,000 each. Other major gifts were received from Mr. Lawrence Flinn, who donated $50,000; Nathan E. Saint-Amand, M.D., who made a gift of $50,000; and the Mary G. Turner Charitable Lead Unitrust, which made a gift of $20,000 to the project. The Seraph Foundation, Inc., also gave a leadership gift of $100,000 to support the new facility.

Architectural rendering of the Genome Research Center.

The BioMedia Addition to the DNA Learning Center

The DNA Learning Center facility was updated and expanded this year, brought to the cutting-edge of technology with a new BioMedia Addition. The campaign was anchored by a generous donation from The Dolan Family Foundation, which made a total commitment of $3,000,000 to finance the renovations, build the new facilities, and equip the new lab areas.

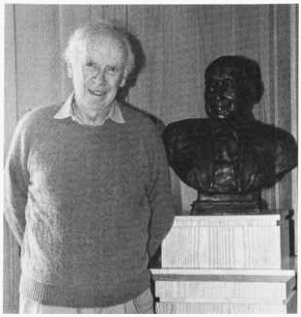
PE Biosystems made a gift-in-kind of $130,570 to furnish the addition with equipment, and Mr. and Mrs. Denis J. O'Kane made a generous gift of $35,000 to further fund the facility. The New York Stock Exchange also made a gift of $10,000 to the project.

The Watson School of Biological Sciences

Cold Spring Harbor Laboratory Trustee David L. Luke III continues to lead the campaign to fund the Watson School of Biological Sciences. This year was a particularly fruitful year for the fund-raising effort. Bristol Myers Squibb, The Charles A. Dana Foundation, and The Florence Gould Foundation each pledged $1,000,000 to support the Watson School. Mr. Alan Goldberg pledged $500,000 to support the School, and Lazard Freres & Company, LLC, and The Koshland Foundation each gave $100,000 to the campaign. Laboratory Trustee Robert D. Lindsay and his wife pledged $500,000, and CSHLA President David H. Deming and his wife pledged $50,000. Mr. and Mrs. Charles E. Harris made a gift of $103,075 to the Watson School Campaign, and The Rathmann Family Foundation made a commitment of $300,000. The Lita Annenberg Hazen Foundation, a long-time Laboratory supporter, made a pledge of $825,000 to continue the School's educational programs. Dr. Michael Wigler, a CSHL professor, made a pledge of $55,000 to support the School, and Mrs. John H. Livingston and the Fairchild Martindale Foundation each gave $50,000 to the Campaign. Among many other donations made, significant commitments and gifts of support were made by the Alan B. Slifka Foundation; Biogen, Inc.; Mrs. Gertrude W. Conner; Mrs. Frances Elder; Mr. and Mrs. Edward Giles; Dr. and Mrs. Walter C. Meier; Mrs. Wendy Vander Poel Russel; and Karen and Mark J. Zoller, Ph.D. All told, nearly $7 million was raised this year to support the Watson School, and we continue to be grateful to our many donors and friends.

Research Support

We have received the following generous gifts in support of research in the year 2000: The Arthur and Barbara Crocker Charitable Trust gave $200,000 to the Young Incoming Scientists Endowment; the Goldring Family Foundation gave $60,000 to support postdoctoral fellowships; and Mr. and Mrs. Edmond J. Nouri gave $84,551.70 to support the work of Dr. Tim Tully, part of a 3-year pledge of more than $130,000.



James Watson standing beside a bust of Arnold O. Beckman, CSHL benefactor.

The Arnold O. and Mabel M. Beckman Foundation

The Arnold O. and Mabel M. Beckman Foundation, a long-time Laboratory supporter, continued its generous support in 2000. The Foundation pledged $1,000,000 to support the students of the Watson School of Biological Sciences, and pledged $250,000 for Core Course support in the School.

Members of 1 in 9: The Long Island Breast Cancer Coalition present a gift to Michael Wigler to fund breast cancer research.

Breast Cancer Support

A new decade did nothing to hamper the efforts of the numerous breast cancer groups who support the Laboratory's cancer program.

1 in 9: The Long Island Breast Cancer Action Coalition—which celebrated 10 years of support to the Laboratory in 2000—presented a check in the amount of $240,000, its largest single gift in the organization's history. To date, the support from 1 in 9 has surpassed $650,000.

Several other groups continued their support of breast cancer research, including The Breast Cancer Research Foundation, The Huntington Breast Cancer Action Coalition, The Long Island Foundation for the Elimination of Breast Cancer, and Breast Cancer Awareness Day in Memory of Elizabeth McFarland. The West Islip Breast Cancer Coalition also made a generous donation to support the work of Dr. Michael Wigler, the first time the group has made a donation to the Laboratory.

We were also pleased to welcome the members of Long Islanders Against Breast Cancer (L.I.A.B.C.). The group announced with its formation that it would raise funds to benefit Cold Spring Harbor Laboratory, and its 2000 gifts—since its October formation—already total more than $65,000.

President's Council

This year's President's Council meeting was on "The Dog and Its Genetics: Breeds, Evolution & Behavior," an unbeatable combination of cutting-edge genetic research and humanity's closest friend. An eminent roster of speakers began with Roger Caras, internationally known for his encyclopedic knowledge of dogs and as Master of Ceremonies at the annual Westminster Club Show. Mr. Caras talked on the special relationship between human beings and animals. Elaine Ostrander (University of Washington) and Greg Acland (Cornell University) then described the dog genome project, and how the dog is afflicted by genetic

disorders that may have a genetic basis identical to disorders of human beings. But perhaps the most fascinating feature of dogs is their behavior. Raymond Coppinger (Hampshire College) has specialized in studies of the behavior of working dogs, and he described the differing characteristics of the sheepdogs that guard flocks and those that herd flocks. All-in-all, the speakers introduced us to a wonderful blend of science and our everyday experiences of the animal that has been associated with human beings for at least 14,000 years.

Gavin Borden Visiting Fellow

Douglas A. Melton, Ph.D., of the Howard Hughes Medical Institute and the Department of Molecular and Cellular Biology at Harvard University was the Laboratory's sixth Gavin Borden Visiting Fellow. Dr. Melton's lecture, titled "Construction of the Pancreas," was held on October 2 in Grace Auditorium. The Lecture series was named in memory of Gavin Borden, a science writer whose work on *Molecular Biology of the Cell* and several other books made a lasting impression on the Laboratory.

Douglas A. Melton, Ph.D.

Building Projects

The historic landscape of Cold Spring Harbor Laboratory changed again this year, with the completion of three new building projects. On May 3, the Laboratory dedicated the Samuel Freeman Building, located adjacent to Urey Cottage. The Freeman Building—part of the Laboratory's neuroscience program—is a state-of-the-art facility for neuroscientists who use computer technologies to aid in the study of the brain. The building was made possible through a generous gift from the Samuel Freeman Charitable Trust, led by CSHL Trustee Bill

Samuel Freeman Building dedication.

Townsend J. Knight (second from right) joins his family at the building that bears his name.

Murray. The building, designed by Jim Childress of Centerbrook architects, is an Adirondack-style structure, accented by dark wood siding and a gabled roof of copper-foil-coated shingles. The Freeman Building—which will house three faculty in the field of computational neuroscience—is reminiscent of the cabins that formerly occupied the site.

On June 10, the Laboratory dedicated the newly renovated Knight House, across Cold Spring Harbor from CSHL's main campus. The 190-year-old structure was an original home of the Jones Family, the Laboratory's earliest benefactors. Named for CSHL Honorary Trustee Townsend J. Knight—a descendant of the Jones family—the historic, two-story white structure is a beacon of the Cold Spring Harbor shoreline. Following extensive renovations, the Knight House is now being used as a residence for students of the Watson School of Biological Sciences. The interior contains six bedrooms, a living area, television room, kitchen, and laundry facilities.

Renovations continue on the Genome Research Center in nearby Woodbury. The building, now near completion, is a 72,000-square-foot facility that houses four research components: a Bioinformatics Center, a Cancer Research Center, a Genome Sequencing Center, and a Plant Genomics Center, as well as new space for the CSHL Press, and other administrative departments. A campaign is under way to fully fund the complex. Its first tenants—CSHL's Purchasing Department and the CSHL Press—are expected to occupy the building in Spring 2001.

Undergraduate Research Program

The 2000 summer Undergraduate Research Program (URP) consisted of 27 students—14 men and 13 women—from 8 countries. They were chosen from among 546 applicants from more than 59 nations.

The objective of the program is to provide a greater understanding of the principles of biology. It instills in the students an awareness of major topics of investigation, helps develop intellectual tools necessary for modern research, exposes students to the process of research, and allows them to meet the top scientists who visit CSHL.

The program received financial support in 2000 from the C. Bliss Memorial Fund, the Burroughs Wellcome Fund, Robert Cummings, Dr. Ira Herskowitz, the Jephson Educational Trust, the JM Foundation, the National Science Foundation, and the URP Endowment Fund (composed of the Emanual Ax Fund, the Garfield Fund, the Glass Fund, the Libby Fund, the Olney Fund, the Read Fund, the Shakespeare Fund, and the Von Stade Fund).

Partners for the Future

The CSHL Partners for the Future program was established in 1990 to give young students a taste of the real world of biomedical research. Each year, six or eight Long Island high school students entering their senior year are chosen from a large pool of nominees to participate in the program. The selected students spend a minimum of 10 hours per week, October through March, doing original research under the guidance of a Cold Spring Harbor Laboratory scientist. At the conclusion of the program, students present their research project to an audience of scientific mentors, high school teachers, friends, and relatives.

The program, now in its 11th year, is an overwhelming success. To date, 53 students have completed the program, and all of them are now or have been enrolled in top-ranked colleges and universities.

Back row: Maria Reimels, Brian Mohr, Daniel McAlvin, Owen Martin
Front row: Eric Van Nostrand, Jacqueline Rho, Deborah Yom, Allison Brincat

Community Outreach

Grateful for the overwhelming support that CSHL receives, a number of employees set out this year to "give back" to the Long Island community.

- CSHL "road-runners" were on-hand at the Cigna 5K Walk/Run, to support 1 in 9: The Long Island Breast Cancer Action Coalition, held at Eisenhower Park in East Meadow on August 24. The CSHL group is grateful for more than 10 years of support from 1 in 9. Lynn Cannon, Administrative Assistant in the Development Office, came in third for her age group in the run. In all, the event raised more than $100,000.
- Members of the W. Richard McCombie lab made a generous contribution to the North Shore Pediatric Oncology Unit to purchase holiday gifts for the patients. In their certificate of appreciation, administrators at North Shore noted how the gifts really touched the children.

Special Events

Neuroscience Lecture Series

This year, the Laboratory continued a series of public education lectures by hosting its first Neuroscience Lecture Series. The three public lectures attracted more than 900 visitors to Grace Auditorium. The first lecture was given by Dr. Michael Merzenich on November 14, titled "Training Strategies for Brain Illnesses and Disabilities in Children." Dr. Merzenich is the Francis A. Sooy Professor of Otolaryngology at the Keck Center for Integrative Neurosciences at the University of California, San Francisco. The second lecture, held November 21, was given by Dr. Charles F. Stevens, a professor from The Salk Institute for Biological Sciences. Dr. Stevens' lecture was titled "Synapses: The Neural Information Highway." The final lecture in the series was given by Dr. Larry Squire, Professor of Psychiatry and Neurosciences at the University of California School of Medicine in San Diego. Dr. Squire's lecture, held November 28, was titled "Memory Systems of the Brain."

Dr. Michael Merzenich

Other Lectures

Cold Spring Harbor Laboratory continued to host the Huntington Hospital Lecture Series, which ran from February to June. This year's topic was "Keeping Your Heart Healthy," part of Huntington Hospital's Heart Health Lecture program.

On June 26, the Laboratory welcomed Viviana Risca, the 2000 winner of the Intel Science Talent Search, and Laura F. Landweber, an assistant professor at Princeton University, for a special public lecture on "DNA Games: From Computing to Espionage." The event attracted a large group of teens and adults interested in DNA and the human genome.

On October 28, Phillip Valentine Tobias, Professor Emeritus at the University of the Witwatersrand in Johannesburg, presented a special public lecture titled "Humanity's Cradle: 75 Years of Evolutionary Studies in Africa." As part of a special Halloween program, "Bones and Strings," Dr. Tobias lectured on his research of the human biology of the peoples of Southern Africa. A special public concert by famed violinist Viviane Hagner followed the lecture.

Concerts

As part of the Laboratory's Millennium Cultural Series, several concerts were held this year. Attended by the participants of CSHL's Meetings program and the public, the free concert series was a huge success. The concerts included:

May 6	Karen Gomyo, violinist
May 20	Adam Neiman, pianist; Stefan Malenkovich, violinist; Ani Aznavoorian, cellist
May 27	Margarita Shevchenko, pianist
August 19	Julia and Irina Elkina, duo piano
August 26	Randall Scarlata, baritone and accompanist
September 9	Makoto Nakura, marimbist
September 16	Eric Johnson, jazz guitarist, with band
October 28	Viviane Hagner, violinist
November 11	Mark Ptashne (CSHL Board of Trustee Member), violinist

Millennium Cultural Series

In addition to the concert series, the Millennium Cultural Series brought several art events to the Laboratory for the year. Dale Chihuly, famed glass sculptor, presented a lecture on May 2 titled "Chihuly on Chihuly: The Jerusalem Project." The lecture, cohosted by the Hecksher Museum of Art, examined the sculptor's year of work in Israel. The Laboratory's second outdoor art show, *Sculpture by the Sea,* ran from May 28 through October 31 and featured 17 original works. On August 31, sculptor Charles Jencks unveiled his sculpture *Spirals Time-Time Spirals* and lectured on his work, particularly how the structure of DNA had inspired this latest piece. On November 4, the Laboratory hosted *Labscapes 2000*, a painting show which featured 25 pieces depicting the beautiful grounds and historic buildings of the Laboratory's property. Many of the exhibits were possible due to the generosity of Jim and Liz Watson.

Stefan Malenkovich, violinist; Ani Aznavoorian, cellist; Adam Neiman, pianist

Viviane Hagner

Sculpture by the Sea

Margarita Shevchenko

First Row (top): Bruce Stillman, James Watson, Harry Wozniak, Frank Carberry
Second Row: Michael Riggs, G. Morgan Browne, Madeline Wisnowksi, David Spector
Third Row: Steven Tang, Linda Rodgers, Marlene Rubino, Patricia Hinton-Stenko
Fourth Row: Andrea Stephenson, Lisa Manche, Bruce Fahlbusch

Laboratory Employees

Long-term Service

On June 29th, employees celebrating milestone anniversaries with the Laboratory were honored at a special poolside dinner at Robertson House, on the Banbury Center property. Congratulations to all! Honorees included:

30 Years	Madeline Wisnewski
20 Years	Bruce Fahlbusch, Michael Riggs, Linda Rodgers, Marlene Rubino and Andrea Stephenson
15 Years	G. Morgan Browne, Frank Carberry, Patricia Hinton-Stenko, Lisa Manche, David Spector, Wanda Stolen, Steven Tang and Harry Wozniak

Awards and Honors

I was honored to be elected to the National Academy of Sciences (NAS) in May, 2000. Membership in the Academy is one of the highest honors a scientist can receive from an American organization.

Changes in Administrative Staff

Annette Gangitano joined us this year as the Executive Director of the Cold Spring Harbor Laboratory Association. Annette came to the Laboratory from a position with the firm of J&W Seligman.

Jeff Picarello joined us as the Director of Public Affairs. Jeff formerly worked at the C.W. Post Campus of Long Island University, where he was Director of Media Affairs.

Charles Prizzi joined the Laboratory staff as Director of Special Events. Charlie was formerly Director of Special Events for Long Island University.

New Faculty and Staff

Z. Josh Huang joined the Laboratory this year as an assistant professor and Bhubaneswar Mishra, as an adjunct professor. Other new hires included Gilbert (Lee) Henry and Terence Strick, who joined the Laboratory as Cold Spring Harbor Laboratory Fellows.

Promotions

Scott Lowe was promoted to professor and deputy director of the CSHL Cancer Center. Greg Hannon, Lincoln Stein, Karel Svoboda, Rui-Ming Xu, and Jerry Yin were each appointed associate professor, and Robert Lucito and Vivek Mittal were promoted to assistant professors.

Departures

After many years of association with Cold Spring Harbor Laboratory, David Beach, an adjunct professor, left the Laboratory to focus on his role as president of Genetica. Bruce Futcher, an associate professor, left Cold Spring Harbor for a position at the State University of New York at Stony Brook.

Visiting Scientists

Five visiting scientists joined us this year: Vincent Colot will spend his sabbatical year in the lab of Robert Martienssen; Anna Giulini joined the lab of David Jackson; Edith Heard will spend her sabbatical year in the lab of David Spector; Jacqueline Vitali joined the lab of Rui-Ming Xu; and Ying-hua Zhu joined the lab of Yi Zhong. Five scientists also wrapped up their stays at Cold Spring Harbor Laboratory: Christine Berthier, Daniel Bogenhagen, Hilde Grasmo-Wendler, Nathalie Pavy, and Fumio Shiobara.

Postdoctoral Departures

The following postdoctoral researchers left Cold Spring Harbor Laboratory in 2000:

Guy Birkenmeier
James Chong
James Dezazzo
Nicholas Edgington
Ludwig Englemeier
Francisco Ferrezuelo
Andrew Groover
Mariko Hayashi
Yasunori Hayashi
Daniel Hoeppner
Jianzhong Jiang
Keiji Kimura
Zhixin Lin
Hong Liu
Tohru Mizushima
Michael Murray
Jairaj Puthenveettil
Keiichi Shibahara
Javor Stolarov
Suzanne Tharin
Venugopal Valmeekam
Daniel Vaughn
Michael Weinreich
Herman Wijnen
Jianlong Zhou
Jian Zhu

Graduate Student Departures

The following graduate students left Cold Spring Harbor Laboratory in 2000:

Eric Gillitzer	Julia Polyakova	Beth Trumbull
Tina Gumienny	Alex Rai	Helena (Xiaohong) Yang
Susan Harrington	Bjorn Schumacher	Lee Zou
Shirly Pinto	Setareh Sepehri	

Concluding Remarks

Constant change has been the theme at Cold Spring Harbor for much of its history, and this past year has been no exception. This dynamism, coupled with results emerging from the various genome projects, has opened new doors and revealed tremendous opportunities for biology and biomedicine, possibilities that were unheard of when I first came to Cold Spring Harbor Laboratory 21 years ago. It is time once again for this institution to rise to the challenges before us, enjoying, but not resting on, our significant past achievements.

Cold Spring Harbor Laboratory is a unique research and educational institution, more like an advanced research university now than ever before. More than many other universities, we have the flexibility to rapidly adapt to the fast pace of modern science. However, to remain at the leading edge of research in areas such as cancer and neuroscience, we will require major new infrastructure, significant new funding, dedication, and much energy from our already efficient and busy staff. This institution has never shied away from a challenge, and I am confident that with the right help, we will go on to an even brighter future.

April 2001 **Bruce Stillman**

ADMINISTRATION

The year 2000 marked the continuation of a period of transition for our Administration Departments. In recent years, the Laboratory has grown very rapidly; virtually all of our research and education programs have expanded and added new activities. We have a new Genome Research Center on an 11-acre campus in Woodbury. The DNA Learning Center has doubled in size with a new BioMedia Addition in the Village of Cold Spring Harbor. Already, the Watson School is recruiting its third class of graduate students, and the first publication will appear later this year from the new textbook division of the Cold Spring Harbor Laboratory Press. The first building of the new Broad Hollow Bioscience Park in Farmingdale is open and filled to capacity, and a second building is already in the planning stage. The Laboratory has, in fact, been transformed into a very diverse small university of advanced research. There are many new staff, new buildings, and new locations for which the Administration is responsible, and so our institution has become a larger and more complex place to manage.

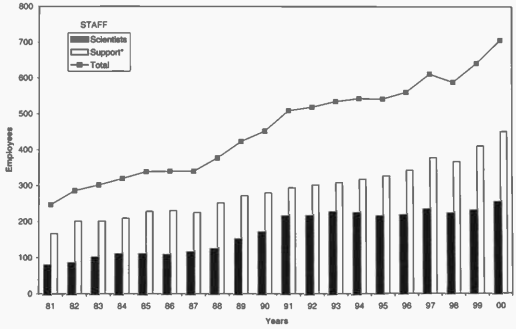
Traditionally, the Administration at Cold Spring Harbor Laboratory has functioned as a very lean and close-knit group of departments with very short lines of communication. Decisions are made quickly, and requisitions, for example, for new equipment or facility renovations, are processed in hours or at most a few days. Overhead costs have been kept low, especially when compared with those of larger institutions. Now, the challenge for Administration is to maintain these traditions while fully keeping pace with the expanding needs of our institution.

One year ago, we initiated an important first step in the transition with the selection, installation, and start-up of new Laboratory-wide business-computing software, which replaced previous technology of many years' vintage. The new software is modern and versatile, with all the growth potential and back-up support that one could wish. During 2000, the administrative departments learned to use its basic features and gained appreciation for the power and flexibility of its more advanced modules, such as electronic order processing and purchasing, to be brought on-line during 2001.

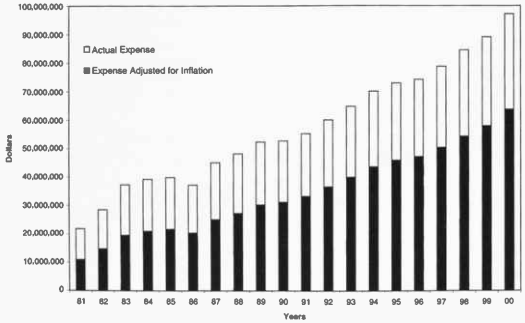
During the year, we added cautiously to administrative staff and created a small amount of additional office space. A space shortage remains a difficult and continuing problem at the Laboratory. The Grants Department, bursting at the seams, was given an office that became available in Wawepex. It was decided to move the Purchasing Department to the new Woodbury Campus this summer, freeing the top floor of the Nichols Building. A beginning was made on the long-delayed project to fully renovate Nichols and to create there a badly needed conference room for Administration and for the Tech Transfer Department. Progress was made in rationalizing pay scales in the Facilities Department and in other areas where we have not been fully competitive with other Long Island businesses.

Perhaps most important, the Board of Trustees approved my recommendation for the planned succession in administrative leadership outlined in last year's Annual Report. Dill Ayres, at the Laboratory since 1998 as Associate Administrative Director, was appointed to lead the Administration with the new title of Chief Operating Officer. We have great confidence in Dill and are pleased to have such talented and younger leadership in this important position. For the next year or so, I will continue at the Laboratory in the role of Chief Financial Officer and be available to help with other matters as needed.

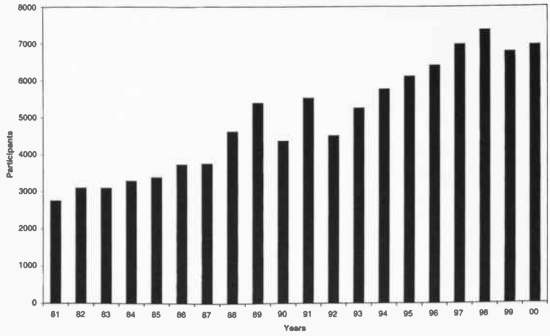
* Consists of Full-time and Part-time Technical Support, Core Services, Publications, Meetings, Library, Public Affairs, Building and Grounds, Administrative, Personnel, Banbury Center, and DNA Learning Center



OPERATING EXPENSE



MEETINGS & COURSE PARTICIPANTS



Meanwhile, we are pleased to report that 2000 was another good year financially for the Laboratory. A strong financial position was maintained, backed up by a budget that was balanced, after allowing for depreciation, for the twelfth consecutive year. Revenues increased to a new high level of just over $66 million. There was an operating excess of $297,000 that will be added to our reserves for start-up expenses at the Genome Research Center and for other future research programs. Overall, the financial results were more than $850,000 better than the original budget for the year. There was positive cash flow from operations of approximately $4.3 million, which as in past years was available to fund capital expenditures, for renewal of capital plant and for the purchase of ever more expensive scientific equipment. The success of our scientists in obtaining federal and other grants was again key to the good results for the year. Other positives were the continued good attendance at the Meetings and Courses Program, a surplus at the DNA Learning Center despite the dislocations resulting from the BioMedia Addition expansion, and good years for Banbury Center and the CSHL Press, which published the eagerly awaited third edition of its all-time best-selling manual, *Molecular Cloning*. The Watson School, completing its second year and benefiting from its growing endowment, succeeded in balancing its revenues and expenses.

It was also not a bad year, relatively speaking, for the Laboratory's permanent endowment, which collectively consists of the Robertson and Cold Spring Harbor Funds. After a string of years of extraordinary gains, the overall equity markets were down in 2000. It was a very difficult year for many investment managers. Nevertheless, at December 31, 2000, the Laboratory's endowment totaled $227 million, an increase of $7 million from the previous year-end. It should be noted that the Laboratory has $45 million of tax-exempt bonds outstanding, issued by the Nassau and Suffolk County Industrial Development Agencies, which mature in the amounts of $3 million in 2023 and $42 million in 2034. Throughout the year, the endowment was invested in a balanced mix of equity, long-term and short-term fixed income securities, and a small commitment to alternative investments. The asset allocation was approximately 55%/45% equity/fixed income during most of the year. Total return was a positive 2.9% as compared to (1.1%) for an index of comparable allocation. Our value and large cap equity managers, U.S. Trust Company and Vanguard PRIMECAP Fund, returned 9.2% and 5.1%, respectively, while small cap growth-oriented Essex Investment Management had a down year. Fixed-income manager Miller Anderson & Sherrerd returned a strong 11.3%. The Laboratory's policy on draw-down from the endowment is a conservative 4% per annum based on a 3-year moving average of year-end market values. This policy has been maintained over a number of years and has served well in supporting our research and education programs while permitting the endowment to increase handsomely.

Worthy of mention again is the Science Fund that was established by our Board of Trustees in 1992 as a permanent component of the endowment, designated specifically for the support of science at the Laboratory. Since 1992, all equity and a major portion of royalties from technology transfer activities have been accumulated in the Science Fund, allowing Cold Spring Harbor Laboratory to lead the way toward assuring that financial gains from research funded by public and other grants are recycled back into more science. It has also been our expectation that this Fund would help to replace substantial funding received in recent years from an important patent used extensively by the biotech industry that expired last June 30. At year-end, the Science Fund had a market value in excess of $30 million, and in 2001, it will provide $1.2 million in support of our science program.

In September, a landmark ceremony was held to celebrate the opening of the new Broad Hollow Bioscience Park located on a 20-acre site at the State University of New York at Farmingdale, just 20 minutes from the main Laboratory campus. The concept for the Park had

come from the realization that a number of fledgling new bioscience companies—spawned from Lab technology—were locating away from Long Island because of the lack of appropriate facilities and an academic/bioscience cluster locally. The Park's impressive 50,000-square-foot first building was fully funded by the State of New York. Many of those whose vision and leadership made the Park possible attended or spoke at the ceremony. These included New York State Senators Kemp Hannon and Carl Fuschillo, SUNY Farmingdale Acting President Michael Vinciguerra, and Long Island businessman Horst Saalbach. In particular, the Laboratory owes a debt of gratitude to Honorary Trustee John Cleary, who shepherded the creation of the Park through the vagaries of State government and who has served as Chairman of the Board of Broad Hollow since its incorporation in 1998. Many from the Laboratory were also present, including Jim Watson, Bruce Stillman, and all of the members of our Tech Transfer Department: Director John Maroney, Carol Dempster, and Barry Braunstein. We had all been confident of the eventual success of the Park, but none of us could have expected that all of its space would be taken and an expansion planned even before the opening ceremony. Currently, there are active discussions under way with the State concerning the urgent need for a second building.

This is my final report as Administrative Director. It has been a great privilege for the past 15 years to be associated with an extraordinary group of individuals who lead and staff our administrative departments. The Laboratory's reputation and success belong to them as well as to our scientists, and they should take great pride in their accomplishments.

G. Morgan Browne
Administrative Director

April, 2001

It is with a good deal of anticipation and excitement, together with a healthy degree of trepidation, that I begin my leadership of the Laboratory's administration and operations. To have a hand in the management of an institution of this caliber, as well as to work in partnership with the likes of Bruce Stillman, Jim Watson, Morgan Browne, and all of the other wonderful professionals here, is an extraordinary opportunity. The challenges are many and daunting. We are in the midst of a substantial expansion of our facilities and our employee base. Management is in the process of planning the long-term future of our research and academic programs. The implications of this plan for our physical infrastructure, administration, and fund-raising are profound.

I have large shoes to fill. Morgan Browne has done an exemplary job as Administrative Director for the last 15 years. He has overseen growth in our employee population of 320 in 1984 to the current count of 825. He has instilled a sense of financial discipline without compromising important expansion. Our Trustees have come to expect operating surpluses, which Morgan has delivered for 12 consecutive years. This is not an insignificant accomplishment. The Laboratory tends to attract perfectionists, whether at the senior faculty level or in the Facilities Department, who demand the best in equipment and resources in order to satisfy their very high standards. Balancing institutional excellence, across all fronts, with fiscal discipline is one of the formidable challenges of this office.

Part of my responsibility is to anticipate problems without being negative. 2001 will be a more challenging year, from a budgetary perspective, than we have recently experienced. The costs associated with bringing the Genome Research Center on-line, in combination with the expiration of certain patent-related income, will make it difficult to achieve a balanced budget this year. The equity markets are in disarray, putting pressure on the value of our endowment funds and creating a more difficult environment for fund-raising should current market conditions persist.

Nevertheless, there is much reason for optimism. The Laboratory continues to excel with its research and academic programs and continues to attract scientific and staff personnel of the very highest quality. I take great comfort in the fact that Morgan has agreed to serve as Chief Financial Officer. His experience and judgment are invaluable to me. Other recent appointments of note are the promotion of Assistant Comptroller Lari Russo to Comptroller; Jeff Picarello from Long Island University to Director of Public Affairs; and Charlie Prizzi, also from LIU, to Director of Special Events. Bill Keen, our veteran Comptroller, has taken on an important new assignment as Finance Director of the Cold Spring Harbor Laboratory Press in addition to acting as Assistant to the Chief Financial Officer. We are also most fortunate to be able to attract prominent and influential Trustees. While we will miss the services of Martha Gerry and John Phelan, I look forward to working with Doug Morris, Jeff Hawkins, and David Deming—all leaders in their respective industries.

Several months ago I was discussing, with Jim Watson, *Time Magazine*'s selection of Albert Einstein as their "Man of the Century." Jim agreed with the choice, saying that the 20th would be thought of as the century of physics but that the 21st would, undoubtedly, be the century of biology. Cold Spring Harbor Laboratory is a place where the best and brightest minds in biology come to work, study, teach, share information, and solve problems critical to mankind. The overriding goal of the Administrative staff is to facilitate this work by creating and maintaining an environment that is conducive to these ends. We undertake this responsibility with great pride, dedication, and seriousness of purpose.

W. Dillaway Ayres, Jr.
Chief Operating Officer

April, 2001


RESEARCH

TUMOR VIRUSES

The investigators in the Tumor Viruses Section are unified by the shared use of viruses to probe cell function and to understand cell transformation. The viruses used include adenovirus, human and bovine papillomaviruses, simian virus 40, herpes simplex virus, and human and simian immunodeficiency viruses.

- Arne Stenlund and Bruce Stillman use papillomavirus and SV40, respectively, to study DNA replication. The Stillman laboratory also studies cellular DNA replication in human and yeast cells, and how DNA replication is linked to the establishment of inherited states of gene expression.
- Winship Herr and Adrian Krainer study the control of gene expression, particularly the control of gene transcription and pre-mRNA splicing.
- William Tansey studies control of gene expression via modulation of transcription factor stability by regulated proteolysis, using the Myc oncoprotein as a model.
- Yuri Lazebnik and Scott Lowe focus their research on the cellular defenses against cancer including programmed cell death or apoptosis and senescence.
- Jacek Skowronski studies how the human and simian immunodeficiency viruses disrupt signal transduction and the expression of cell surface receptors in infected T cells.

See previous page for photos of the following scientific staff.
Row 1: Mila McCurrach; Masashi Narita; Julie Thomas; Dmitri Chklovskii; Rebecca Ewald, Mirjana Maletic-Savatic
Row 2: Kurt Haas; Terence Strick; Anitra Auster; Said Ahmad Zia; Joseph West
Row 3: Vivek Mittal; Kimberly LaVine; Marja Timmermans; Linda Van Aelst, Ming Ming Zhao
Row 4: Barbara Mish; Michelle Cilia; Tzu-Ching Meng; David Jackson
Row 5: Ira Hall, Zachary Lippman; Robert Lucito, Pablo Rabinowicz; Kenneth Seidenman, Songhai Shi
Row 6: Lidia Nascimento; Lidia Serina; Ahmet Denli; Varie Vil

TRANSCRIPTIONAL REGULATION

W. Herr D. Aufiero P. Reilly
A. Bubulya T. Tubon
E. Julien J. Wysocka
S. Lee X. Zhao

We are interested in the mechanisms of transcriptional regulation in human cells. We use the human herpes simplex virus (HSV) as a probe to uncover these mechanisms and to study virus–host cell interactions. Viruses provide simple regulatory networks in which the cellular transcriptional machinery is altered 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 carried in 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, a 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 through a potent transcriptional activation domain.

Our research continues to focus 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?

Transcriptional Activation

T. Tubon, X. Zhao

Our current studies on how transcriptional regulators activate the basal transcriptional machinery focus on the activities of two basal transcription factors, the TATA-box-binding protein TBP and TFIIB. TBP is interesting because it is involved in transcription by all three eukaryotic RNA polymerases: pol I, pol II, and pol III. We study the activity of TBP in transcription by both pol II and pol III from promoters containing and lacking TATA boxes by using four types of promoters: (1) promoters that direct the synthesis of mRNAs by pol II and contain a TATA box core promoter element (e.g., adenovirus major late and c-*fos* promoters); (2) promoters that direct the synthesis of small nuclear RNAs (snRNAs) by pol II and lack a TATA box (e.g., U1 and U2 snRNA gene promoters); (3) a promoter that directs the synthesis of an snRNA by pol III and contains a TATA box (i.e., U6 snRNA gene promoter); and (4) a TATA-less pol III promoter (e.g., the adenovirus VAI promoter).

We are currently studying the activity of TBP in these varying promoter contexts to understand how it can support transcriptional activation in such varying promoter contexts. Unlike TBP, TFIIB is only known to be involved in transcription by pol II, but it is interesting because it has a central role in the transcription initiation process by associating with the TATA-box-bound TBP molecule at the core promoter and interacting with pol II. As with TBP, we are studying the different activities of TFIIB in various promoter contexts for both mRNA-type and snRNA-type pol II transcription.

VP16-induced Complex

D. Aufiero, R. Babb, A. Bubulya

We study how transcription factors modify their transcriptional activity through selective protein-protein and protein-DNA interaction, by studying the structure and function of the VP16-induced complex. On

its own, VP16 displays little if any sequence-specific DNA-binding activity: It is only through association with Oct-1 and HCF-1 that it can bind DNA well and specifically. Thus, whether VP16 binds DNA directly in the VP16-induced complex has been controversial. Last year, we described the three-dimensional crystal structure of the core region of VP16 that is sufficient for VP16-induced complex assembly. The results revealed a novel, concave seat-like protein structure. We have now shown that, within the VP16-induced complex, the VP16 core has an important role in DNA binding. Mutation of basic residues on the surface of the VP16 core reveals a novel DNA-binding surface involving a conserved region of the concave seat-like surface. These results illuminate how, through association with DNA, VP16 is able to interpret *cis*-regulatory signals in the DNA to direct assembly of a multiprotein-DNA transcriptional regulatory complex.

Cellular Functions of HCF-1 and Oct-1

A. Bubulya, E. Julien, S. Lee, P. Reilly, J. Wysocka

HSV must maintain an intimate relationship with the host cell to remain latent for many years and yet retain the ability to grow lytically. Because VP16 associates with the cellular proteins HCF-1 and Oct-1 to initiate HSV gene expression during lytic infection, we hypothesize that HCF-1 and Oct-1 are key regulators of which mode of infection—latent or lytic—HSV enters. We are therefore interested in the natural cellular roles of HCF-1 and Oct-1, and how these roles may influence HSV infection. We focus primarily on the cellular functions of HCF-1 because HCF-1 has been highly conserved during evolution and is involved in cell proliferation.

Human HCF-1 is synthesized as a large approximately 2000-amino-acid precursor protein, which is proteolytically cleaved to generate a family of associated amino ($HCF\text{-}1_N$)-terminal and carboxy ($HCF\text{-}1_C$)-terminal polypeptides. The majority of HCF-1 in the cell is a complex of associated $HCF\text{-}1_N$ and $HCF\text{-}1_C$ subunits. In collaboration with K. Johnson and A. Wilson (New York University), we have characterized the structure and function of sequences required for $HCF\text{-}1_N$- and $HCF\text{-}1_C$-subunit association. HCF-1 contains two matched pairs of self-association sequences called SAS1 and SAS2. One of these matched association sequences—SAS1—consists of a short 43-amino-acid region of the $HCF\text{-}1_N$ subunit, which associates with a carboxy-terminal region of the $HCF\text{-}1_C$ subunit that is composed of a tandem pair of fibronectin type-3 repeats, a structural motif known to promote protein-protein interactions. Unexpectedly, a related protein called HCF-2, which is not proteolyzed, also contains a functional SAS1 association element, suggesting that this element does not function solely to maintain $HCF\text{-}1_N$- and $HCF\text{-}1_C$ -subunit association.

We have also continued our studies of a functional homolog of HCF in the worm *Caenorhabditis elegans* (called CeHCF). We have now demonstrated developmental regulation of CeHCF phosphorylation: A hyperphosphorylated form of CeHCF is present in embryos, whereas a hypophosphorylated form is present in L1 larvae. The phosphorylation patterns of

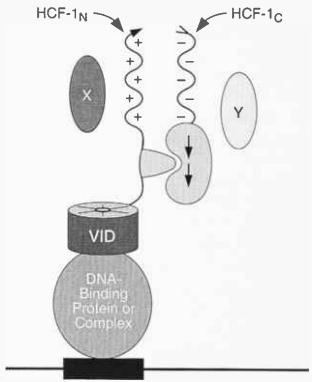


FIGURE 1 Hypothetical structure of HCF-1 bound to chromatin. In this model, HCF-1 is targeted to the DNA (*black box*) in chromatin by its VP16-interaction domain (VID) through protein-protein interaction with a DNA-binding protein or protein complex. Other regions of HCF-1, basic (+), acidic (–), and the $HCF\text{-}1_N$ and $HCF\text{-}1_C$ SAS1 association regions, are shown. Hypothetical effectors of HCF-1, proteins X and Y, are also shown.

endogenous CeHCF in worms and ectopically synthesized CeHCF in mammalian cells are remarkably similar, suggesting that the way CeHCF can be recognized by kinases is conserved in animals. Phosphorylation site mapping of endogenous CeHCF, however, revealed that phosphorylation occurs at four clustered sites in the region of the protein that is not highly conserved among HCF proteins and is not required for VP16-induced complex formation. Indeed, phosphorylation of either CeHCF or human HCF-1 appears dispensable for association with VP16. All four CeHCF phosphorylation sites match the consensus recognition site for the cell-cycle kinases CDC2 and CDK2. Consistent with this similarity and with the developmental phosphorylation of CeHCF in *C. elegans* embryos, CeHCF phosphorylation is cell-cycle-regulated in mammalian cells.

Finally, during the past year, we have discovered that HCF-1 is an abundant cellular protein that is naturally tethered to chromatin in uninfected cells through its amino-terminal 380-amino-acid VP16-interaction domain as illustrated in Figure 1. A single proline-to-serine substitution in the HCF-1 VP16-interaction domain, which is known to cause a temperature-induced cell proliferation arrest in hamster tsBN67 cells and prevent transcriptional activation by VP16, interferes with HCF-1 association with chromatin: At permissive temperature, HCF-1 is chromatin-bound in tsBN67 cells, but it dissociates from chromatin before tsBN67 cells stop proliferating at nonpermissive temperature, suggesting that loss of HCF-1 chromatin association is the primary cause of the temperature-induced tsBN67 cell proliferation arrest. We propose that, as during HSV infection, the role of HCF-1 in cell proliferation is to regulate gene transcription, in this instance, by associating with a multiplicity of DNA-bound transcription factors through its VP16-interaction domain.

PUBLICATIONS

Wilson A.C., Boutros M., Johnson K.M., and Herr W. 2000. HCF-1 amino- and carboxy-terminal subunit association through two separate sets of interaction modules: Involvement of fibronectin type 3 repeats. *Mol. Cell. Biol.* **20:** 6721–6730.

In Press

Wysocka J., Reilly P.T., and Herr W. 2001. Loss of HCF-1 chromatin association precedes temperature-induced growth arrest of tsBN67 cells. *Mol. Cell. Biol.* (in press).

Wysocka J., Liu Y., Kobayashi R., and Herr W. 2001. Developmental and cell-cycle regulation of *C. elegans* HCF phosphorylation. *Biochemistry* (in press).

RNA SPLICING

A.R. Krainer M. Aaron, L. Cartegni, M. Hastings, L. Manche, M. Murray, X. Roca, S. Shaw, Q.-S. Zhang, J. Zhu

MECHANISMS OF CONSTITUTIVE AND ALTERNATIVE PRE-mRNA SPLICING

RNA splicing is an essential step for the expression of most eukaryotic protein-coding genes. The selection and pairing of authentic splice sites within the spliceosome occur with a very high degree of fidelity, requiring precise interpretation of limited and dispersed sequence information present throughout introns and exons. The expression of many cellular and viral genes occurs via alternative splicing, which involves substantial flexibility in the choice of splice sites, allowing the expression of multiple protein isoforms from individual genes. 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 unexpectedly small number of genes uncovered by genome-sequencing projects.

Both constitutive and alternative splicing mechanisms involve multiple protein components, as well as RNA components that are part of small nuclear ribonucleoprotein (snRNP) particles. Our lab has focused on the identification, purification, and molecular characterization of protein factors that are necessary for the catalysis and fidelity of splicing and/or for the regulation of alternative splice site selection.

RS DOMAIN STRUCTURE AND FUNCTION

SR proteins are a conserved family of protein splicing factors, which consist of one or two RNA-recognition motifs and a characteristic arginine/serine-rich carboxy-terminal RS domain. The RS domain, which is extensively phosphorylated, mediates subcellular localization and shuttling of individual SR proteins and also functions as a splicing activation module, apparently by engaging in protein-protein interactions. The RS domain of SF2/ASF is dispensable for the concentration-dependent effects of this prototype SR protein on alternative splice site selection. However, this RS domain is highly conserved phylogenetically and was shown to be required for constitutive splicing in vitro and for cell viability. J. Zhu has now demonstrated that the RS domain of SF2/ASF is, in fact, dispensable for splicing of several substrates, including constitutive and enhancer-dependent pre-mRNAs. The requirement for this RS domain is substrate-specific and correlates with the strength of the splicing signals. When the 3′ splice site is weak, both the SF2/ASF RS domain and its interacting partner U2AF[35] (the small subunit of the splicing factor U2AF) are required for splicing. These results demonstrate the existence of an RS-domain-independent function of SR proteins in constitutive and enhancer-dependent splicing, and suggest mechanisms for their role in enhancer function besides U2AF recruitment. J. Zhu further showed that in the case of pre-mRNAs that do require the SF2/ASF RS domain for in vitro splicing, it is possible to replace the natural 51-residue RS domain by ten RS dipeptide repeats. This simplified RS domain is efficiently phosphorylated in vitro during the early stages of splicing, suggesting that the critical feature of an RS domain, at least for splicing in vitro, is the alternating charge structure.

EXONIC SPLICING ENHANCER MOTIFS AND EXON SKIPPING

We have continued to study the nature and function of exonic elements that stimulate the removal of adjacent introns. These elements are thought to be involved in exon definition, splicing fidelity, splicing efficiency, and regulation of alternative exon inclusion. Splicing enhancers are recognized specifically by individual members of the SR protein family. L. Cartegni has been pursuing the analysis of SR protein recognition motifs previously initiated by H.-X. Liu (in collaboration with M. Zhang). We analyzed a previously identified nonsense mutation in exon 18 of the *BRCA1* gene that causes skipping of that exon, thereby inactivating the gene and resulting in breast and ovarian cancer predisposition. We demonstrated that the single-base change disrupts an exonic splicing enhancer (ESE) that is recognized by the SR protein SF2/ASF. We were able to reproduce the nonsense-associated exon

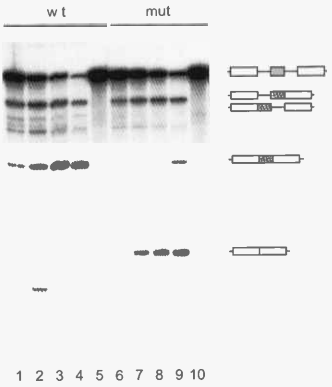


FIGURE 1 Nonsense-associated exon skipping of BRCA1 exon 18 can be reproduced in vitro. Radiolabeled *BRCA1* three-exon minigene transcripts were prepared by in vitro transcription, spliced in HeLa nuclear extract, and analyzed by denaturing PAGE and autoradiography. Time courses of in vitro splicing are shown for the wild-type transcript (lanes *1–4*) and a transcript with a nonsense mutation at position +6 in the middle exon (lanes *6–9*). The respective input RNAs are shown in lanes *5* and *10*. (*Unshaded boxes*) Exons 17 and 19; (*shaded box*) exon 18. Introns 16 and 17 have internal deletions.

skipping phenomenon in vitro, using *BRCA1* minigene transcripts (Fig. 1). Using our previous SR protein motif-scoring matrices, in conjunction with in vitro splicing assays, we further showed that a missense mutation in exon 18 that also disrupts the SF2/ASF-recognition motif likewise causes skipping of the exon, whereas a nonsense mutation that maintains a high-score SF2/ASF motif does not result in exon skipping.

Nonsense, missense, and silent mutations that cause skipping of the exon harboring the mutation are very common among disease-associated point mutations. Our analysis of SR protein motif scores in 50 such mutations in human genes suggested that approximately half of the mutations result in disruption of recognition motifs for the SR proteins SF2/ASF, SC35, SRp55, or SRp40. We conclude that protein-coding exons comprise interspersed signals that are very important for splicing fidelity. Point mutations in exons that are identified only at the genomic DNA level may be misclassified, unless the mRNA splicing patterns are also determined. L. Cartegni is currently applying this type of analysis to other clinically important genes, in particular the survival-of-motor-neuron genes, *SMN1* and *SMN2*, which are important in spinal muscular atrophy.

AT-AC PRE-mRNA SPLICING PATHWAY

M. Hastings is pursuing the characterization of AT-AC pre-mRNA splicing. AT-AC introns represent approximately 0.1% of introns in mammals. AT-AC introns have conserved signals that differ from those of the major class of introns, and their processing requires a distinct set of snRNAs. We found that this pathway requires the same set of SR proteins that are involved in conventional splicing. SR proteins are required for

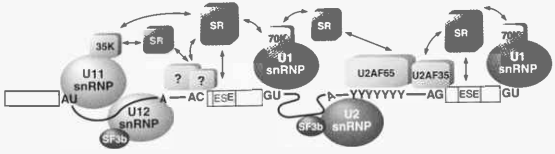


FIGURE 2 Model of exon-definition interactions involving an AT-AC intron and a conventional intron. A pre-mRNA with three exons (*boxes*) and two introns (*lines*) is shown. The first intron is of the AT-AC class, and the second intron is a conventional one. Their conserved 5′ splice site, 3′ splice site, and branch site sequence elements are indicated. The 5′ splice site of a third, downstream conventional intron is also shown. The second and third exon have exonic splicing enhancers (ESEs), which are recognized by SR proteins. Selected protein and snRNP components of the major and minor spliceosomes are shown, with the arrows denoting presumptive protein-protein or protein-RNA interactions.

the basal AT-AC splicing reaction and also appear to participate in AT-AC splicing stimulation via exonic enhancers or exon-definition interactions with adjacent conventional introns (Fig. 2). Thus, SR proteins function in both major and minor splicing pathways and in coordinating the activities of both spliceosomes by exon definition.

An AT-AC intron in a sodium channel pre-mRNA failed to splice in S100 extract complemented by SR proteins, unless an additional nuclear fraction was also present. In contrast, another AT-AC intron from the P120 gene did not require the additional fraction. Moreover, the two AT-AC introns differed with respect to their ability to splice in the presence of individual recombinant SR proteins. Analysis of chimeric sodium channel/P120 transcripts showed that partially redundant information dispersed throughout the introns and flanking exons dictates SR protein specificity and the requirement for the additional nuclear fraction. These and other results show that, despite the substantial differences in intron consensus sequences and in four of the five snRNPs in each spliceosome, at least some of the interactions involving SR proteins are conserved between the two pathways.

PUBLICATIONS

Boudrez A., Beullens M., Groenen P., Van Eynde A., Vulsteke V., Jagiello I., Murray M., Krainer A.R., Stalmans W., and Bollen M. 2000. NIPP1-mediated interaction of protein phosphatase-1 with CDC5L, a regulator of pre-mRNA splicing and mitotic entry. *J. Biol. Chem.* **275:** 25411–25417.

Dirksen W.P., Li X., Mayeda A., Krainer A.R., and Rottman F.M. 2000. Mapping the SF2/ASF binding sites in the bovine growth hormone exonic splicing enhancer. *J. Biol. Chem.* **275:** 29170–29177.

Eperon I.C., Makarova O.V., Mayeda A., Munroe S.H., Cáceres J.F., Hayward D.G., and Krainer A.R. 2000. Selection of alternative 5′ splice sites: Role of U1 snRNP and models for the antagonistic effects of SF2/ASF and hnRNP A1. *Mol. Cell. Biol.* **20:** 8303–8318.

Liu H.-X., Zhang M.Q., Chew S.L., and Krainer A.R. 2000. Exonic splicing enhancer motif recognized by human SC35 under splicing conditions. *Mol. Cell. Biol.* **20:** 1063–1071.

Pollard A.J., Sparey C., Robson S.C., Krainer A.R., and Europe-Finner G.N. 2000. Spatio-temporal expression of the *trans*-acting splicing factors SF2/ASF and heterogeneous ribonuclear proteins A1/A1^B in the myometrium of the pregnant human uterus: A molecular mechanism for regulating regional protein isoform expression *in vivo*. *J. Clin. Endocrinol. Metab.* **85:** 1928–1936.

van der Hooven van Oordt W., Diaz-Meco M.T., Lozano J., Krainer A.R., Moscat J., and Cáceres J.F. 2000. The $MKK_{3/6}$-p38-signaling cascade alters the subcellular distribution of hnRNP A1 and modulates alternative splicing regulation. *J. Cell Biol.* **149:** 307–316.

Van Scoy S., Watakabe I., Krainer A.R., and Hearing J. 2000. Human p32: A coactivator for Epstein-Barr virus nuclear antigen-1-mediated transcriptional activation and possible role in viral latent cycle DNA replication. *Virology* **275:** 145–157.

Zhu J. and Krainer A.R. 2000. Pre-mRNA splicing in the absence of an SR protein RS domain. *Genes Dev.* **14:** 3166–3178.

In Press

Hastings M. and Krainer A.R. 2001. Functions of SR proteins in the U12-dependent AT-AC pre-mRNA splicing pathway. *RNA* **7:** (in press).

Hastings M. and Krainer A.R. 2001. Pre-mRNA splicing in the new millenium. *Curr. Opin. Cell Biol.* (in press).

Liu H.-X., Cartegni L., Zhang M.Q., and Krainer A.R. 2001. A mechanism for exon skipping induced by nonsense or missense mutations in *BRCA1* and other genes. *Nat. Genet.* **27:** 55–58.

Nilsson C.E., Petersen-Mahrt S., Durot C., Shtrichman R., Krainer A.R., Kleinberger T., and Akusjärvi G. 2001. The adenovirus E4-ORF4 splicing enhancer protein interacts with a subset of phosphorylated SR proteins. *EMBO J.* **20:** (in press).

REGULATION OF APOPTOSIS IN CANCER CELLS

Y. Lazebnik D. Duelli X. Opitz-Araya
L. Faleiro J. Raychaudhuri
P. Lassus J. Rodriguez
S.-C. Lin

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 can 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 a 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 in 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. During the last year, we investigated how E1A regulates caspase-9 activation and continued to study basic mechanisms of caspase functioning.

Caspases Break the Nuclear Cytoplasmic Barrier

L. Faleiro

During apoptosis, caspases disassemble a cell by cleaving a set of proteins. Caspase-3 has a major role in disassembly of the nucleus by processing several nuclear substrates. The question was how caspase-3, which is usually cytoplasmic, gains access to its nuclear targets. This problem was part of a general puzzle: How does the apoptotic machinery, which is activated in the cytoplasm, reach the nucleus. It was suggested that caspase-3 is actively transported to the nucleus through nuclear pores. In contrast, we found that caspase-9, which is activated before caspase-3, directly or indirectly inactivates nuclear transport and increases the diffusion limit of the nuclear pores. This allows caspase-3 and other molecules that cannot normally pass through the nuclear

pores in living cells to enter or leave the nucleus by diffusion. Hence, we concluded that caspases enter the nucleus because caspase-9, directly or indirectly, disrupts the nuclear-cytoplasmic barrier. Interestingly, we found that caspase-8, another initiator caspase, is unable to change the diffusion limit of the nuclear pores and disrupt nuclear transport; this argues that the current model, that initiator caspases directly converge on the common effector caspases, is an oversimplification.

Compensatory Activation of Caspases

Y. Lazebnik [in collaboration with R. Flavell, Yale University]

Caspases are considered attractive targets for therapeutic intervention, even though it is not known with certainty how these enzymes are regulated. One question is to what degree are caspase activities redundant. For example, would eliminating one caspase result in compensatory activation of another caspase? We collaborated with the laboratory of Dr. Richard Flavell to address this question experimentally, using an animal model of apoptosis induced by Fas, a receptor whose activation leads to cell death. In this model, mice are injected with an antibody that acts as a Fas agonist. Activation of Fas, in turn, leads to activation of caspase-8, which is thought to initiate two pathways. One involves direct processing of caspase-3, and the other, processing of Bid. The truncated Bid then promotes the release of cytochrome *c*, thus activating caspase-9. As a result, injection of Fas antibody leads to massive apoptosis in the liver, causing death of the animal. A deficiency in Bid prevents death, predicting that a deficiency in caspase-9 should have the same effect. However, we found that a deficiency in caspase-9 failed to protect animals from death. Biochemical analysis revealed that the set of caspases activated in mice lacking caspase-9 differed from that observed in wild-type mice. Although we did not identify the caspase that compensates for the capase-9 deficiency, our unexpected observations provided direct experimental evidence for compensatory pathways of caspase activation. These observations provided another reminder that developing caspase inhibitors and activators for clinical applications must be preceded by an effort to better understand caspase regulation.

E1A and Myc Promote Apoptosis by Suppressing Inhibitors of This Process

D. Duelli

We have found that adenovirus oncogene E1A sensitizes cells to pro-apoptotic drugs by facilitating activation of caspase-9. In principle, E1A can promote activation of caspase-9 in three ways. The prevailing model (Fig. 1a) is that E1A directly or indirectly enables expression of pro-apoptotic proteins, such as Bax. An alternative possibility is that normal cells are protected from apoptosis by inhibitors that are inactivated in response to E1A expression (Fig. 1b). The third possibility is a combination of the first two (Fig. 1c). We tested these models using a cell-fusion complementation assay. In this assay, we fused primary human fibroblasts with fibroblasts that express E1A and immediately treated the resulting heterokaryons with etoposide, an anti-cancer drug that induces DNA damage. We reasoned that if the induction of dominant pro-apoptotic proteins is sufficient for apoptosis, then the heterokaryons would undergo apoptosis, as would the cells expressing E1A. However, if expression of the apoptosis inhibitors were repressed by E1A, then the heterokaryons would be resistant to the drug treatment. We found that the heterokaryons remained resistant to drug for at least 40 hours after fusion. We concluded that primary cells contain inhibitors of apoptosis which we called IODA (inhibitor of oncogene-dependent apoptosis). Our subsequent studies indicated that IODA inhibits apoptosis by preventing cytochrome *c* release, whereas additional preliminary results suggest that other activities may prevent the subsequent steps of caspase-9 activation. Our current

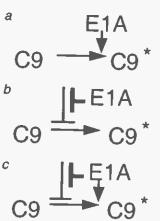


FIGURE 1 In principle, E1A can promote activation of caspase-9 in three ways: (*a*) by inducing pro-apoptotic proteins; (*b*) by inactivating inhibitors of apoptosis; and (*c*) by a combination of both.

model is that oncogenes suppress several activities that prevent caspase-9 activation at multiple steps. Of interest, IODA is repressed not only by E1A, but also by Myc, which suggests that this activity is regulated by multiple oncogenic stimuli. Here, we propose to identify IODA, as our preliminary results place it as a link between oncogenes and the apoptotic machinery.

E1A Enables a Link between DNA Damage and Translocation of Bax to Mitochondria

D. Duelli

IODA could be a direct inhibitor of cytochrome *c* release or could prevent E1A from activating pro-apoptotic factors that are required for release. To distinguish between these two possibilities, we focused on the Bax protein. Bax can release cytochrome *c* by binding to mitochondria. It has been reported that induction of Bax by E1A contributes to sensitization to cytotoxic drugs. We found that Bax was distributed diffusely both in primary cells and in cells expressing E1A. Following etoposide treatment, Bax concentrated at the mitochondria of E1A-expressing cells, but remained diffuse in primary cells. Hence, expression of E1A establishes the link between DNA damage, which is the primary effect of etoposide, and Bax translocation. Bax also translocated in heterokaryons between primary and E1A-expressing cells following treatment with etoposide, indicating that IODA does not sever the link between DNA damage and Bax translocation. Thus, we concluded that expression of E1A sensitizes cells to an anti-cancer drug by at least two pathways (as in Fig. 1c). One establishes a link between the drug and Bax, whereas the second suppresses IODA, which blocks cytochrome *c* release even if Bax is translocated to mitochondria (Fig. 2).

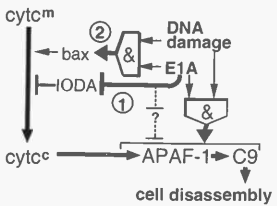


FIGURE 2 E1A facilitates apoptosis by at least two pathways: (1) repressing inhibitors (IODA) and (2) promoting activators (Bax) of apoptosis.

Is Caspase-9 Required for Chemotherapy-induced Apoptosis in All Tumor Cells?

X. Opitz-Araya, Y. Lazebnik [in collaboration with S. Lowe, Cold Spring Harbor Laboratory, and S. Kaufmann, Mayo Clinic]

The strict requirement for caspase-9 for E1A-dependent apoptosis makes fibroblasts a good model for studying the basic mechanisms that link oncogenic transformation and the apoptotic machinery. However, an important question is whether conclusions reached studying E1A-transformed fibroblasts can be applied to tumor cells. Studies by Dr. Scott Lowe, in which we participated, indicated that a deficiency in APAF-1 is linked to drug resistance in some melanomas. However, studies by Dr. Scott Kaufmann, to which we also contributed, found that in acute myelogenous leukemia (AML) and acute lymphocytic leukemia (ALL), levels of caspases and APAF-1 did not correlate with prognosis or with response to chemotherapy. These observations suggested several possibilities: that chemotherapy kills these tumor cells in a caspase-independent way, that as yet unidentified caspases are involved, or that regulation of known caspases is affected in drug-resistant cells in ways that we still need to understand.

PUBLICATIONS

Bellows D.S., Chau B.N., Lee P., Lazebnik Y., Burns W.H., and Hardwick J.M. 2000. Antiapoptotic herpesvirus Bcl-2 homologs escape caspase-mediated conversion to proapoptotic proteins. *J. Virol.* **74:** 5024–5031.

Duelli D.M. and Lazebnik Y.A. 2000. Primary cells suppress oncogene-dependent apoptosis. *Nat. Cell Biol.* **2:** 859–862.

Faleiro L. and Lazebnik Y. 2000. Caspases disrupt the nuclear-cytoplasmic barrier. *J. Cell Biol.* **151:** 951–960.

Rodriguez J., Chen H., Lin S., and Lazebnik Y. 2000. Caspase phosphorylation, cell death, and species variability. *Science* **287:** 1363a.

Svingen P.A., Karp J.E., Krajewski S., Mesner, Jr., P.W., Gore S.D., Burke P.J., Reed J.C., Lazebnik Y.A., and Kaufmann S.H. 2000. Evaluation of apaf-1 and procaspases-2, -3, -7, -8, and -9 as potential prognostic markers in acute leukemia. *Blood* **96:** 3922–3931.

Zheng T.S., Hunot S., Kuida K., Momoi T., Srinivasan A., Nicholson D.W., Lazebnik Y., and Flavell R.A. 2000. Deficiency in caspase-9 or caspase-3 induces compensatory caspase activation. *Nat. Med.* **6:** 1241–1247.

REGULATION OF APOPTOSIS AND SENESCENCE BY CANCER GENES

S. Lowe E. de Stanchina, G. Ferbeyre, J. Fridman, W. Jiang, J. Jin, A. Lin, M. McCurrach, Z. Nahle, M. Narita, J. Polyakova, E. Querido, C. Rosenthal, A. Samuelson, C. Schmitt, M. Soengas

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.

Much of our research stems from our interest in the p53 tumor suppressor. p53 functions as a key component of several cellular stress responses and as such acts at a variety of levels to protect against cancer. For example, p53 can be activated by DNA damage to trigger cell cycle checkpoints or apoptosis, such that cells lacking p53 are prone to certain forms of mutation and genomic instability. This implies that p53 can *indirectly* suppress tumorigenesis by acting as a "Guardian of the Genome," i.e., to promote the repair or elimination of cells that have sustained potentially deleterious mutations. Remarkably, since most current anticancer agents damage DNA, the integrity of this p53 response may contribute to the outcome of cancer therapy. In addition, certain mitogenic oncogenes can activate p53 to promote apoptosis or senescence. Loss of p53 prevents these homeostatic responses, leading to oncogenic transformation or tumor progression. This implies that p53 can *directly* suppress tumorigenesis by acting in a fail-safe mechanism to counter hyperproliferative signals. We currently study many aspects of p53, including how oncogenes or DNA-damaging agents signal p53, how p53 executes a biological response, and the factors that influence whether p53 induces a cell cycle checkpoint, cellular senescence, or apoptosis.

Oncogene-induced Apoptosis

E. de Stanchina, J. Jin, M. McCurrach, Z. Nahle, J. Polyakova, A. Samuelson [in collaboration with Y. Lazebnik, Cold Spring Harbor Laboratory]

Our laboratory was the first to demonstrate that oncogenes can activate p53 to promote apoptosis. Our current research focuses on how oncogenes sensitize cells to apoptotic stimuli and the execution of apoptosis in these oncogene-primed cells. Using murine fibroblasts as a model, we have produced a working framework for this process (Fig. 1). Current questions include: What are the cellular targets of E1A in promoting apoptosis? How does $p19^{ARF}$ couple oncogenes to p53? How do oncogenes promote apoptosis independently of p53? To what extent does disruption of oncogene-induced apoptosis contribute to tumor development in vivo? To address these questions, we continue to use the murine fibroblast system, which has proven effective in the past, but we are also investing in new gene expression and transgenic technologies that will impact this project in future years.

E1A promotes apoptosis using at least two separable functions: The first involves inactivation of Rb, and the second requires an amino-terminal E1A region. Although our previous studies have suggested that it is the p300/CBP interaction which is the relevant amino-terminal target, our recent work shows that

FIGURE 1 The p53 apoptotic pathway. Shown is a model for the p53 apoptotic program as determined from genetic studies using primary murine fibroblasts. Although the regulation of this pathway is clearly more complex than indicated here, this model provides a framework for much of our current investigation.

the regions of E1A required to promote apoptosis are distinct from those required to bind p300/CBP (A. Samuelson, in prep.) and instead correlate with those required to associate with a poorly characterized E1A-binding protein known as p400. In parallel, we have shown that E1A induces several caspases through a transcriptional and translational mechanism that involves deregulation of the Rb pathway (J. Polyakova, in prep.). Hence, caspase induction strictly depends on the E1A-Rb interaction and occurs both in Rb-deficient fibroblasts and in normal fibroblasts overexpressing E2F-1. Interestingly, E1A also induces caspases in fibroblasts lacking ARF or p53, implying that caspase induction occurs independently of the ARF-p53 tumor suppressor pathway. Although pro-caspase induction by E1A cannot solely explain its pro-apoptotic activity, it seems likely that this contributes to the p53-independent apoptosis induced by oncogenes. We hope that our efforts to understand caspase regulation will lead to a better understanding of this E1A action in apoptosis.

Effector Mechanisms of p53-dependent Apoptosis

W. Jiang, M. McCurrach, M. Soengas [in collaboration with L. Attardi and T. Jacks, Massachusetts Institute of Technology; D. McCombie, Cold Spring Harbor Laboratory; M. Esteller and J. Herman, Johns Hopkins University; W. Gerald and C. Cordon-Cardo, Memorial Sloan-Kettering Cancer Center]

We have had a long-standing interest in how p53 promotes apoptosis and the importance of this process for its tumor suppressor functions. This year, we worked with L. Attardi and T. Jacks (MIT) to identify a new p53 transcriptional target that is specifically induced by p53 during apoptotic cell death (Attardi et al. 2000). This gene, called PERP (*p53* apoptosis *e*ffector *r*elated to *P*MP-22), was identified from a differential gene expression screen using fibroblasts expressing or lacking E1A. It encodes a plasma membrane protein with sequence similarity to the tetraspan membrane protein PMP-22/gas3 and is a candidate tumor suppressor. How this molecule might promote apoptosis will be the subject of future research by Dr. Attardi, and we will continue to contribute to her analysis.

Last year, we showed that the cell death effectors Apaf-1 and Casp9 mediate the p53 apoptotic signal in oncogene-expressing cells, such that loss of either gene substituted for p53 loss in facilitating the oncogenic transformation (Soengas et al., *Science 284:* 156 [1999]). As such, this study identified Apaf-1 and Casp9 as candidate tumor suppressors. This year, we showed that metastatic melanomas (which rarely have *p53* mutations) often lose Apaf-1, a cell death effector that acts with cytochrome *c* and caspase-9 to mediate p53-dependent apoptosis (M.S. Soengas et al., in press). Loss of Apaf-1 expression is accompanied by allelic loss in metastatic melanomas, but it can be recovered in melanoma cell lines by treatment with the methylation inhibitor 5-aza-2′-deoxycytidine (5azaCdR). Apaf-1-negative melanomas are invariably chemoresistant and unable to execute a typical apoptotic program in response to p53. Restoration of physiologic Apaf-1 levels through gene transfer or 5azaCdR treatment markedly enhanced chemosensitivity and rescued the apoptotic defects associated with Apaf-1 loss. Therefore, these studies demonstrate that Apaf-1 is inactivated in metastatic melanomas leading to defects in the execution of apoptotic cell death. We suspect that Apaf-1 loss may contribute to the low frequency of *p53* mutations observed in this highly chemoresistant tumor type. We are continuing to explore the basis of Apaf-1 inactivation in melanoma and are interested in extending our understanding of Apaf-1 loss on the biology of melanoma.

Control of Cellular Senescence

G. Ferbeyre, A. Lin, E. de Stanchina, M. McCurrach, M. Narita, E. Querido

We previously showed that expression of oncogenic *ras* in primary human or rodent fibroblasts results in a

permanent arrest accompanied by accumulation of p53, p16, and $p19^{ARF}$ and is phenotypically indistinguishable from cellular senescence. We hypothesize that this program limits the transforming potential of oncogenic *ras*. Currently, we are addressing several questions: How does *ras* signaling activate a permanent cell cycle arrest? How do tumor suppressors such as p53, p16, and $p19^{ARF}$ initiate and maintain this arrest? What is the relationship between *ras*-induced arrest and replicative senescence? How do epithelial cells respond to *ras*?

We previously used cDNA array technology to compare *ras*-induced arrest to replicative senescence. In collaboration with W. Funk (Geron Corporation), we showed that the transcriptional profiles of cells arrested by oncogenic *ras* or by serial passaging ("senescent") are quite similar (Shelton et al., submitted). Independently, we characterized some of these changes in more detail. For example, we noted that one of the genes up-regulated during *ras*-induced arrest was the promyelocytic leukemia (PML) protein, a potential tumor suppressor that encodes a component of nuclear structures known as promyelocytic oncogenic domains (PODs) (Ferbeyre et al. 2000). We have shown that PML levels increase during both *ras*-induced arrest and replicative senescence, leading to a dramatic increase in the size and number of PODs. And importantly, in a series of genetic experiments, we have provided strong evidence that PML contributes to premature senescence. Precisely how this works remains to be established, but we have noted that a portion of p53 migrates to the PODs in *ras*-arrested cells. Together, our data imply that PML acts with Rb and p53 to promote *ras*-induced senescence and provides new insights into PML regulation and activity.

Although our analysis of fibroblasts clarifies aspects of senescence control, an important goal of our research is to demonstrate that this process actually suppresses tumor development. One potentially relevant setting is during chemically induced skin carcinogenesis in mice. In this paradigm of epithelial neoplasia, oncogenic *ras* mutations precede *p53* and *INK4a/ARF* mutations during the progression toward malignancy. This year, we have shown that, as in fibroblasts, oncogenic *ras* induces keratinocyte cell cycle arrest in a manner involving the $p19^{ARF}$, $p16^{INK4a}$, and p53 tumor suppressors. This arrest clearly limits tumor development, since ARF-null keratinocytes expressing oncogenic *ras* rapidly formed carcinomas in immunocompromised mice, whereas their wild-type counterparts were not tumorigenic. Importantly, the mechanism of *ras*-induced arrest in cultured keratinocytes can explain some of the mutational events observed during carcinoma progression in vivo: Specifically, selective pressure to override *ras*-induced arrest may enrich for cells with *p53* or *INK4a/ARF* mutations. Future work will continue to explore this hypothesis.

Molecular Genetics of Drug Sensitivity and Resistance

J. Fridman, Z. Nahle, C. Rosenthal, C. Schmitt

A major goal of our research is to understand the biological and molecular basis of drug sensitivity and resistance in tumors. 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. Last year, we established this model as a tractable system to study drug action and showed that tumors with disrupted *p53* or *INK4a/ARF* genes were severely compromised in their ability to respond to conventional chemotherapeutic agents. We wish to use this system to identify additional factors that affect treatment sensitivity.

This year, we have developed new methods to manipulate the genetics of these lymphomas to further dissect treatment response (Schmitt et al. 2000; C.S. Schmitt et al., in press; C.S. Schmitt and S. Lowe, in press]. Our approach uses primary *Eμ-myc* lymphomas and retroviral gene transfer to rapidly generate a series of spontaneous tumors differing only in a gene of interest, allowing us to study the impact of the test gene on the treatment sensitivity in a highly controlled but physiological context. Using this system, we have demonstrated that the Bcl-2 oncoprotein produces multidrug resistance when assessed in primary lymphomas. In contrast, this effect was dramatically reduced when the primary lymphomas were subjected to long-term culture and completely missed in the standard clonogenic survival assay. Hence, by comparing results from our in vivo system to more conventional assays, we have shown that some determinants of treatment sensitivity are missed

in vitro or when using established lymphoma cell lines. We will continue to use this system to improve our understanding of drug action and, ultimately, to provide relevant preclinical settings to test new anti-cancer agents.

We are also taking more nonbiased approaches to studying treatment sensitivity. To this end, we have produced a series of lymphomas that are resistant to different therapies and are beginning to use genome-wide approaches to identify the molecular basis for drug resistance (C.S. Schmitt et al., in press). In one approach, drug-sensitive and drug-resistant lymphomas are analyzed by transcriptional profiling. To this end, we are working with V. Mittal here at the Laboratory to produce a microarray consisting of approximately 7000 murine genes and expressed sequence tags. Larger microarrays are in production, and preliminary results indicate that high-quality transcriptional profiles can be obtained using lymphoma RNA (Z. Nahle, V. Mittal, C. Schmitt, unpubl.). We fully expect this and other genome-wide approaches to provide important new insights into drug sensitivity and resistance.

PUBLICATIONS

Attardi L.D., Reczek E.E., Demicco E.G., Cosmas C., McCurrach M.E., Lowe S.W., and Jacks T. 2000. PERP, an apoptosis-associated target of p53, is a novel member of the PMP-22/gas3 family. *Genes Dev.* **14:** 704–718.

Ferbeyre G., de Stanchina E., Querido E., Baptiste N., Prives C., and Lowe S.W. 2000. PML is induced by oncogenic *ras* and promotes premature senescence. *Genes Dev.* **14:** 2015–2027.

Lowe S.W. and Lin A.W. 2000. Apoptosis in cancer. *Carcinogenesis* **21:** 485–495.

Nakaya N., Lowe S.W., Taya Y., Chenchik A., and Enikolopov G. 2000. Specific pattern of p53 phosphorylation during nitric oxide-induced cell cycle arrest. *Oncogene* **19:** 6369–6375.

Rocha S., Soengas M.S., Lowe S.W., Glanzmann C., Fabbro D., Winterhalter K., Bodis S., and Pruschy M. 2000. Protein kinase C inhibitor and irradiation-induced apoptosis: Relevance of the cytochrome *c*-mediated caspase-9 death pathway. *Cell Growth Differ.* **11:** 491–499.

Schmitt C.S., Rosenthal C.T., and Lowe S.W. 2000. Genetic analysis of chemoresistance in primary murine lymphomas. *Nature Med.* **6:** 1029–1035.

Soengas M.S. and Lowe S.W. 2000. p53 and p73: Seeing double? *Nature Gen.* **26:** 391–392.

Yang X., Sham J.S., Ng M.H., Tsao S.W., Zhang D., Lowe S.W., and Cao L. 2000. LMP1 of Epstein-Barr virus induces proliferation of primary mouse embryonic fibroblasts and cooperatively transforms the cells with a p16-insensitive CDK4 oncogene. *J. Virol.* **74:** 883–891.

In Press

Lin A.W. and Lowe S.W. 2001. Oncogenic ras activates the ARF-p53 pathway to suppress epithelial cell transformation. *Proc. Natl. Acad. Sci.* (in press).

McCurrach M.E. and Lowe S.W. 2001. Methods for studying pro- and antiapoptotic genes in nonimmortal cells. *Methods Cell Biol.* (in press).

Schmitt C.A. and Lowe S.W. 2001. Bcl-2 mediates chemoresistance in matched pairs of primary *Eμ-myc* lymphomas *in vivo*. *Blood Cells Mol. Dis.* (in press).

Schmitt C.A., Wallace-Brodeur R.R., Rosenthal C.T., McCurrach M.E., and Lowe S.W. 2001. DNA damage responses and chemosensitivity in the *Eμ-myc* mouse lymphoma model. *Cold Spring Harbor Symp. Quant. Biol.* (in press).

Soengas M.S., Capodieci P., Polsky D., Mora J., Esteller M., Optiz-Araya X., McCombie R., Herman J.G., Gerald W.L., Lazebnik A., Cordon-Cardo C., and Lowe S.W. 2001. Inactivation of the apoptosis effector *Apaf-1* in malignant melanoma. *Nature* (in press).

CELL SIGNALING IN HIV PATHOGENESIS

J. Skowronski M.E. Greenberg B. Hill A. Janardhan N. Shohdy T. Swigut

Our interest is in understanding the molecular mechanisms underlying the pathogenesis of AIDS and, in particular, in understanding the functional consequences of the 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. Viruses with defects in Nef can replicate poorly in the host, and this correlates with attenuated development of AIDS. Deleting the *nef* gene is a common strategy to generate attenuated immunodeficiency viruses for use as live vaccines.

NEF FUNCTIONS

Natural Nef protein isolates have three conserved classes of effects on signal transduction and protein-sorting machineries that probably enhance viral virulence in vivo. One effect of Nef is to disrupt the molecular machinery mediating normal T-cell responses to antigens. This is accomplished by down-regulating the cell surface CD4 molecule, an essential component of the T-cell antigen receptor (TCR) on class II major histocompatibility complex (MHC)-restricted T lymphocytes, and by blocking signal transduction via the CD3 signaling component of the TCR. Another effect of Nef is to down-regulate expression of class I MHC complexes at the cell surface. Class I MHC molecules are critical elements of the immune system detection machinery that allow elimination of virally infected cells. Thus, this effect of Nef may enable infected cells to evade the immune response in the infected host. Finally, Nef also stimulates infectivity of SIV and HIV virions and stimulates replication in natural T cells.

In previous years, we probed and described the molecular mechanisms that mediate the effects of HIV-1 Nef on CD4 and class I MHC expression and initiated a structure-function analysis of Nef protein encoded by a pathogenic strain of SIV. These experiments identified mutant SIV Nef proteins that were selectively defective for subsets of functions and paved the way to experiments addressing the contribution of these individual functions toward enhancing viral virulence in a rhesus macaque model of human AIDS. These experiments continued to be the major focus of our recent research.

SURFACES OF NEF REQUIRED FOR DOWN-REGULATION OF CD4 AND FOR THE ENHANCEMENT OF VIRION INFECTIVITY ARE CRITICAL FOR EFFICIENT SIV REPLICATION IN VIVO

We demonstrated previously that the effects of Nef on CD4 expression, class I MHC expression, and the signal transduction machinery are genetically separable and map to different surfaces in the SIV Nef molecule. During the last year, we completed a series of experiments that addressed the individual roles of SIV Nef surfaces involved with CD4 down-regulation, enhancement of SIV infectivity, and in vitro replication on SIV replication in rhesus macaques. These experiments were performed in collaboration with Dr. Frank Kirchhoff and Dr. Christiane Hennig-Stahl (University of Erlangen and German Primate Center, Germany).

We constructed an SIVmac239 variant containing three amino acid substitutions in Nef that disrupted its ability to down-regulate CD4 (EDR mutation). This mutant Nef protein also lost the ability to stimulate SIV infectivity and replication in simian peripheral blood mononuclear cells, suggesting that these functions are linked to the CD4 down-regulation function of Nef. Importantly, the EDR mutation had no detectable effect on other Nef effects such as down-regulation of TCR-initiated signaling and the class I MHC.

Six rhesus macaques were infected with an SIV variant carrying the EDR mutation in Nef, and additional animals were infected with control viruses con-

taining either an intact or a nonfunctional *nef* gene. Interestingly, in all animals inoculated with SIV containing the EDR-mutated Nef, viral loads early in infection were unusually low and similar to those seen in infection with SIV containing the nonfunctional *nef* gene. Subsequent increases in viral loads coincided with the selection of amino acid changes that restored Nef functions disrupted by the EDR mutation. Our results indicate that surfaces of the SIV Nef protein that mediate molecular interactions important for CD4 down-regulation and in vitro replication are important for optimal in vivo replication early in the infection.

ANTIVIRAL IMMUNE RESPONSE AND SIV VIRULENCE

Presentation of antigens derived from viral proteins by class I MHC molecules to competent effector T cells is the key event required for the recognition and elimination of infected cells by the immune system of the host. Notably, EDR-mutated Nef retained the ability to down-regulate class I MHC molecules from the cell surface and was therefore likely to promote survival of infected cells in the face of an active immune response. On the other hand, SIV with EDR-mutated Nef replicated poorly in the infection. These findings indicate that class I MHC down-regulation by Nef is not sufficient for SIV virulence early in infection, possibly because the immune response against SIV is not yet fully developed, or because other factors are limiting viral replication at this early phase of infection.

MUTATIONS IN SIV NEF THAT SELECTIVELY DISRUPT CLASS I MHC DOWN-REGULATION

Down-regulation of class I MHC expression could be very important for the establishment of persistent SIV/HIV infection once strong antiviral responses develop in the infected host. To directly address the role of this Nef function as well as the broader issue of the role of the immune response during immunodeficiency virus infection and progression to AIDS, we studied class I MHC down-regulation by SIV Nef in detail. Initially, we compared the mechanisms used by SIVmac239 Nef and HIV-1 Nef to down-regulate class I MHC and found that the ability of SIV Nef to down-regulate class I MHC requires a unique carboxy-terminal region of the mac239 Nef molecule, which is not found in HIV-1 Nef. However, similar to HIV-1 Nef, we found that down-regulation of class I MHC by SIV Nef requires a conserved tyrosine in the cytoplasmic domain of the class I MHC heavy chain and involves accelerated endocytosis of class I complexes. Thus, although SIV and HIV-1 Nef proteins use a similar mechanism to down-regulate class I MHC expression, they have evolved different surfaces for molecular interactions with cell factors that regulate class I MHC traffic. Notably, mutations in the carboxy-terminal domain of SIVmac239 Nef selectively disrupt class I MHC down-regulation, having no detectable effect on other functions of Nef such as the down-regulation of CD4 and CD3 surface expression, or the stimulation of SIV virion infectivity and induction of SIV replication from peripheral blood mononuclear cells infected in the absence of stimulation. Finally, we contructed several mutant Nef proteins with small deletions in the carboxy-terminal region. These mutations likewise selectively disrupt only class I MHC down-regulation, but they are less likely to revert in vivo than point mutations. We hope that these mutants will be useful reagents to study the importance of class I MHC down-regulation for SIV replication in the context of a functional immune system in rhesus macaques.

PUBLICATIONS

Carl S., Iafrate A.J., Lang S.M., Stolte N., Stahl-Hennig C., Matz-Rensing K., Fuchs D., Skowronski J., and Kirchhoff F. 2000. Simian immunodeficiency virus containing mutations in N-terminal tyrosine residues and in the PxxP motif in Nef replicates efficiently in rhesus macaques. *J. Virol.* **74:** 4155–4164.

Iafrate A.J., Carl S., Bronson S., Stahl-Hennig C., Swigut T., Skowronski J., and Kirchhoff F. 2000. Disrupting surfaces of Nef required for downregulation of CD4 and for the enhancement of virion infectivity attenuates simian immunodeficiency virus replication in vivo. *J. Virol.* **74:** 9836–9844.

Swigut T., Iafrate A.J., Muench J., Kirchhoff F., and Skowronski J. 2000. Simian and human immunodeficiency virus Nef proteins use different surfaces to downregulate class I major histocompatibility antigen expression. *J. Virol.* **74:** 5691–5701.

In Press

Swigut T., Shohdy N., and Skowronski J. 2001. Mechanism for downregulation of CD28 by Nef. *EMBO J.* (in press).

MOLECULAR BIOLOGY OF PAPILLOMAVIRUSES

A. Stenlund C. Sanders H. Maats
E. Gillitzer G. Chen
A. Lee

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 to generate initiation complexes. Our studies demonstrate that the E1 protein has all the characteristics of an initiator protein, including ori recognition, DNA-dependent ATPase activity, and DNA helicase activity. The transcription factor E2, whose 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, and the assembly and loading of the E1 replication 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.

Initiator Complex Assembly

C. Sanders

An intriguing aspect of viral initiator proteins is their ability to perform several different, seemingly unrelated, biochemical functions. Our model to explain how these activities can reside in one single polypeptide is that different oligomeric forms of the protein may have different activities. In agreement with such a model, we have previously demonstrated that a dimeric form of E1 recognizes the ori together with E2 and that the form of E1 which has helicase activity is a hexamer. Our current understanding of the assembly pathway of these complexes is that a sequence-specif-

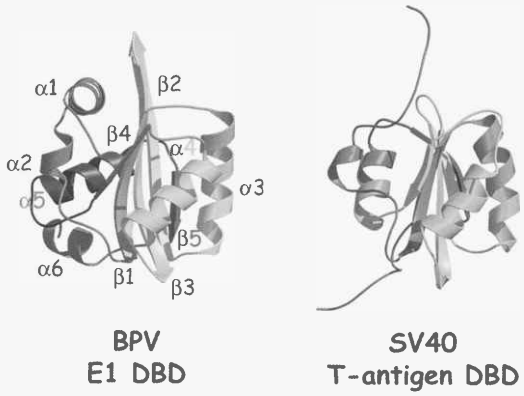


FIGURE 1 Ribbon diagrams of the three-dimensional structures of E1 DBD and the SV40 T antigen DBD showing the similarity of the fold.

ic complex of a dimer of E1 and a dimer of E2 together bind to the ori and initially recognize the ori. In an ATP-dependent manner, the E2 dimer is displaced and additional E1 molecules are added to the complex. These are bound sequence specifically, forming a tetramer on the four paired E1-binding sites in the core.

The Crystal Structure of the E1 DBD

G. Chen [in collaboration with E. Enemark, D. Vaughn, and L. Joshua-Tor, Cold Spring Harbor Laboratory]

To understand the DNA-binding properties of the E1 DNA-binding domain (DBD), we undertook structural analysis of the E1 DBD in collaboration with L. Joshua-Tor here at the Laboratory. The E1 DBD has a central five-stranded β-sheet and flanking α helices on either side of the sheet. Previous mutational analyses have revealed that a number of positively charged residues are critical for DNA binding. These residues are present in two conserved regions, which form a continuous area on the surface of the protein. The first region is located on an extended loop between α2 and β1 (which we refer to as the DNA-binding loop) and the second is amino-terminal to helix α4 (which we refer to as the DNA-binding helix). The DNA-binding loop (residues 180–190), which contains the several of the residues shown to be critical for DNA binding, is a very striking feature of the structure. Although this loop does not contain internal secondary structure, it is very well-defined in the crystal and adopts an extended path.

As mentioned earlier, both full-length E1 and E1 DBD are monomers in solution, although they bind to the ori as a dimer together with a dimer of E2. One of the E1/E1 interactions that is present in the crystal places the DNA-binding surfaces of the two monomers approximately 34 Å apart on the same face of this putative dimer. This distance corresponds to one helical turn of a B-DNA double helix. Since the E1-binding sites on DNA are positioned one helical turn apart, this dimeric form is particularly appealing. To test whether this interaction was relevant for dimerization, we substituted two small hydrophobic residues at the dimer interface with the large charged residue arginine. Since E1 DBD can bind to a half-site ori as a monomer in the presence of E2 DBD, E1 defective for dimerization should retain the DNA-binding activity but bind DNA as a monomer. The mutant proteins and wild-type E1 DBD were compared for DNA binding, and as predicted, both

mutants had significant dimerization defects, although DNA binding and interaction with E2 DBD were not impaired. Finally, comparison of the structure of the E1 DBD to the NMR (nuclear magnetic resonance) structure of the DBD of SV40 T antigen shows quite remarkable similarity, despite only 6% sequence identity (Fig. 1). Given that the homology in other regions of the two proteins is similar or greater than that observed for the DBD, it is likely that E1 and T antigen have highly related structures.

Structural Changes in the ori Induced by Cooperative Binding of E1 and E2

E. Gillitzer

Cooperative binding of the E1 and E2 proteins to the ori is essential for viral DNA replication in vivo. We have previously demonstrated that the interaction between the E1 and E2 proteins has two separate components. The DBDs of the two proteins interact with each other, and the activation domain of E2 interacts with the helicase domain of the E1 protein. The interaction between the activation domain of E2 and E1 is the productive interaction, whereas the interaction between the two DBDs serves to facilitate this interaction. To determine how the physical interaction between the two DBDs triggers the productive interaction, we have analyzed the structure of the ori in response to binding of the two DBDs. Both the E1 and E2 DBDs individually generate modest bends in the DNA upon binding. However, when the two proteins are bound cooperatively, a much sharper bend centered between the two binding sites results. This sharp bend (which approaches 90°) could result either from the combined intrinsic bends contributed by the individual proteins or from the interaction between the two proteins. To distinguish between these possibilities, we utilized mutants in the E2 DBD that fail to interact with the E1 DBD. Interestingly, although the intrinsic bend generated by the mutant E2 DBDs was of a magnitude similar to that generated by wild-type E2 DBD, the mutant E2 DBDs failed to generate a sharp bend in combination with the E1 DBD. This suggested that the physical interaction between E1 and E2 DBDs gives rise to the bend between the two sites. To determine whether bending of the DNA also was a requirement for cooperative interaction, we changed the bendability of the DNA sequence between the E1- and E2-binding sites. It is well established that homopolymeric A and T stretches are intrinsically stiff. We therefore changed two A-T base pairs to T-A base pairs, creating a T4 sequence that is intrinsically stiff as measured by DNase cleavage. As expected, these mutations did not have a significant effect on binding of E1 or E2 alone. However, the double mutation with increased stiffness resulted in a severe reduction of both cooperative binding and bending, consistent with the idea that bendability of the DNA sequence is important for the interaction between the two DBDs and that bending of the DNA is a prerequisite for cooperative DNA binding. The interaction between the E1 and E2 DBDs therefore seems to serve a predominantly architectural role, analogous to the function of cellular factors such as HMG proteins, which can modify the architecture of a protein-DNA complex by introducing bends in DNA.

PUBLICATIONS

Chen G. and Stenlund A. 2000. Two patches of amino acids on the E2 DNA binding domain define the surface for interaction with E1. *J. Virol.* **74:** 1506–1512.

Enemark E.J., Chen G., Vaughn D.E., Stenlund A., and Joshua-Tor L. 2000. Crystal structure of the DNA binding domain of the replication initiation protein E1 from papillomavirus. *Mol. Cell* **6:** 149–158.

Gillitzer E., Chen G., and Stenlund A. 2000. Separate domains in E1 and E2 proteins serve architectural and productive roles for cooperative DNA binding. *EMBO J.* **19:** 3069–3079.

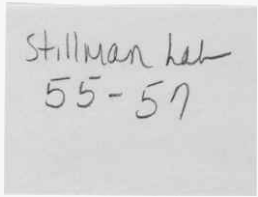
Sanders C.M. and Stenlund A. 2000. Transcription factor-dependent loading of the E1 initiator reveals modular assembly of the papillomavirus origin melting complex. *J. Biol. Chem.* **275:** 3522–3534.

In Press

Chen G. and Stenlund A. 2001. The E1 initiator recognizes multiple overlapping sites in the papillomavirus origin of DNA replication. *J. Virol.* **75:** 292–302.

DNA SYNTHESIS

B. Stillman K. Braun, J. Chong, S. Dike, V. Ellison, J. Mendez, T. Mizushima, Y.-J. Sheu, K. Shibahara, C. Speck, M. Weinreich, Z. Zhang, Y.N. Du, X.H. Yang, L. Zou, P. Wendel



Our studies on the inheritance of DNA and chromatin continue, with an emphasis on the proteins that are required for DNA replication and how they coordinate with processcs that mediate inheritance of chromatin and that control cell cycle progression. In prior years, wc havc identified the proteins that are required for synthesis of DNA at a replication fork and for initiation at origins of replication. Although the repertoire of proteins is well known, it is very likely that we still do not fully understand how replication occurs. But more importantly from thc view of genome stability, there is still much to determine how DNA replication is monitored during the cell division cycle to ensure accurate inheritance.

INITIATION OF DNA REPLICATION

One of the key protein complexes in initiation of DNA replication is the origin recognition complex (ORC) bccause it recognizes origins of replication and recruits key proteins at different times during the cell cycle to facilitate initiation of DNA replication. One of these key proteins is Cdc6p that we have demonstrated in yeast to interact with ORC and help recruit the minichromosome maintenance (MCM) protein complex to form what has been called a pre-replication complex (pre-RC). This recruitment renders chromosomes competent for initiation of DNA replication when cells are committed to undergo cell division. In thc yeast *Saccharomyces cerevisiae*, Cdc6p is loaded onto the chromosome-bound ORC as cells exit from mitosis and remains there until it is degraded at the G_1- to S-phase transition. Thus, Cdc6p is bound to chromosomes only during the G_1 phase of the cell cycle.

During the past few years, we have completed the cloning and characterization of the human ORC genes and have demonstrated that it is possible to reconstitute a six-subunit ORC complex. Unlike yeast, however, we found that the human ORC complex is dynamic, with the largest Orc1 subunit assembled onto chromatin as cells exit mitosis and enter early G_1 phase. The human Orc1 subunit is then degraded by ubiquitin-mediated proteolysis at the G_1- to S-phase transition, creating the need to reassemble the full ORC complex after mitosis. Thus, in terms of protein stability, human Orc1 behaves more like the yeast Cdc6p. All the other ORC subunits are bound to chromatin throughout the cell division cycle, except during mitosis.

The human Cdc6 protein is also subject to cell-cycle-regulated, ubiquitin-mediated protcolysis, but in this case, we found that Cdc6 is synthesized as cells passage through late G_1 phase and binds to chromatin during the remainder of the cell cycle. The excess Cdc6 protein is degraded during mitotic exit. Although we do not know the biochemical functions of Orc1 and Cdc6 in humans like we do in yeast, it is possible that the two proteins have reversed roles in human cells compared to yeast cells.

In tissues, most normal human prolifcrating cells pause for long periods of time in the G_1 phase of the cell cycle, and the Cdc6 and Orc1 proteins would be present at very low levels. The genes encoding these proteins are regulated by the E2F transcription factors, and thus, when cells are stimulated to go through cell division, Orc1 and Cdc6 are synthesized in late G_1 phase. The pre-RC can thus be formed either immediately as cells exit mitosis or just before the G_1- to S-phase boundary when cells are mitogenically stimulated to re-enter the cell division cycle.

Thc process of initiation of DNA replication is therefore a highly regulated event, and it is likely that many other proteins have a role in this process. In the last year, another protein called Cdt1 was found by the laboratories of Paul Nurse and Marcel Méchali in *Schizosaccharomyces pombe* and *Xenopus*, respective-

ly. Cdt1p is required for the loading of the MCM proteins onto chromatin. Independently, we have searched for novel proteins that interact with ORC with the expectation that they will have a role in either the establishment or regulation of initiation of DNA replication. Using a panel of monoclonal antibodies directed against *S. cerevisiae* ORC, we have immunoprecipitated the ORC complex and identified by mass-spectrometry-mediated protein sequencing (in collaboration with Ryuji Kobayashi) a number of proteins that appear to interact with ORC. One of these has been the focus of our attention during the past 2 years because it contains a BRCT motif that is also found in the breast cancer tumor suppressor protein BRCA1. This protein is also very similar to a protein in zebrafish called pescadillo, which, when altered by mutation, causes a severe proliferation phenotype in embryos. We have demonstrated that the yeast pescadillo protein associates in a very large complex with ORC and also with a number of other proteins involved in DNA replication and cell cycle and checkpoint control. The function of this complex and the essential pescadillo protein is under intense study.

CHROMATIN ASSEMBLY AND EPIGENETIC INHERITANCE

During the last 2 years, we have made significant progress in understanding how chromatin is inherited during cell division and how this process is linked to DNA replication. A central protein complex, called chromatin assembly factor-1 (CAF-1), is bound to newly synthesized histones H3 and H4 and loads them onto replicating DNA during S phase. CAF-1 also facilitates the assembly of the mature nucleosome containing histones H2A and H2B, as well as the inheritance of epigentically determined states of gene expression. The latter complexes in yeast include the silent mating-type loci and telomere-associated silencing complexes. We recently reported that CAF-1 connects with the replication fork by a direct interaction with the proliferating cell nuclear antigen (PCNA) and that PCNA is also required for epigenetic inheritance.

The histones that are associated with CAF-1 are modified by acetylation on lysine residues in the amino-terminal tail of histones H3 and H4. Since the pattern of acetylation on these newly synthesized histones is unique, there has been considerable speculation that the acetylation is required for assembly and for marking the newly replicated chromatin. Although in yeast, mutation of the amino-terminal tail of either histone H3 or H4 has no obvious effect, mutation of both tails simultaneously is lethal, suggesting an important role in chromatin.

We tested whether the amino-terminal tails of histones H3 and H4 are required for nucleosome assembly by reconstituting the CAF-1-histone H3•H4 complex with recombinant proteins, including histones that either retained or lacked both amino-terminal tails. Unexpectedly, neither tail was required for DNA-replication-dependent assembly of nucleosomes. These results suggest that the amino-terminal tails of histones H3 and H4, both of which can be modified by acetylation and possibly methylation, are required after formation of the mature nucleosome. Furthermore, since the pattern of modification on these tails can change after replication, acetylases and deacetylases must be recruited to the replicated DNA, although how this happens is not known. One clue might be the finding that the small subunit of CAF-1 is also a subunit of known nucleosome remodeling complexes, histone deacetylases, and histone acetylases. Perhaps the CAF-1 small subunit remains associated with the newly formed nucleosome and recruits the chromatin modifying enzymes.

CAF-1 was originally identified from human cell extracts, and later, the identification of the yeast protein complex led to discovery of its role in inheritance of epigentically determined states of gene expression. In the last year, in collaboration with Takahasi Araki's group at the University of Kyoto, we have shown that CAF-1 is conserved in plants (and therefore most likely all eukaryotes). The cloning of the genes encoding the two large subunits of CAF-1 led to the interesting discovery that the *FASCIATA* genes in *Arabidopsis thaliana* encode CAF-1 subunits. The genes encoding the two largest subunits of CAF-1 were expressed in cells in the shoot apical meristem and root apical meristem, both of which contain the rapidly proliferating stem cells. Mutations in these genes caused an unusual thickening and shortening of the shoot or root and miscexpression of genes that determine cell fate in these two growing parts of the plant. As a consequence, genetically identical plants display a highly varied appearance, reminiscent of the variegated phenotype of mutation in the PCNA gene in both yeast and *Drosophila*. Thus, it is likely that CAF-1-mediat-

ed inheritance of chromatin will greatly influence development in all multicellular eukaryotes.

PUBLICATIONS

Chong J.P., Hayashi M.K., Simon M.N., Xu R.M., and Stillman B. 2000. A double-hexamer archaeal minichromosome maintenance protein is an ATP-dependent DNA helicase. *Proc. Natl. Acad. Sci.* **97:** 1530–1535.

Méndez J. and Stillman B. 2000. Chromatin association of human origin recognition complex, cdc6, and minichromosome maintenance proteins during the cell cycle: Assembly of prereplication complexes in late mitosis. *Mol. Cell. Biol.* **20:** 8602–8612.

Mizushima T., Takahashi N., and Stillman B. 2000. Cdc6p modulates the structure and DNA binding activity of the origin recognition complex in vitro. *Genes Dev.* **14:** 1631–1641.

Shibahara K., Verreault A., and Stillman B. 2000. The N-terminal domains of histones H3 and H4 are not necessary for chromatin assembly factor-1-mediated nucleosome assembly onto replicated DNA in vitro. *Proc. Natl. Acad. Sci.* **97:** 7766–7771.

Zhang Z., Shibahara K., and Stillman B. 2000. PCNA connects DNA replication to epigenetic inheritance in yeast. *Nature* **408:** 221–225.

Zou L. and Stillman B. 2000. Assembly of a complex containing Cdc45p, replication protein A, and Mcm2p at replication origins controlled by S-phase cyclin-dependent kinases and Cdc7p-Dbf4p kinase. *Mol. Cell. Biol.* **20:** 3086–3096.

In Press

Kaya H., Shibahara K., Taoka K., Iwabuchi M., Stillman B., and Araki T. 2001. *FASCIATA* genes for chromatin assembly factor-1 in *Arabidopsis* maintain the cellular organization of apical meristems. *Cell* **104:** 131–142.

Ola A., Waga S., Ellison V., Stillman B., McGurk M., Leigh I.M., Waseem N.H., and Waseem A. 2001. Human-*Saccharomyces cerevisiae* proliferating cell nuclear antigen hybrids: Oligomeric structure and functional characterization using in vitro DNA replication. *J. Biol. Chem.* **276:** 10168–10177.

Y.-J. Sheu

TRANSCRIPTION AND PROTEOLYSIS

W.P. Tansey A. Caudy, J. Chenowith, S. Hart, A. Herbst, S.Y. Kim, S. Salghetti, K. Tworkowski

The maintenance of normal cellular growth and differentiation ultimately depends on mechanisms that regulate cell cycle progression. Two of the most important of these regulatory mechanisms are transcriptional activation and proteolytic destruction, which together control the appropriate appearance and disappearance of key cell cycle players. Work in our laboratory explores the intersection between these two processes to understand how oncogenc transcription factors are regulated at the levcl of their own destruction.

REGULATION OF MYC DESTRUCTION

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 within minutes of its synthesis. The rapid destruction of Myc has an important rolc in keeping intracellular levels of the Myc protein low and responsive to environmental stimuli. Our laboratory studies how cells control Myc activity by controlling Myc destruction, and how this process goes wrong in cancer.

We have previously shown that Myc is an unstable protein because it is destroyed by a process known as ubiquitin-mediated proteolysis. In this process, the covalent attachment of Myc to the protein ubiquitin (Ub) signals its destruction by a large protease complex called the proteasome. Protcin ubiquitylation is a highly specific process that begins when an element within the target protein—termed a degron—is recognized by the cellular ubiquitylation machinery. After the degron is recognized and bound, Ub is then transferred to a lysine residue within the target protein. This process is then repeated many times to produce a highly ubiquitylated substrate that is rapidly destroyed by the proteasome. Because protcasomal destruction depends on prior substrate ubiquitylation, selectivity in degron recognition by the ubiquitylation machinery—and regulation of this process—is central to the control of Ub-mediated proteolysis.

We have characterized scveral elements in Myc that regulate its stability. Our previous studies identified two of these elements (see Fig. 1): (1) Degl, which signals Myc destruction by Ub-mediated proteolysis, and (2) Stabl, which promotes Myc stability, probably by association with a protcin called Miz-1. Degl is an interesting element because it overlaps with the transcriptional activation domain (TAD) of Myc, and because it is the major site of cancer-associated mutations within Myc. Indeed, we have previously shown that cancer-associated mutations within Degl, which are observed in a very high percentage of lymphomas, subvert the Ub-mediated destruction of Myc and allow the protein to accumulate, suggesting that enhanced protein stability is a mechanism of Myc activation in cancer.

In the past year, we have identified a second degron, Deg2, that lies within the interior of the Myc protein (see Fig. 1). Deg2 displays a number of interesting characteristics. First, although Deg2 is required for rapid Myc turnover, it does not appear to signal Myc ubiquitylation. On the contrary, deletion of Deg2 promotes Myc ubiquitylation at the same time as it

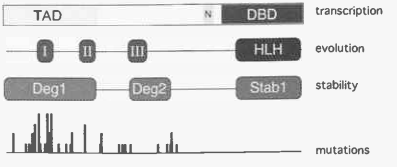


FIGURE 1 Myc. The cartoon shows the domain structure of the Myc protein and indicates the relationship between different functional elements within Myc. "Transcription" refers to elements required for transcriptional activation: the TAD, DNA-binding domain (DBD), and nuclear localization signal (N). "Evolution" refers to the highly conserved sequence elements within Myc: the three Myc boxes (I, II, and III), and the helix-loop-helix (HLH) DNA-binding motif. "Stability" refers to elements that regulate Myc destruction (see text for details). "Mutations" shows the position and frequency with which residues within Myc are mutated in lymphoma.

stabilizes the protein. This unusual behavior has led us to speculate that Deg2 specifically has a role in the destruction of ubiquitylated Myc; we are currently exploring this possibility. Second, Deg2 appears to function in a cell-specific manner. We have used our recently developed HAM-epitope tag—which improves the sensitivity of in vivo metabolic labeling experiments—to probe the destruction of Myc in a variety of primary and established cell lines. These studies have revealed that Deg2 is active only in a subset of cell types. This activity of Deg2 reveals that Myc stability is regulated differently in different cell types and implies that the destruction of Myc is a highly controlled event that is modulated to control specific aspects of Myc activity. Finally, Deg2 is interesting because it overlaps structurally and functionally with a highly conserved sequence element within Myc called Myc box III (MbIII). MbIII was discovered many years ago as an element present within almost all Myc proteins, yet its function has remained elusive. Our demonstration that MbIII has a role in Myc destruction provides a function for this "orphan" element within Myc and suggests that the proteolytic destruction of Myc has a major role in directing the conservation of this element. We are currently exploring how Deg2 works and attempting to understand the basis of its cell-specific function.

ACTIVATION DOMAIN-DIRECTED PROTEOLYSIS

Our studies of Myc proteolysis not only have contributed to our understanding of the biology of Myc, but have also given insight into more general aspects of how transcriptional activators are targeted for Ub-mediated destruction. Many transcription factors, particularly those involved in the control of cell growth (e.g., p53, E2F, etc.), are unstable proteins that are destroyed by Ub-mediated proteolysis. Our demonstration that Deg1 of Myc overlaps with the Myc TAD prompted us to further explore the relationship between these two types of elements. We have since learned that the overlap of TADs and degrons is common, intimate, and reciprocal: TADs and degrons overlap in most unstable transcription factors, we cannot separate the two activities by mutagenesis of these domains, and degrons, when fused to a DNA-binding domain, can activate transcription. On the basis of these observations, we conclude that activation domains are a type of degron and have coined the term DADs (destruction and activation domains) to refer to these elements. The realization that TADs and degrons overlap has practical value—it can aid in the discovery and analysis of instability determinants in transcription factors—and it implies that a common mechanism underlies transcription factor destruction; perhaps transcription factors share a common set of ubiquitylating enzymes.

In the past year, we have attempted to understand why it is that these two (apparently) very different biological activities have converged in DADs. Our approach to this problem has been to analyze the activity of DADs in yeast, where genetics makes it possible to manipulate the factors that control DAD activity. In our yeast studies, we have not focused on Myc, but instead have analyzed the function of another DAD, the prototypical acidic activation domain from the herpes simplex virus activator VP16. We have used genetics to identify the cellular machinery that targets the VP16 DAD for destruction and have shown that the

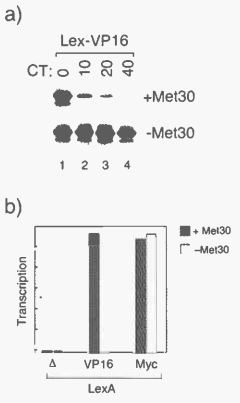


FIGURE 2 Degron function is required for the VP16 activation domain to activate transcription. (*a*) Lex-VP16 destruction requires the Ub-ligase Met30. The VP16 DAD was fused to the LexA DBD and expressed in yeast either carrying (+Met30) or lacking (–Met30) a wild-type Met30 gene. The figure shows the results of pulse-chase analysis, measuring the stability of Lex-VP16 in the two different yeast backgrounds. "CT" refers to chase-time (in minutes). (*b*) LexA-VP16 activity requires the Ub-ligase Met30. The figure shows the results of transcriptional analyses, measuring the ability of Lex-VP16 to activate a reporter gene in the presence or absence of Met30, as indicated. Also shown are the transcriptional activities of the LexA DBD alone (Δ) and LexA fused to the Myc DAD.

degron function of the VP16 DAD requires a ubiquitylating enzyme called Met30. In yeast deficient for Met30, the VP16 DAD cannot signal protein destruction, as shown in Figure 2. Surprisingly, in yeast deficient for Met30, we also find that the VP16 DAD cannot activate transcription. Thus, both the degron and activation domain function of the VP16 DAD require Met30. This result further cements the tight relationship between activation domains and degrons and suggests that, in order to activate transcription, transcription factors need to be unstable.

But why would activation domain function require proteolysis? We suspect that the intimate relationship between transcriptional activation and protein destruction reflects a mechanism of "activator licensing," in which transcriptional activation domains are permitted to activate transcription only under conditions in which they will be destroyed. This mechanism makes efficient use of the proteolytic machinery (by ensuring that only active proteins are destroyed) and could serve as an important regulatory step to limit uncontrolled gene activation. Future studies will test this hypothesis and will explore the mechanistic link between TADs and degrons.

PUBLICATIONS

Salghetti S.E., Muratani M., Wijnen H., Futcher B., and Tansey W.P. 2000. Functional overlap of sequences that activate transcription and signal ubiquitin-mediated proteolysis. *Proc. Natl. Acad. Sci.* **97:** 3118–3123.

Tansey W.P. 2000. Transcriptional regulation: RUPture in the ER. *Nat. Cell Biol.* **2:** E175–177.

In Press

Herbst A. and Tansey W.P. 2001. HAM: A new epitope-tag for in vivo protein labeling. *Mol. Biol. Rep.* (in press).

D. Siolas

- Dr. Hengartner's group continued their effort to understand how *C. elegans* responds to DNA damage. They published this year the first report showing that DNA damage response pathways in *C. elegans* were likely to be conserved with those found in humans and yeasts.
- Dr. Hannon's lab has succeeded in identifying a combination of viral and cellular oncogenes that can successfully transform normal human cells into cancer cells. They are now investigating the cellular pathways that are altered by these oncoproteins to effect transformation. In addition, they have made substantial progress in understanding the mechanism by which double-stranded RNA induces gene silencing in organisms ranging from plants to *Drosophila* and, under some circumstances, mammals. A detailed picture of this mechanism may allow the development of novel experimental tools and possibly even new therapeutic approaches.
- Dr. Jackson's lab is studying the mechanisms of cell-to-cell communication in the shoot meristem and has characterized mutations in a receptor that is involved in controlling stem cell proliferation and may be a target for improving crop yields. They have also shown that the cell-to-cell movement of a homeodomain protein can be tracked using fusions to the green fluorescent protein, providing a tool to characterize this novel pathway of cell-to-cell communication.
- Dr. Martienssen's group has completed the first plant genome sequence, that of the laboratory model *Arabidopsis thaliana* (mouse-ear cress), clearly the highlight of the year in plant genetics. The sequence is being used at CSHL to perform systematic functional genomics, to investigate the epigenetic properties of heterochromatin, and to positionally clone genes important in development. One example is *asymmetric leaves 1*, a gene required for leaf patterning and stem cell fate that encodes a homolog of the maize gene *roughsheath2*.
- Dr. Lucito's group is designing a genomic microarray to identify regions of the genome that undergo copy number fluctuations in cancer and is exploring the use of microarrays for gene mapping. This method relies heavily on the technique of genomic representations, populating the array with representational fragments and hybridizing genomic representation samples to these microarrays.
- Dr. Mittal's group, with collaboration from Wigler's lab, have used an ecdysone-inducible mammalian expression system and human cDNA microarrays (containing 15,000 elements) to analyze changes in patterns of gene expression upon induction of either the wild-type or mutant PTEN tumor suppressor in the glioblastoma cell line U87MG (which lacks a functional PTEN). Also, in collaboration with Wigler and Lucito's groups, they have been exploring the use of cDNA microarrays for gene mapping.
- Dr. Hamaguchi's lab has isolated six genes from the deleted region at human chromosome 8p22, including *TRAILR2, DBC1,* and *DBC2*. *TRAILR2* has been screened for mutations in both familial and sporadic breast cancer cases and no significant genetic alterations have been detected. They have discovered that *DBC2* is inactivated in many breast cancer cells by deletions, somatic mutations, or transcriptional inactivation, which makes *DBC2* a strong candidate for a tumor suppressor gene involved in breast cancer development.
- Dr. Wigler's group has been exploring the use of microarrays for functional genomics, gene mapping, and mutation detection in cancer and spontaneous genetic disease. They have continued the analysis of the PTEN tumor suppressor, confirming its role in the phosphatidyl inositol signaling pathways, and confirming its role as a tumor suppressor. Additionally, the group has been developing algorithms for automated anotation of the human genome.

CELL CYCLE CONTROL IN *SACCHAROMYCES CEREVISIAE*

B. Futcher J. Donovan T. Volpe
N. Edgington H. Wijnen
F. Ferrezuelo J. Zhou
S. Honey

Our main interest is the regulation of Start and mitosis in *Saccharomyces cerevisae*. We are most interested in the tethering of division to cell growth and in the generation of the basic cell cycle oscillation. Many key cell cycle events are regulated by protein kinase complexes formed between Cdc28 and one of nine cyclins. These fall into two broad groups: the G_1 cyclins, which include Cln1, Cln2, and Cln3, that regulate Start; and the mitotic, B-type cyclins Clb1, Clb2, Clb3, and Clb4. Two other cyclins, Clb5 and Clb6, are very important for DNA replication, but they also have roles at Start and perhaps also in early mitosis. A second interest is yeast and human telomerase, and how telomere length relates to cell senescence.

A Network for Exit from Mitosis

J. Donovan

High Cdc28 kinase activity is required for mitosis. At the end of anaphase, this Cdc28 kinase must be inactivated to allow the mitotic spindle to dissolve. This inactivation can be thought of as consisting of two steps: (1) a signaling step, in which the completion of anaphase triggers some unknown signal, and (2) an inactivation step, in which Cdc28 kinase is inactivated by destruction of cyclins, production of the CDK inhibitor Sic1, and possibly by other mechanisms. A network of genes including *CDC5, CDC15, TEM1, DBF2, DBF20*, and *CDC14* is required for this signaling and inactivation pathway. The typical loss-of-function phenotype is a large budded cell with divided nuclei and a long mitotic spindle.

In a screen for high-copy suppressors of the temperature-sensitive *dbf*2 mutation (a mutation that blocks exit from mitosis), we isolated the novel gene *SLK19*. Slk19 is a centromere- and spindle-associated protein and has a role in the maintenance of spindle integrity. Although *SLK19* is not essential, we have shown that Slk19 has a role in completion of M phase in concert with the mitotic exit network kinases Dbf2 and Cdc15. Furthermore, *SLK19* becomes essential when cells are defective in Clb proteolysis, or otherwise have an excess of Clb2-Cdc28 kinase activity. We believe that Slk19 may use its position at the centromere to help signal to the cell when anaphase has been completed, and so may have a role in triggering the events that end mitosis, such as destruction of the Clb cyclins.

Dissection of Nuclear and Cytoplasmic Roles of Cyclins

N. Edgington

Nine different yeast cyclins bind to and activate the Cdc28 protein kinase. Although the enzymatic properties of these complexes are similar, they have very different biological roles. We asked how many of these differences could be accounted for by differences in location. We have localized various G_1 and mitotic cyclins by various methods and, in addition, have created "forced localization" tags, which, when appended to a protein, force it to be either cytoplasmic or nuclear. These allow the functional importance of any particular location to be evaluated.

For instance, the G_1 cyclin Cln2 is found in both the cytoplasm and the nucleus. The cytoplasmic Cln2 is mostly at the sites of bud growth or polarized growth, a very interesting location given that Cln2 is known to be important for budding.

Neither cytoplasmic nor nuclear Cln2 can fully complement *cln2*, showing that the Cln2 in both locations has important functions. The *cln1 cln2 clb5 clb6* quadruple mutant and the *cln1 cln2 kar3* triple mutant

are inviable, and these two mutants are rescued by the nuclear form of Cln2 but not the cytoplasmic form, suggesting that these functions of Cln2 are nuclear. On the other hand, the inviable *pcl1 pcl2 cln1 cln2* and *bud2 cln1 cln2* mutants are rescued by the cytoplasmic form of Cln2 but not the nuclear form, suggesting that these functions of Cln2 are cytoplasmic. Finally, the *swi4 swi6* mutant is rescued by wild-type Cln2, but it is not rescued by cytoplasmic Cln2, or by nuclear Cln2, or by the simultaneous combination of cytoplasmic and nuclear Cln2. We suggest that shuttling of Cln2 from nucleus to cytoplasm and back again is essential for some role of Cln2.

Whi3 Binds the mRNA of the G_1 Cyclin *CLN3* to Modulate Cell Fate in Budding Yeast

T. Volpe, B. Futcher [in collaboration with E. Gari, H. Wang, C. Gallego, and M. Aldea, Universitat de Lleida, Spain]

WHI3 is a novel size control gene. Mutant *whi3* cells have small cell sizes, and this is because they undertake Start and cell division at smaller cell sizes than wild-type cells. We have shown that Whi3 is a negative regulator of the G_1 cyclin Cln3, which promotes Start. Whi3 contains an RNA-recognition motif that is essential for Whi3 function. This RNA-recognition motif allows Whi3 protein to bind specifically to the *CLN3* mRNA. This seems to restrict *CLN3* mRNA to discrete cytoplasmic foci, which also contain Whi3 protein. In the absence of Whi3, *CLN3* mRNA is not restricted to these foci, *CLN3* activity is increased, the transcription factors SBF/MBF become hyperactivated, and cells go through Start prematurely. The physiological role of *WHI3* seems to be to restrain *CLN3* activity in preparation for meiosis, filamentous growth, and mating, and perhaps also when certain kinds of nutrient deprivations occur.

Mechanisms of Transcriptional Activation by Cyclin-Cdc28 Complexes

H. Wijnen

The Cln3-Cdc28 complex promotes passage through the G_1/S transition by activating two transcription factors, SBF and MBF, which in turn induce the transcription of more than 200 genes. We are trying to discover the mechanism of SBF/MBF activation.

One model for activation is that *CLN3* inhibits an inhibitor of SBF/MBF. This would be parallel to the system in mammalian cells, where cyclin D/*CDK4* (analogous to Cln3/Cdc28) inhibit Rb, an inhibitor of E2F (analogous to SBF). To look at this model, we have selected for mutants in which SBF is active even in the absence of *CLN3* (and the coactivator *BCK2*). Several such mutants have been found. One is in the *CHD1* gene, a chromatin remodeling protein containing chromodomains. The *CHD1* gene is highly conserved throughout evolution, with homologs in humans. It seems to be involved in repression. Interestingly, Rb recruits a similar chromatin remodeling protein to E2F-regulated promoters. Other mutations allowing SBF activity in the absence of *CLN3* and *BCK2* were also found. Strikingly, several of these, like *chd1*, were mutations in genes involved in chromatin modification or remodeling. Furthermore, some of these mutants were nonresponsive to *CLN3*, consistent with the idea that in the mutants, the pathway from Cln3 to SBF/MBF is no longer functional.

Use of Multiple Affinity Purification Tags

S. Honey, B. Schneider

We have designed a small multiple affinity purification tag. It consists of (1) the calmodulin-binding peptide, which allows binding to calmodulin resin in the presence of calcium, and allows elution by EGTA; (2) six histidines, which allow binding to a nickel column, and allow elution by imidazole; and (3) a triple hemagglutinin (HA) tag, which allows immunoprecipitation by antibody 12CA5. When this "CHH" (Calmodulin, Histidine, HA) tag is appended to any protein, the protein can then be purified by two affinity steps and an immunoprecipitation step can be applied in tandem. In practice, we have found that application of any two of the steps in tandem leads to essentially pure protein, plus associated proteins. We have tagged the yeast mitotic cyclin Clb2 with the CHH tag and have purified Clb2 and associ-

ated proteins. The associated proteins were identified by mass spectrometry. They included Cdc28, Sic1, and Cks1.

Identification of Bdf1 as a Transcription Factor for Ribosomal Proteins

J. Zhou, K. Hidaka, B. Futcher [in collaboration with M. Tyers, Samuel Lunenfeld Research Institute, University of Toronto]

Bdf1 is a bromodomain protein that helps regulate telomere length. In addition, it is a basal transcription factor. S. Buratowski (Harvard Medical School, Boston) and colleagues have recently shown that Bdf1 is functionally similar to the carboxy-terminal domain of mammalian TAF(II)250, whereas the yeast protein Taf145 is functionally similar to the amino-terminal domain of TAF(II)250. That is, in yeast, Taf145 plus Bdf1 form the equivalent of mammalian TAF(II)250.

Bdf1 has a homolog called Bdf2, and the *bdf1 bdf2* double mutant is inviable. We constructed a conditional allele of *bdf1* and carried out microarray analysis to see what genes were controlled by Bdf1/Bdf2. A striking result was that most genes encoding ribosomal proteins, and many other genes involved in protein synthesis, were strongly down-regulated when Bdf1 activity was lost. Few other genes responded. However, among the other genes that did respond were genes involved in secretion. It is known from the work of J. Warner (Albert Einstein College of Medicine, New York) and colleagues that defects in secretion quickly lead to defects in the transcription of ribosomal protein genes. We are now asking whether the loss of transcription of ribosomal protein genes in the *bdf1* mutant is a direct effect (i.e., due to Bdf1 acting at the promoters of these genes) or an indirect effect via the secretion genes. We are also assaying the presence of Bdf1 at telomeres.

PUBLICATIONS

Futcher B. 2000. Microarrays and cell cycle transcription in yeast. *Curr. Opin. Cell Biol.* **12:** 710–715.

Futcher B., Pines J., and Lafont F. 2000. Web alert. *Curr. Opin. Cell Biol.* **12:** 653–654.

Salghetti S.E., Muratani M., Wijnen H., Futcher B., and Tansey W.P. 2000. Functional overlap of sequences that activate transcription and signal ubiquitin-mediated proteolysis. *Proc. Natl. Acad. Sci.* **97:** 3118–3123.

Zhou J., Hidaka K., and Futcher B. 2000. The Est1 subunit of yeast telomerase binds the Tlc1 telomerase RNA. *Mol. Cell. Biol.* **20:** 1947–1955.

Zhu G., Spellman P.T., Volpe T., Brown P.O., Botstein D., Davis T.N., and Futcher B. 2000. Two yeast forkhead genes regulate the cell cycle and pseudohyphal growth. *Nature* **406:** 90–94.

In Press

Nash R.S., Volpe T., and Futcher B. 2001. Isolation and characterization of WHI3, a size-control gene of *Saccharomyces cerevisiae*. *Genetics* **157:** 1469–1480.

Honey S., Schneider B.L., Schieltz D.M., Yates J.R., and Futcher B. 2001. A novel multiple affinity purification tag and its use in identification of proteins associated with a cyclin-CDK complex. *Nucleic Acids Res.* **29:** E24.

TUMOR SUPPRESSOR GENES INVOLVED IN BREAST CANCER

M. Hamaguchi D. King T. Odawara
J. Meth M. Reimels

TUMOR SUPPRESSOR GENES AND BREAST CANCER

Cancer cells have multiple genetic alterations such as amplifications, deletions, insertions, translocations, single nucleotide substitutions, and chromosomal abnormalities. These alterations affect two classes of genes: oncogenes that are activated or altered in function and tumor suppressor genes that are down-regulated or abolished in cancer cells. Our interest lies in tumor suppressor genes because we believe that analysis of tumor suppressor genes will provide further insights into the relationship between cancer development and cellular function. We also believe that understanding the biological function of tumor suppressor genes will have an impact on several aspects of cancer treatment. A new screening test might facilitate risk assessment, precise molecular characterization of cancers might facilitate diagnosis and therapeutic choice, and a new treatment can be established on new knowledge about tumor suppressor genes.

In the case of breast cancer, isolation of *BRCA1* and *BRCA2* has resulted in a better understanding of hereditary breast cancer. *BRCA1* and *BRCA2* genes are utilized for identification of those who are at high risk of developing the breast cancers. Sequence alterations in *BRCA1* and *BRCA2* account for approximately 85% of hereditary breast cancer cases. It is possible that other genes responsible for inherited breast cancer exist. Furthermore, the majority of breast cancer is nonhereditary, and the somatic mutations responsible for these cases remain to be elucidated.

The short arm of human chromosome 8 frequently suffers loss of heterozygosity (LOH) in sporadic breast cancer cases, which means a part of a chromosome is missing and only one copy (instead of a pair) of genes remains in the affected region. Frequent LOH has been a clue to the presence of tumor suppressor genes on that chromosomal arm. In addition, we discovered homozygous deletion (HD) of 8p22 at a frequency of 3.5% in surgical specimens. HD results in elimination of genes in the region. It is a good indicator of tumor suppressor genes because loss of tumor suppressors is advantageous to malignant cells and is observed commonly in many types of cancer. Analysis of HD at 8p22 has led to isolation of six genes from the commonly deleted region.

Deleted Genes

M. Hamaguchi, J. Meth, T. Odawara [in collaboration with R. McCombie and M. Wigler, Cold Spring Harbor Laboratory]

We have isolated six genes from the deleted region at human chromosome 8p22. Sequence analysis of the six genes revealed that three were known genes, another partial sequence was registered in GenBank with no information regarding biological function, and the other two were new genes. We named the new genes *DBC1* (GenBank accession number: AF293335) and *DBC2* (GenBank accession number AY009093). DBC stands for *d*eleted in *b*reast *c*ancer. The three known genes were *TRAILR2*, *Decoy Receptor*, and *EGR3*, among which *TRAILR2* had been demonstrated to have mutations in lung cancer. We conducted mutation analysis of *DBC1*, *DBC2*, and *TRAILR2*.

MUTATION ANALYSIS

Several methods are available for mutation analysis. The protein truncation test (PTT) is a rapid way to screen frameshifts and nonsense mutations, which are most likely to result in alteration of the protein function. By PTT, an entire open reading frame (ORF) can be screened in a reaction. The drawback, however, is its inability to detect missense mutations. Additionally, it requires RNA as a starting material, which makes analysis more expensive and tedious than using DNA as the starting material. Single-strand conformation

polymorphism (SSCP) analysis is one of the most commonly used methods for mutation detection. SSCP is based on the mobility shift of mutants in electrophorcsis due to their conformational changes. It is conccivable that not all mutations will result in the mobility shift, which limits the sensitivity of SSCP. Another mcthod is denatured high-performance liquid chromatography (DHPLC), which relies on a difference of retention time in a column between mutant and wild-type fragments under a half-denatured condition. DHPLC is high-throughput, but not all genomic regions are suitable for the analysis. It is difficult to determine a running condition for a DNA fragment with uneven distribution of bascs becausc a selected condition will be optimized for a part of the fragment and mutations in the other part will not be revealed. We utilized all three methods for mutational analysis to supplement each other.

Mutation Analysis of *DBC1*

M. Hamaguchi, J. Meth

We searched for mutations of *DBC1* in 60 cell lines by PTT and DHPLC. Neither analysis detected a mutation. Expression analysis by rcverse transcriptase–polymerase chain reaction (RT-PCR) and Northern blot analysis revealed that *DBC1* was expressed ubiquitously not only in normal tissues, but also in many tumors including breast cancer. We believe that *DBC1* has a remote possibility of being a tumor suppressor gene involved in breast cancers.

Mutation Analysis of *TRAILR2*

M. Hamaguchi, J. Meth [in collaboration with M.C. King, University of Washington, and M. Wigler, Cold Spring Harbor Laboratory]

Since *DBC2* and *TRAILR2* were found to reside in the deletion epicenter, they were considered stronger candidates for being tumor suppressor genes. Further-more, *TRAILR2* was shown to be involved in the apoptosis pathway. In fact, several groups have published mutational analyses of *TRAILR2*. One group demonstrated frequent mutations in the "death domain" in non-small-cell lung cancer. Another group reported mutational analysis of colorectal cancer, but found no mutations. A germ line mutation of *TRAILR2* that caused premature termination of translation was demonstrated in a head and neck cancer. HD of *TRAILR2* has been reported in nasopharyngeal cancer.

TRAILR2 was analyzed for mutations in cancer cells, using PTT, DHPLC, and SSCP. In 60 cell lines (including 20 breast cancer cell lines) and 90 primary breast tumors, we found two previously identified polymorphisms and two new single-nucleotide intronic alterations, but no new mutations in coding regions. Because linkage to this locus has been reported by other investigators in one family with a predisposition to breast cancer, we determined the status of the *TRAILR2* gene in 427 breast cancer patients and 372 normal controls. Two new rare missense mutations were identified in the germ line DNA of breast cancer patients, and one of these in normal controls. Analysis of germ line DNA of probands of 44 high-risk families (unlinked to *BRCA1* or *BRCA2*), however, revealed only previously known common polymorphisms in *TRAILR2*. Our findings tend to eliminate mutation of *TRAILR2* in somatic breast cancer development.

Analysis of *DBC2*

M. Hamaguchi, D. King, J. Meth, T. Odawara, M. Reimels [in collaboration with M. Zhang and M. Wigler, Cold Spring Harbor Laboratory]

***Mutation Analysis of* DBC2.** Elimination of *TRAILR2* leaves *DBC2* as a leading candidate for a tumor suppressor in the deletion epicenter. A cDNA clone composed of ten exons was isolated from a cDNA library of a normal breast tissue (Genbank accession number AY009093). This transcript is found in the placenta, colon, and stomach and is considered a major transcript. An alternatively spliced transcript was found in a lung tissue. It lacks exon 1 and a part of exon 2 but contains three unique exons derived from further upstream to the promoter region of the major transcript. Although the ORFs of both transcripts seem to be identical, their transcriptional regulation may be

different since CpG islands for the major transcript are located in an intron of the variant transcript.

We first performed mutation analysis of the common exons (exons 3 through 10) of *DBC2*. We applied PTT on 60 tumor cell lines and found no truncating mutations. Exons 3–10 were also analyzed by DHPLC on 96 cell lines, resulting in identification of three missense mutations and six polymorphisms/silent mutations.

Expression Analysis. We investigated the expression of three genes, *DBC1*, *DBC2*, and *TRAILR2*, in 59 tumor cell lines by RT-PCR. All three were expressed in the normal breast tissues we examined. The *DBC2* transcript was detected only in 44% of breast tumors and in 50% of lung cancer, but in as much as 90% of cancer of the other origins such as colon and kidney. In contrast, both *DBC1* and *TRAILR2* were expressed in at least 80% of any tumor. Some of the results were confirmed by Northern blot analysis.

Computational Analysis of **DBC2.** Analysis of a novel gene can be initiated with computational analysis of the sequence. Similar sequences to the query can be searched by the basic local alignment search tool (BLAST) (Altschul et al., *Nucleic Acids Res. 25:* 3389 [1997]). Motif and promoter searches are also available. These results will sometimes provide a clue to hypothesize the function of the gene product, but a gene in an entirely new class will produce only insignificant output and call for other approaches.

We utilized a program, CpGpromoter, to locate CpG islands of the major transcript and found one about 500 bp upstream of exon 1. The BLAST search of *DBC2* revealed that amino acids 12 to 308 of *DBC2* had homology with *RAC1* with 38% identities (76/200), 53% positives (106/200), and 3/200 gaps. It also showed homology with an anonymous gene, KIAA0740. The conserved protein regions were also searched for by Pfam HMM (Bateman et al., *Nucleic Acids Res. 28:* 236 [2000]). Two BTB/POZ (254–472 and 485–598) domains and a *ras* (16–258) domain were located (Zollman et al., *Proc. Natl. Acad. Sci. 91:* 10717 [1994]). *DBC2* is a gene of new class because a BTB/POZ domain is often observed with MATH, Kelch repeats, or a C2H2-type zinc finger, none of which are present in *DBC2,* and because no known gene has both *ras* and BTB/POZ domains.

DBC2 ***as a Candidate for a Tumor Suppressor.*** We believe that the 8p22 locus is likely to contain a new tumor suppressor gene on the basis of our findings: Homozygous deletion at 8p22 is observed in 7 of 200 breast tumors; *DBC2* is the most frequently deleted gene located in the deletion epicenter; it suffers mutations, although the frequency is not high, and it is silenced in more than half of the breast cancer cell line. These results make *DBC2* a good candidate for a tumor suppressor gene involved in breast cancer and urge us to scrutinize it further.

GROWTH CONTROL IN MAMMALIAN CELLS

G. Hannon S. Boettcher J. Du
D. Conklin M. Carmell
S. Hammond A. Caudy
Y. Seger A. Denli

My laboratory focuses on two general areas. First, we probe the mechanisms by which normal cells evolve into cancer cells. Our approaches to this problem are varied. The mainstay of our cancer program is the ability to manipulate cultured cells and to confer upon those cells the properties of tumor cells. This past year, we achieved the goal of beginning with normal, human diploid cells and converting these into cancer cells through a series of three genetic alterations. This opens the door to an investigation of cellular pathways, which can be altered to create a cancer cell de novo. We are presently attempting to extend this initial finding to the creation of cell-type-specific cancer models for a variety of human neoplasms. Second, we are interested in the mechanisms by which double-stranded RNA can silence gene expression in organisms ranging from plants to worms, flies, and mammals. We are using biochemical approaches to understand the mechanism underlying this phenomenon. During the past year, we have made substantial progress developing in vitro systems that recapitulate double-stranded RNA (dsRNA)-induced gene silencing and identifying some of the proteins that participate in this process. In addition, we have begun to weave together the two threads in the laboratory, initiating a project to harness dsRNA-induced silencing in high-throughput "phenomics." Using cultured cells from *Drosophila* as a model system, we hope to investigate genetic pathways that have proven to be central to the transformation process in humans. Our ultimate goal is to extend the ability of dsRNA to silence gene expression to human cells, enabling cancer-related questions to be asked in the relevant organism, rather than in a model system.

Human Cancer Cells

Y. Seger

Cellular transformation processes require a series of genetic and epigenetic events that result in a normal cell being converted into one that is cancerous. This process is most commonly initiated by an increase in proliferative signals coupled with the inactivation of tumor suppressor mechanisms. Although the use of rodent model systems has provided a significant amount of information regarding precise genetic alterations involved in cellular transformation, it is apparent that these models do not directly reproduce the processes, which lead to human cancer. Namely, whereas primary rodent cells can be transformed by the combined overexpression of the *myc* and *ras* oncogenes, human cells expressing this same gene combination undergo irreversible growth arrest or senescence.

We have found that normal human diploid fibroblasts (BJ cells) expressing the combination of E1A and Ha-RasV12 are capable of soft agar colony formation. This reflects one property of transformed cells, a decreased reliance on proper attachment to an extracellular substrate. These coinfected cells, however, were incapable of tumor formation in nude mice. Previous analysis of this oncogene combination in rodent cells indicated that tumor formation was aided by a concomitant hit in the p53 pathway. We therefore chose *MDM2*, the negative regulator of p53, as a candidate third oncogene in our transformation equation. BJ cells expressing the combination of adenovirus E1A, *MDM2*, and Ha-RasV12 are capable of both soft agar colony formation and tumor formation in nude mice.

Substitution of wild-type E1A with deletion mutants has allowed us to characterize the functional interactions that may be important for both transformation processes and evasion of normal mortality controls in this human fibroblast model. Interactions at the amino terminus of E1A, namely, those with the transcriptional coactivator p300/CBP and with p400, appear to be vital for transformation. The CR2 region, which directs interactions with Rb, is also indispensable. We are particularly interested in the ability of cellular oncogenes such as c-*myc* to rescue the transformation defects of these mutants with the hope of establishing a transformation equation based solely on cellular oncogene expression.

Mechanisms That Contribute to Genetic Instability in Cancer

J. Du

An almost invariant characteristic of human tumors is that they display genetic instability. This has been touted as an obligate step in the genesis of a tumor and dismissed as simply a consequence of the transformation process. In fact, reality probably lies between these extreme views. We have begun to investigate the mechanistic basis of chromosome instability by focusing on the organelle that organizes the mitotic spindle. Mitotic spindles are responsible for correctly partitioning the genome between two daughters that are generated by cell division. The spindle is nucleated by an organelle known as the centrosome, which serves to position the spindle for proper segregation of the genome. Replication of the centrosome is tightly regulated during the cell cycle so that a single centrosome in early G_1-phase cells is duplicated to yield the two centrosomes that anchor the bipolar spindle. However, in human cancer cells, centrosome numbers are often abnormal, leading occasionally to multipolar spindles and possibly to improper chromosome segregation. We have begun to investigate the mechanisms that regulate centrosome duplication in an attempt to understand how abnormal centrosome numbers are generated in cancer cells and to probe the relationship between deregulation of centrosome duplication and genomic instability in cancer.

We have investigated the ability of oncogene expression to provoke centrosome amplification in NIH-3T3 cells. Among many oncogenes and other tumor-related genes that were tested, AKT and Jun are capable of inducing centrosome amplification. Other centrosome defects observed in these cells include aberrant centrosome morphology, stronger microtubule nucleating capacity (abnormally strong staining with anti-β-tubulin antibodies), and abnormal centrosome localization (away from nuclear envelope). These defects may be primary effects of these oncogenes because just 48 hours after introducing the genes into cells by infection, centrosome numbers are well above controls. Live-cell imaging shows that cells with multiple, green fluorescent protein (GFP)-tagged centrosomes go through mitosis and occasionally divide into more than two daughter cells. This strongly suggests that the presence of multiple centrosomes can induce aberrant division to generate cells with potentially abnormal chromosome contents.

We are also taking a biochemical approach, focusing on a kinase that has been linked to the centrosome cycle in yeast, worms, and mammals. Using anti-STK15 antibodies, we have isolated an STK15 complex, which is composed of at least six components (SAF160, SAF55 [CDC20], SAF46 [STK15/aurora], SAF37, SAF35, and SAF31). We are currently purifying the complex and identifying these proteins. With two-hybrid screening and IP Western, we also find that STK15 associates with Nm23-H1, a human metastasis suppressor protein that is localized at the centrosome. Its *Drosophila* counterpart, *awd*, is microtubule-associated, and null mutations are arrested in metaphase of cell cycle. In the coming year, we will investigate the role of these proteins in regulating centrosome duplication and the relevance of this process to genomic instability in human cancer.

Genetic Investigation of Calcium Regulation of Cellular Proliferation

D. Conklin

Although calcium ion is arguably the most important second messenger in excitable cell signaling, a considerable amount of work has indicated an important role for Ca^{++} signaling in the regulation of proliferation in nonexcitable cells. At present, both the mechanisms of calcium release and the downstream effects during the cell cycle are poorly understood. The importance of these signals to cellular proliferation, however, is underscored by the ability of calcium antagonists to inhibit cell cycle progression in a number of eukaryotic cells.

In an effort to identify gene products involved in the calcium regulation of cell proliferation, genetic screens have been carried out in eukaryotic cells to identify cellular components with roles in Ca^{++} regulation of mitogenesis. Thirty independently isolated yeast mutants have been found that are resistant to calcium antagonists. Interestingly, all of these strains exhibit a late G_1 cell cycle arrest when grown under normal conditions. When grown in media containing elevated concentrations of Ca^{++}, these strains grow

normally. Genetic analysis indicates that the lesions which give rise to this phenotype are duplication and translocation mutations within the yeast genome. Current efforts are directed at discerning which genes contribute to the Ca^{++} remedial arrest phenotype in these strains.

In mammalian cells, retroviral cDNA libraries have been screened for genes that, when overexpressed, allow cells to escape calcium channel antagonist-induced preneoplicative cell cycle arrest. Several known genes involved in signal transduction have been isolated in these screens. One gene selected for the ability to confer resistance to the inorganic calcium channel blocker cobalt also conferred resistance to the 1,4 dihydropyridine calcium channel antagonist, nifedipine. This gene, *HXCT1*, encodes a member of the CD98 light-chain family of amino acid transporters. Functional analysis indicates that *HXCT1* encodes the human x_c^- transport system, which carries out cystine uptake. Overexpression of this gene results in increased cystine uptake and increased intracellular reduced thiol levels, which in turn affect the impact of drugs on Ca^{++} signaling. Since HXCT1 overexpression does not confer increased resistance to a number of other drugs and xenobiotics that are known to inhibit proliferation, the increased calcium antagonist resistance does not result from increased levels of general stress response or drug detoxification. Current efforts are directed at investigating links between cellular thiol status and Ca^{++} release.

Dicer Is a Nuclease That Generates Guide RNAs for PTGS

E. Bernstein, A. Caudy, S. Hammond

The phenomenon of RNA interference (dsRNA-induced gene silencing or PTGS) is a gene silencing mechanism that is not well understood. dsRNAs induce gene silencing, in part, by provoking degradation of homologous endogenous mRNA. RNA interference (RNAi) has been demonstrated in a diverse group of organisms including *Caenorhabditis elegans, Drosophila*, trypanosomes, plants and fungi, and the early mouse embryo.

Various genes involved in RNAi have been identified through genetic screens in plants and worms, although none have been given a definitive role in the RNAi pathway. Recently, the identification of a small RNA species of about 25 nucleotides was linked to RNAi in plants undergoing silencing. We have demonstrated that these small RNAs form a part of the effector nuclease that degrades mRNAs and most likely act as the specificity subunit of the enzyme. Another group discovered that *Drosophila* embryo extracts contained an activity that cleaved dsRNA to generate these small "guide" RNAs.

Our lab has previously established a very efficient biochemical system to probe the mechanism of RNAi in *Drosophila* S2 cells, which has allowed for the identification of an RNA nuclease capable of degrading the target mRNA (RNA-induced silencing complex [RISC]). We have since created similar in vitro systems from *Drosophila* embryos. Biochemical purification and candidate gene approaches led to the identification of Dicer, the enzyme responsible for cleaving the dsRNAs that provoke interference into 22-nucleotide "guide" RNAs. This enzyme is quite unique in its structure in that it contains two RNase III domains and an RNA helicase domain. It also contains a motif of unknown function called the PAZ (piwi/argonaute/zwille) domain, which is also present in the argonaute family of proteins, which have also been implicated in RNAi. Dicer probably acts at the top of the pathway by creating small guide RNAs that allow the RISC protein complex to find its homologous target mRNA. It is the first protein to be given a precise role in the mechanism of RNAi.

Argonaute Is a Component of the RNAi Effector Nuclease

S. Hammond, S. Boettcher

Our studies have indicated that RNAi is mediated by a sequence-specific nuclease which destroys mRNAs homologous to the inducing dsRNA. During the past year, we have taken a biochemical approach to understanding this effector nuclease. We have purified the nuclease substantially but not to homogeneity from *Drosophila* cultured cells. This effector nuclease, which we termed RISC, is a stable entity containing all of the necessary information for the targeting and destruction of appropriate mRNAs. The activity is a

ribonucleoprotein complex of approximately 500 kD, consisting of (four to five so far) protein components, and one RNA component. Microsequencing reveals that one of the protein components is a homolog of *C. elegans* rde-1 and *Arabidopsis thaliana* argonaute. This gene family has a role not only in RNAi, but also in development and stem cell maintenance. This is the first assignment of a previously identified mutation to a specific biochemical point in the RNAi pathway.

PUBLICATIONS

Carnero A., Hudson J.D., Hannon G.J., and Beach D.H. 2000. Loss-of-function genetics in mammalian cells: The p53 tumor suppressor model. *Nucleic Acids Res.* **28:** 2234–2241.

Hammond S.M., Bernstein E., Beach D., and Hannon G.J. 2000. An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cell extracts. *Nature* **404:** 293–296.

Wang J., Hannon G.J., and Beach D.H. 2000. Risky immortalization by telomerase. *Nature* **405:** 755–756.

In Press

Bernstein E., Caudy A.A., Hammond S.M., and Hannon G.J. 2001. Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature* **409:** 363–366.

Hammond S.M., Caudy A.A., and Hannon G.J. 2001. Post-transcriptional gene silencing by double-stranded RNA. *Nat. Rev. Genet.* (in press).

E. Bernstein, Y. Seger

CAENORHABDITIS ELEGANS DEVELOPMENTAL GENETICS

M. Hengartner A. Calixto, A. Elewa (URP), L. Englmeier, S. Granat, T. Gumienny, D. Hoeppner, R. Hofmann, J. Kinchen, S. Milstein, B. Schumacher, M. Spector, S. Tharin, J. Ye

Our laboratory uses *Caenorhabditis elegans* to study two basic biological problems: programmed cell death (apoptosis), and nervous system development and function. The value of *C. elegans* for such studies derives from its simplicity at the anatomical and developmental levels, from its powerful genetics and reverse genetics, and from the ready availability of genetic and molecular reagents (mutant collections, genome sequence, and so forth).

Despite its humble dimensions and simple anatomy, one should not lose sight of the fact that *C. elegans* is a metazoan—a multicellular animal—and as such shares with humans many fundamental molecular and cellular programs. It is this similarity at the level of genes and biochemical pathways that allows the nematode worm to be a useful model system.

PROGRAMMED CELL DEATH

Programmed cell death (PCD), or apoptosis, is a highly regulated program of cell suicide. Apoptosis is ubiquitous in humans. It is used during development to remove unnecessary cells; in adults, it contributes to the maintenance of cell populations and to the elimination of potentially dangerous (e.g., cancer-prone) cells. Defects in cell death have been implicated in the pathogenesis and/or etiology of many human diseases including cancer, various neurodegenerative diseases, and AIDS. A better understanding of the molecular mechanisms underlying the regulation and execution of cell death may thus lead to significant improvements in how these diseases are treated.

The proximal cause of apoptosis in *C. elegans* is the activation of the caspase homolog CED-3 from the inactive zymogen (proCED-3) into the mature protease. This activation is mediated by the Apaf-1 homolog CED-4. In cells that should survive, CED-3 and CED-4 are kept in check by the Bcl-2 family member CED-9. All three proteins are structurally and functionally conserved between nematodes and mammals, strongly suggesting that all metazoans share a common mechanism of apoptotic cell killing.

Physiological Cell Death in the* C. elegans *Germ Line: How is the apoptotic machinery regulated? In mammals, cells must integrate a large number of inputs (coming from various pro-death and pro-survival signals) before a decision to live or die can be taken. To understand how individual cells control activation of the death pathway in *C. elegans*, we set out to determine how a single cell type—the germ cell—makes this fateful decision.

PCD is very common in the adult hermaphrodite germ line: More than half of the germ cells that differentiate along the oogenic pathway undergo PCD. To identify genes that control the extent of germ cell death, we screened for mutations that result in increased *g*erm *l*ine *a*poptosis (*gla* mutations). So far, we have identified at least six *gla* genes; mutations in these genes specifically affect germ cell death, but not developmental death. To understand why mutations in *gla* genes lead to increased germ cell apoptosis, we are now cloning these genes, using a combination of positional cloning and genome-wide RNA interference (RNAi).

***DNA-damage-induced Apoptosis in* C. elegans:** We reported last year our discovery that *C. elegans*, like mammals, possesses DNA damage checkpoints that promote the elimination of damaged cells via apoptosis. DNA damage response pathways are intimately implicated in tumor development and in response to chemotherapy and radiotherapy. Thus, a better insight into the molecular basis of DNA-damage-induced apoptosis might lead to new avenues for prevention or treatment of cancer.

To date, we have identified mutations in three checkpoint genes that are required for DNA-damage-dependent cell proliferation arrest and apoptosis. One of these genes, *mrt-2*, encodes a homolog of the conserved *Schizosaccharomyces pombe rad1*+/*Saccharomyces cerevisiae RAD17* checkpoint gene. In the last year, we have focused our attention on a second checkpoint gene, *hus-1*, which encodes the *C. elegans* homolog of the *S. pombe* checkpoint protein Hus1. We isolated mutations in *hus-1* using a forward genetic

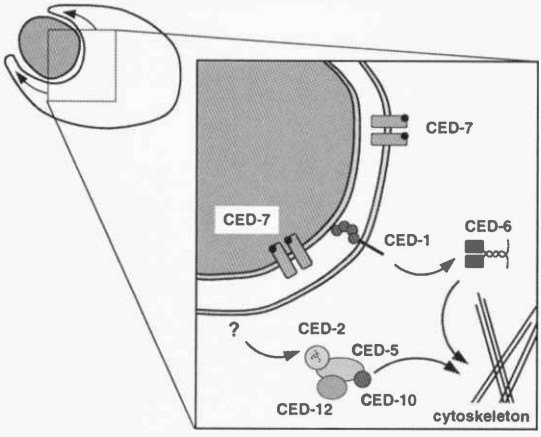


FIGURE 1 Getting rid of the bodies. Two partially redundant pathways, comprising CED-2, CED-5, CED-10, and CED-12 and CED-1, CED-6, and CED-7 mediate the removal of apoptotic cell corpses in *C. elegans*. Arrows represent proposed order of action. Known physical interactions are shown. (Adapted from Hengartner 2001.)

approach as well as through a directed reverse genetic effort. We found that loss of *hus-1* function leads to a defect in both cell proliferation and apoptosis following DNA damage. These results confirm the conserved nature of the DNA damage response pathway between yeasts and metazoans and encouraged us to develop tools that will allow us to perform large forward genetics screens and to properly classify the mutations that we will obtain in these screens.

Engulfment Genes Can Kill: Once a cell turns on the apoptotic machinery, a number of downstream "subprograms" are activated, with the goal of rapidly breaking down the cell and removing it from the body. One important subprogram results in the generation of signals that promote recognition and phagocytosis of the dying cell by other cells. At least seven genes are required for the recognition and efficient removal of apoptotic cells in *C. elegans* (Fig. 1). Inactivation of any one of these seven genes delays clearance of apoptotic cells, resulting in the accumulation of persistent cell corpses.

Surprisingly, a functional engulfment program appears to also be required for efficient cell killing: In collaboration with Ralf Schnabel (TU Braunschweig, Germany), we found that mutations that lead to reduced engulfment result in a significant decrease in apoptotic cell death. In other words, engulfment genes not only remove cells that are already dead, but can also kill cells that are still alive! This observation stands in stark contrast to the classic view (previously shared by us) that phagocytosis functions solely to remove already dead or doomed cells. We are now investigating the mechanism by which the phagocytic machinery might be able to influence the fate of cells at the edge of death.

Because much of the cell death pathway is conserved between *C. elegans* and vertebrates, we predict that the ability of the phagocytic program to kill cells may be a general phenomenon in metazoans. This realization might have important clinical implications. For example, in mammals, the phagocytosis process might contribute to the elimination of cells under pathological conditions, in which cells are exposed to sublethal pro-apoptotic stimuli. Such conditions can often be found in chronic neurodegenerative diseases, following a stroke, or in developing tumors. Modulating the activity of the engulfing machinery in neighboring cells might thus offer a new target for therapies to treat these diseases.

NERVOUS SYSTEM FUNCTION AND DEVELOPMENT

A second area under investigation in the lab is the nervous system in *C. elegans*. The small size and relative simplicity of the nematode nervous system allows us to readily address complex questions at the genetic and molecular levels.

Cell Death in the Developing Nervous System: As is the case in mammals, programmed cell death is very common in the developing *C. elegans* nervous system. To understand how developing neurons decide between differentiation and death, we initiated a detailed study of one particular motor neuron—the sexually dimorphic hermaphrodite-specific neuron (HSN). The HSN drives egg laying in hermaphrodites but dies by PCD in males. We have isolated more than a dozen mutations in at least three genes that specifically rescue the HSN from death. Two of these genes, which we have called *sue-1* and *sue-2* (for *su*ppressor of *egl-1*), share a number of pleiotrophic defects, suggesting that they act in a common pathway. Not only do mutations in *sue-1* and *sue-2* strongly and specifically rescue HSNs from apoptotic death and restore egg-laying function in *egl-1* mutants, but they also affect the differentiation and/or function of several other neurons. We expect that a genetic and molecular analysis of these genes will lead to a better understanding of how the HSN controls activation of the apoptotic machinery, and how life-vs-death decisions integrate into the larger problem of neuronal differentiation and nervous system development.

PUBLICATIONS

Chung S., Gumienny T.L., Hengartner M.O., and Driscoll M. 2000. A common set of engulfment genes mediates removal of both apoptotic and necrotic cell corpses in *C. elegans*. *Nat. Cell Biol.* **2:** 931–937.

Gartner A., Milstein S., Ahmed S., Hodgkin J., and Hengartner M.O. 2000. A conserved checkpoint pathway mediates DNA damage-induced apoptosis and cell cycle arrest in *C. elegans*. *Mol. Cell* **5:** 435–443.

Hengartner M.O. 2000. The biochemistry of apoptosis. *Nature* **407:** 770–776.

Hengartner M.O. and Bryant J.A. 2000. Apoptotic cell death: From worms to wombats... but what about the weeds? In *Programmed cell death in animals and plants* (ed. J.A. Bryant et al.), pp. 1–12. BIOS Scientific Publishers, Oxford, United Kingdom.

Hofmann E.R., Milstein S., and Hengartner M.O. 2000. DNA-damage-induced checkpoint pathways in the nematode *Caenorhabditis elegans*. *Cold Spring Harbor Symp. Quant. Biol.* **65:** 467–474.

Su H.P., Brugnera E., Van Criekinge W., Smits E., Hengartner M., Bogaert T., and Ravichandran K.S. 2000. Identification and characterization of a dimerization domain in CED-6, an adapter protein involved in engulfment of apoptotic cells. *J. Biol. Chem.* **275:** 9542–9549.

In Press

Hengartner M.O. 2001. Apoptosis: Corralling the corpses. *Cell* **104:** 325–328.

PLANT DEVELOPMENT

D. Jackson S. Archer-Evans, V. Bapat, G. Birkenmeier, M. Cilia, A. Giulini, J.Y. Kim, G. Sabino, F. Taguchi Shiobara, J. Thomas, Z. Yuan

Developmental patterning of multicellular organisms usually requires that cells communicate with each other to coordinate their final fate. Genetic analysis of plant development indicates a predominance of non-cell autonomy, implying the importance of cell-to-cell signaling. Our research is aimed at understanding the molecular mechanisms of plant development, with the eventual goal of improving crop yields. Unique features of plants include a complex and expansive extracellular matrix—the cell wall—and cytoplasmic channels or plasmodesmata that interconnect plant cells. These features suggest that mechanisms of cell-to-cell communication in plants may differ from those that control animal development.

Plants make new body parts—leaves, flowers, and roots—throughout their lifetime from groups of undetermined stem cells called meristems. The activity of the meristems is paramount in creating the basic architecture of the root and shoot systems. Although meristems are tiny structures, they control many of the basic aspects of plant form. Our studies in plant development focus on genes that regulate the shoot apical meristems, including those that control stem cell identity, cell proliferation, and the geometry of leaf initiation patterns.

Intercellular Trafficking of GFP Fusion Proteins

Z. Yuan, M. Cilia

Cell-to-cell trafficking of regulatory proteins, such as transcription factors, is a novel mechanism by which plant cells communicate during development. We are interested in how trafficking is regulated, in particular which proteins are able to traffic through plasmodesmata, and whether there exist specific trafficking target signals. To this end, we have made fusions of the green fluorescent protein (GFP) to several proteins and are testing their ability to traffic between cells following biolistic bombardment of constructs into single cells in the leaf. We and others have noticed that GFP itself is able to move freely through plasmodesmata that connect leaf epidermal cells, suggesting that the size exclusion limit is larger than previously thought. GFP has a molecular mass of 27 kD, implying that proteins of up to this size can diffuse through the plasmodesmal pore. We found that a fusion of GFP to the yellow fluorescent protein (YFP) is unable to traffic through plasmodesmata, suggesting that proteins of this size (~55 kD) or larger probably use a targeted mechanism for trafficking. For example, a GFP-KN1 fusion, which is 69 kD, is able to traffic between cells in the *Arabidopsis* leaf. We are currently making deletion derivatives of KNOTTED1 (KN1) to look for plasmodesmal targeting signals, and our initial studies suggest that one region of KN1 is able to promote trafficking of an inert reporter protein. Although an increasing number of plant and viral proteins have been found to move through plasmodesmata, no targeting signals have been identified, and we will therefore pursue these experiments with KN1 to see if we can identify a conserved targeting sequence or domain.

Cell-to-cell Trafficking of GFP-KNOTTED1 in the Shoot Apical Meristem

D. Jackson [in collaboration with P. Benfey, New York University]

KN1 normally functions in the shoot meristem to maintain cells in an undifferentiated state, and evidence from localization studies in maize suggests that cell-to-cell movement of KN1 protein may be important for communication between meristem cells. To directly test whether KN1 protein can move in the meristem, we used the *SCARECROW* (*SCR*) promoter, which drives expression in the outer layers of the

meristem, to express our GFP-KN1 fusion protein. In plants carrying the *SCR* promoter driving a cell, autonomous endoplasmic-reticulum-localized GFP, we observed GFP fluorescence only in the L1 and L2 layers of the meristem. However, when the same promoter drives GFP-KN1 expression, we observe fluorescence additionally in the underlying L3 cells, suggesting that GFP-KN1 can indeed move between cell layers in the meristem. Studies performed at Cold Spring Harbor Laboratory in the 1930s first identified lineage restrictions in the outer layers of shoot meristems and indicated that communication between cell layers in the meristem must be important to coordinate the development of the different layers. Intercellular trafficking of KN1 could be one such communication pathway, and we hope to directly test this idea by making trafficking-defective mutants of KN1.

Genetic Complementation of *shootmeristemless* by *KNOTTED1*

J. Thomas, Z. Yuan [in collaboration with K. Barton, University of Wisconsin, Madison]

The *KNOTTED1* or "knox" class of genes was the first of the homeobox gene family to be characterized in plants, although function has been assigned only to a small number of family members. Higher plants have about three to eight of the class I knox genes that are expressed specifically in shoot meristems and down-regulated in lateral determinate organ primordia such as leaves. Two of the class I genes, *SHOOTMERISTEMLESS* (*STM*) from *Arabidopsis* and *KN1* from maize, function to maintain the meristem in an indeterminate state; mutations in each of these genes cause premature termination of the shoot and loss of the stem cells. Together with their similar patterns of expression, it seems likely that these genes are functional homologs. However, sequence analysis suggests that *KN1* is orthologous to a different class I gene from *Arabidopsis*, *knat1*. To test directly whether maize *KN1* is a functional homolog of *STM*, we used the *STM* promoter to drive *KN1* expression and introduced this construct into *stm* mutants; we transformed heterozygotes because *stm* homozygotes are seedling-lethal. Transformants were selfed to recover *stm* homozygotes carrying the *STM* promoter-*KN1* transgene and scored for rescue of the *stm* phenotype. Most transgenic lines showed some degree of rescue, and one line flowered and produced seed, suggesting that *KN1* can complement for *STM* function throughout vegetative shoot and flower development. These results help to clarify functional relationships between knox genes of maize and *Arabidopsis* and also rationalize our use of *Arabidopsis* for function studies of cell-to-cell trafficking of *KN1*.

An Excision Screen for New *abphyl1* Alleles

A. Giulini, Z. Yuan

The *abphyl1* mutation causes a switch in phyllotaxy, or leaf patterning, from alternate to opposite and is rather unique because no other mutations that cause a simple change in the phyllotactic pattern have been isolated. The correct arrangement of leaves is an important trait for photosynthetic efficiency, as it ensures efficient light capture. During the past 3 years, we have isolated three new alleles of *abph1*, using active *Mutator* (*Mu*) transposon lines. The *Mu* transposon system has the advantage of a high forward mutation rate and near random insertion patterns. We are presently using these alleles in an attempt to clone the *abph1* gene. However, it would be useful to have alleles tagged with other types of transposons, such as those of the *Ac/Ds*, *Spm*, or *Bergamot* families, because these have a higher somatic and germinal excision rate and are useful for the generation of derivative alleles and for mosaic analysis. These transposons generally hop to sites closely linked to the original transposon donor site.

We mapped *abph1* eight map units proximal to the *B* (*Booster*) color locus on 2S, and therefore chose to use transposons at *B* because we can select for excisions from *B* by reversion to full color in the kernels. To make a useful *B* allele for screening of excisions, we made an intragenic recombinant between *B-Peru*, a stable allele that colors the aleurone layer of the seed, and *B-V* (*Bergamot*), an unstable allele that colors the leaves and stems but not the aleurone. The resulting recombinant has color sectors in the aleurone and a germinal excision frequency of about 1%. We generated approximately 300,000 F_1 seeds in an open pollination plot where active transposon plants were pollinated by *abph1* males and picked the full color revertant seed for screening. In an initial screen of approximately 1000 revertants, we recovered several candidates and are growing them for genetic analysis. Any novel alleles we recover will be useful for cloning and perhaps for mosaic analysis of *abph1*.

Isolation of a New *fasciated ear2* Allele and Confirmation of the *fae2* Candidate Gene

F. Taguchi Shiobara, G. Sabino, Z. Yuan

We previously reported the isolation of a candidate gene for the *fasciated ear2* (*fae2*) locus of maize. The *fae2* mutation affects the proliferation of stem cells in the ear inflorescence apical meristem, leading to an enlarged and fasciated or flattened ear with an increased number of rows of seed. To prove that the candidate gene we cloned was the correct one, it was necessary to isolate a novel allele of *fae2* and to demonstrate independent disruption of the same candidate gene. To this end, we screened 30,000 plants from a cross of *fae2* to lines carrying active *Mu* transposons. Screening in the F_1, we expected to recover the recessive *fae2* phenotype only if a novel mutation in *fae2* had occurred in the *Mu* parent. After 4 days of screening, we identified a single plant with a strong fasciated ear phenotype, and the DNA from this plant had a novel insertion into the single large exon of *fae2*. To show that this was a new allele, we also needed to demonstrate a fasciated phenotype when homozygous. This last summer, we planted the families at Uplands Farm, and as hoped, the homozygotes for the novel allele, *fae2-846*, had a strong fasciated ear phenotype. Therefore, the clone that we isolated is the correct gene. Molecularly, the new allele contains an insertion of the *Mu7* transposable element toward the 5′ end of the coding sequence, and it is likely a null allele. We have also isolated the complete *fae2* cDNA and have confirmed its identity as a putative transmembrane protein with predicted extracellular leucine-rich repeats (LRRs). Therefore, we predict that *fae2* acts as a cell surface receptor for an unidentified ligand that together function to regulate stem cell proliferation in the maize ear. We also mapped *fae2* and found that it maps close to a quantitative trait locus (QTL) for row number, a measure of the number of vertical rows of seeds on the ear. An association with this QTL is intriguing, because *fae2* ears make many more rows of seed than normal, between 30 and 36 vertical rows compared to 16–18 in normal sibs. In addition, in the tassel, *fae2* mutants are not fasciated, but they do have an increase in the number of flowers ("spikelet density"), an important agronomic trait in other cereals such as rice and wheat. We are planning experiments to test whether *FAE2* is responsible for the row number QTL, which could indicate a use for this gene in controlling crop yields.

Subcellular Localization of the FAE2 Protein

S. Archer-Evans, D. Jackson

Targeting prediction programs indicate that FAE2 is likely a transmembrane protein, with the bulk of the protein- including the leucine-rich repeats—being extracellular, and only a short cytoplasmic tail of about 15 amino acids. To test this hypothesis, we made a fusion of FAE2 to the GFP, attaching GFP at the carboxyl terminus so that it would be cytoplasmic if our predictions were correct. In general, GFP is not fluorescent when targeted to the plant extracellular matrix because the pH is too low. We introduced the construct by microprojectile bombardment into onion leaf epidermis and observed GFP localization by fluorescence confocal microscopy. We observed fluorescence in a peripheral location, fully consistent with the predicted plasma membrane localization. We are currently preparing FAE2 antibodies, which will be used to confirm this localization.

Isolation of New Fasciated Mutants from the MTM Collection

D. Jackson

The MTM (*Mutator*-targeted mutagenesis) system, developed here at the Laboratory by Rob Martienssen and collaborators from other institutions, is a large collection of selfed *Mu* transposon plants for forward and reverse genetics. During the last 2 years, we and others (M. Running, Berkeley) have screened the population for new mutants and have recovered several new fasciated ear mutants. I have shown that at least six of these are heritable, and I am now in the process of introgression into standard genetic backgrounds for complementation test and developmental studies. Of particular interest are a couple of new mutants that affect tassel as well as ear development; many of the existing fasciated mutants exert their effect predominantly in the ear. Therefore, the MTM collection is proving to be an excellent resource for forward as well as reverse genetics.

PUBLICATIONS

Jackson D. 2000. Opening up the communication channels: Recent insights into plasmodesmal function. *Curr. Opin. Plant Biol.* **3:** 394–399.

GENOMIC MICROARRAY ANALYSIS

R. Lucito J. Alexander
A. Buglino
S. Rostan

MEASURING GENOME COPY NUMBER FLUCTUATIONS IN BREAST CANCER

Progression of breast cancer, as for all cancers, is a multistep process. Mutations occur in the genome of precancerous cells and accumulate until growth goes unchecked. The large majority of these mutations occur sporadically, i.e., they are not inherited. Only a small percentage of breast cancers occur due to an inherited predisposition. Therefore, the genes responsible for sporadic breast cancer must be identified if we are to understand why a cell becomes cancerous; yet, to date, few of these mutations have been identified. To identify and characterize genes affected by sporadic mutation, we can look to the genome of the cancer cell itself. Often, mutations in the genome result in increased or decreased gene-copy number, namely, amplifications or deletions, and these changes can be used as markers to locate oncogenes or tumor suppressor genes, respectively, that are involved in the promotion of cancerous growth. The function of these oncogenes and tumor suppressors, once identified, can be investigated to understand the role of these genes in the progression to tumorigenesis. To identify genome copy-number fluctuations in breast cancer, we have developed a genomic microarray technique, borrowing the methodology of representations developed for representational difference analysis (RDA). A representation is a reproducible sampling of the genome, produced by cleavage of the genome with a restriction enzyme, ligation of adaptors, and subsequent polymerase chain reaction (PCR) amplification. The representation produced is composed of fragments from the genome in the size range of 200–1200 bases. The representation produced is reduced in complexity to approximately 3% of the genome, allowing increased hybridization efficiency to the microarray.

Although representations have previously been shown to be accurate in the reporting of copy-number changes between two genomes, a significant amount of time and effort was spent demonstrating that representations are accurate enough to be used with our microarray format. A pilot microarray was designed that contained representational probes, namely, small *Bgl*II fragments, either from specific regions or randomly from the human genome. As a first test, representations were compared to representations prepared separately, or in parallel. Ideally, we should obtain a ratio of 1.0 when comparing identical samples. The ratio of features from these experiments rarely went above 1.5, demonstrating that there was no more significant noise when comparing a representation to itself than when comparing a representation prepared in parallel. This demonstrates not only that the representational procedure is accurate, but also that there is little variation in our labeling with the two fluorochromes. To determine if the microarrays reported accurate copy-number changes, *Bgl*II representations were prepared from two breast cancer cell lines and compared by hybridization. Comparison of experiments performed independently showed little variation, testament to the reproducibility of our procedure. Copy-number values obtained from microarray results were verified by Southern blotting, and we found close concordance.

As proof of the utility of microarrays for analysis of primary breast tumors, a tumor sample was sorted based on ploidy into diploid and aneuploid nuclei, representing normal and tumor, respectively, and representations of this material were compared by array hybridization. A significant amount of genome copy-number information has been accumulated for this particular tumor as it has been analyzed previously by other methods, such as RDA, quantitative PCR, and Southern blotting. From our analysis, we have determined that there is close concordance between these methods. In fact, the microarray results were found to be more accurate than RDA. This experiment displays our ability to extract copy-number information from primary tumors.

We are now constructing an array for genome-wide scanning of tumor samples. Within the next year, we should have an array of 30,000 probes with a resolution of 1 probe for every 100 kb. This array will be used for the hybridization and analysis of a large set of primary breast tumors. By comparing invasive tumors with the noninvasive tumors, we hope to identify the regions of the genome that, when mutated, cause the

progression to invasiveness. The epicenter or common region of mutation will be delimited for the loci identified to undergo genome copy-number fluctuations, and these data will be used along with the information available from the genome sequencing project for the identification of gene candidates. Future knowledge of the function of these genes will lead to a better understanding of the disease and improvements in therapy and diagnosis.

RAC SIGNALING

This work was done in collaboration with Linda Van Aelst and Arndt Schmitz here at the Laboratory. We have used cDNA-RDA as a procedure to identify genes that are regulated by the Rac protein. This method is based on the genomic technique RDA, established in Michael Wigler's laboratory. This technique is a subtractive hybridization method used to compare two mRNA populations. The technique relies on making a representation of the samples being compared. Representations are prepared from the mRNA by converting to cDNA, digestion with a restriction enzyme, ligation of adaptors, and PCR amplification. The representations are compared by hybridization, and the differences are identified by PCR amplification and then subcloning. A cell line with an inducible RacV12 gene, a constitutively active form of the gene, was used comparing uninduced Rac to the induced Rac. To increase the throughput of screening the clones isolated as differences, approximately 1500 clones were grown in 96-well plates processed and arrayed on glass slides. These slides were screened for true differences by hybridizing differentially labeled representations from the uninduced and induced cells. We are currently characterizing the genes that show expression changes due to the presence of the *rac* gene.

MAIZE MUTAGENESIS LIBRARY SCREENING

This work, being done in collaboration with Robert Martienssen, Pablo Rabinowicz, and Erik Vollbrecht here at the Laboratory, is the development of a high-throughput 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 much of the maize genome is methylated repeat sequences, but the gene-rich regions are not methylated. This method enriches the genes represented by subcloning unmethylated genomic regions. The mutant plant library was produced by random insertion of a transposable element. The samples are produced by first pooling the individual plant DNAs in a plate-wise fashion, accumulating 50 individual plants per pool. Representations are prepared from these samples by PCR amplification of this pool using one oligo to the transposable element inserted and a second random oligo. 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.

PUBLICATIONS

Lucito R., West J., Reiner A., Alexander J., Esposito D., Mishra B., Powers S., Norton L., and Wigler M. 2000. Detecting gene copy number fluctuations in tumor cells by microarray analysis of genomic representations. *Genome Res.* **10:** 1726–1736.

In Press

Schmitz A., Lucito R., and Van Aelst L. 2001. Identification of Rac regulated genes using cDNA-RDA and microarraying. *Methods Mol. Biol.* (in press).

PLANT DEVELOPMENTAL GENETICS AND FUNCTIONAL GENOMICS

R. Martienssen J. Arroyo, A. Dabrowski, D. Roh, T. Singer, B. Bartelle, A. Groover, A. Landman, M. Ronemus, E. Vollbrecht, M. Byrne, B. May, T. Volpe, V. Colot, C. Kidner, P. Rabinowicz, J. Simorowski, C. Yordan, R. Shen, Y. Liu, C. Kopec, L. Brodsky

The first sequence of a plant genome, *Arabidopsis thaliana*, was completed at the end of 2000. We are taking advantage of this landmark achievement in our study of epigenetics and plant development. Endogenous transposons are heavily methylated in maize and *Arabidopsis*, and we have demonstrated that methylation and activity are dependent on chromatin remodeling. We are using transposons to develop libraries of maize and *Arabidopsis* from which mutant seed can be selected *in silico*. We also study the genetic basis for plant architecture. In *Arabidopsis*, *asymmetric leaves1* affects the proximodistal axis in leaves and floral organs, whereas *argonaute* disrupts dorsoventral polarity. In maize, *ramosa1* affects higher-order branching in the inflorescence. Each of these genes encodes a protein involved in transcriptional regulation or RNA interference (RNAi), and genetic interactions implicate each gene in stem cell fate. We are using *Schizosaccharomyces pombe* as a simple genetic system to study the mechanism of RNAi. In 2000, we were joined by postdocs Mike Ronemus and Tom Volpe, as well as visiting scientist Vincent Colot from the Evry Plant Genome Center in Paris. Donna Roh and Ben Bartelle joined our lab as research technicians, and Chuck Kopec as our intrepid URP. We said goodbye to Andrew Groover who left for a faculty position at the University of California, Davis.

Differential Methylation of Genes and Retrotransposons in Complex Plant Genomes

P. Rabinowicz, C. Yordan, R. Martienssen
[in collaboration with W.R. McCombie, Cold Spring Harbor Laboratory]

It has been proposed that, regardless of the large range of genome sizes, all diploid higher-plant genomes share essentially the same set of genes. This set, called the "gene space" includes approximately 25,000 genes, as estimated for the recently completed *Arabidopsis* genome sequence. The difference in diploid plant genome sizes is explained by the difference in the content of highly repetitive DNA. Repetitive DNA is composed mainly of transposable elements, many of which are heavily methylated. On the other hand, genes are usually located in nonmethylated regions of the genome. We have shown that repeat sequences in maize, because of their sensitivity to bacterial restriction-modification systems, can be largely excluded from genomic shotgun libraries by the selection of an *mcr*$^+$ host strain. In contrast, unmethylated genic regions are preserved in these genetically filtered libraries if the insert size is less than the average size of genes.

We are currently applying this approach to shotgun-sequence other complex plant genomes. Preliminary results show that low-copy regions can be efficiently selected from the large hexaploid wheat genome. This further supports the hypothesis that the gene space can be selectively isolated from any complex plant genome.

Epigenetic Regulation of Transposable Elements in *A. thaliana*

T. Singer, C. Yordan, R. Martienssen

Fifteen percent of the *Arabidopsis* genome consists of transposable elements (TEs) or their remnants, most of which are found in intergenic and heterochromatic regions (The *Arabidopsis* Genome Initiative 2000; Consortium, C.W.P.A.S., 2000). Despite their high-copy number, most TEs are silenced, highly methy-

lated at cytosine residues, and not active in wild-type plants. We are interested in the epigenetic mechanisms involved in TE regulation, which may be important in gene silencing and genome stability. We have identified more than 200 *Mutator*-like elements (MULEs) in the *Arabidopsis* genome with similarity to the MURA transposase of the Robertson's *Mutator* elements from maize; 21 elements with well-conserved terminal inverted repeats (TIRs) and *murA* transposase genes (*AtMu* elements) became demethylated in *ddm1* mutant plants. Two *AtMu* elements were also shown to transpose under these conditions, reinserting at unlinked positions in the genome. *DDM1* encodes an SWI2/SNF2-like chromatin remodeling protein (Jeddeloh et al., *Nat. Genet. 22:* 94 [1999]), and loss of *DDM1* gene function results in genome-wide demethylation of heterochromatic repeats (Vongs et al., *Science 260:* 1926 [1993]). Thus, MULEs are regulated by chromatin remodeling and methylation. Plants carrying newly transposed *AtMu* elements were outcrossed to wild-type plants and will be analyzed for *AtMu* methylation and activity. We are analyzing other TEs in mutants impaired in cytosine methylation and chromatin remodeling.

Construction of Chromosomal Microarrays in *Arabidopsis*

V. Colot, C. Yordan, R.A. Martienssen [in collaboration with V. Mittal, N. Dedhia, and W.R. McCombie, Cold Spring Harbor Laboratory]

We are studying the effects of DNA/chromatin modification and genome organization on transposon activity and transposon-mediated regulation of genes, which could account for many of the epigenetic phenomena observed in plants. More specifically, we want to examine chromosome-wide expression differences in polyploid *Arabidopsis* as well as in mutants that affect DNA methylation, chromatin remodeling, and histone acetylation. To this end, we are making DNA microarrays that will cover the entire 17 Mb of chromosome IV in contiguous or nearly contiguous fragments of approximately 1 kb. Most of the predicted 3825 protein-coding genes present on chromosome IV are more than 2 kb long and will therefore be covered by three or more contiguous fragments. Intergenic regions will also be represented and should provide a means to identify DNA sequences that are associated with specific proteins by the use of chromatin immunoprecipitation assays. Finally, chromosomal microarrays should allow us to discover new genes (such as those encoding untranslated RNAs) that cannot be identified by current gene-finding programs.

Primer pairs specific to each of the 17,000 fragments are being determined using the PRIMER3 program and verified by electronic polymerase chain reaction (PCR) using the whole *Arabidopsis* genome sequence as template. At present, we have constructed a microarray that covers 1.5 Mb around the heterochromatic knob located on the short arm of chromosome IV and are conducting tests of its many potential applications.

Systematic Transposon Mutagenesis in Maize and *Arabidopsis*

B. May, A. Groover, E. Vollbrecht, P. Rabinowicz, C. Yordan, J. Simorowski, J. Arroyo, D. Roh, R. Shen, B. Bartelle, Y. Liu, R. Martienssen [in collaboration with N. Dedhia, J. Healy, H. Liu, A. Reiner, L. Stein, and W.R. McCombie, Cold Spring Harbor Laboratory]

We have established large collections of maize and *Arabidopsis* plants that have been mutagenized by transposons. In maize, we have a collection of 50,000 plants each carrying hundreds of *Mutator* transposons, from which mutants in any gene can be selected by a simple PCR procedure (http://mtm.cshl.org). We are working on methods to systematically determine the genes disrupted in each line. In *Arabidopsis*, we have developed a large population of seeds carrying a single enhancer trap or gene trap Ds (Dissociation) transposon. DNA is made from each line as it is selected, and the flanking sequence is determined using PCR. The sequence data are automatically processed and annotated using the completed *Arabidopsis* genome as a guide. We are using this population to construct a database of gene knockouts that can be searched by text or sequence similarity (www.cshl.org/genetrap). The results of individual screens were described in more detail in last year's Annual Report.

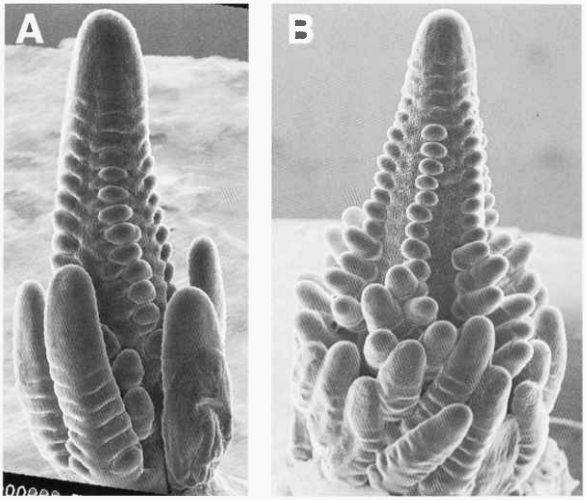


FIGURE 1 *ramosa1* imposes determinacy on branches in the tassel. (*A*) In normal tassels, most branches toward the tip are short and determinate. (*B*) In *ra1* mutants, all branches become indeterminate.

asymmetric leaves1 Mediates Leaf Patterning and Stem Cell Function in *Arabidopsis*

M. Byrne, J. Arroyo, M. Dunham, R. Martienssen

Meristem function in plants requires both the maintenance of stem cells and the differentiation of founder cells from which lateral organs arise. Coincident with their initiation and growth, lateral organs are patterned along proximodistal, dorsoventral, and mediolateral axes. The *Arabidopsis* mutant *asymmetric leaves1* (*as1*) disrupts this process. *AS1* encodes a Myb domain transcription factor, closely related to *PHANTASTICA* in *Antirrhinum* and *ROUGH SHEATH2* in maize, both of which negatively regulate knotted-class homeobox genes. *AS1* negatively regulates the homeobox genes *KNAT1* and *KNAT2* and is, in turn, negatively regulated by the meristematic homeobox gene *SHOOT MERISTEMLESS*. This genetic pathway defines a mechanism for differentiating between stem cells and founder cells within the shoot apical meristem and demonstrates that genes expressed in organ primordia interact with meristematic genes to regulate shoot morphogenesis. We are investigating these interactions further using second-site suppressor screens, enhancer and gene-trap mutagenesis, as well as microarrays to identify downstream targets.

ramosa1 Regulates Branching in the Maize Inflorescence

E. Vollbrecht, C. Kopec, R. Martienssen

Regulated branching patterns define plant architecture. Branches may be determinate, in which the apical meristem is capable of initiating only a few organs before ceasing to function, or indeterminate, in which the meristem may iterate many initiation cycles during

its lifetime. The maize tassel and ear have similar underlying architecture, but differential activity of second-order meristems results in differing morphologies. In the ear, all second-order meristems are determinate, whereas in the tassel, second-order meristems may be indeterminate and form long branches, or determinate as in the ear. In *ramosa1* mutants, ears and tassels are fully branched and architecturally equivalent (Fig. 1). We cloned *ra1* by transposon tagging with *Spm* and have identified molecular lesions in ten mutant alleles, including weak alleles with point mutations. The *ra1* gene is expressed in developing tassels and ears but not in vegetative shoots and encodes a 19-kD protein with a single TFIIIa-type zinc finger, suggesting that RA1 functions as a transcriptional regulator. *ra1* is expressed in a narrow spatiotemporal window consistent with a function in second-order meristems. Mutation in a distinct gene, *ramosa2*, is epistatic to the *ra1-R* mutation in the tassel, and *ra1* is expressed at lower levels in *ra2-R* mutants, placing *ra1* genetically downstream from *ra2*. These data indicate that *ra1* gene function imposes determinacy to inhibit second-order meristems from becoming long branches. At least two genetically separable pathways regulate branching in the tassel. We have begun to examine the evolutionary conservation of branching programs by looking at *ra1*-related genes in selected grasses such as sugar cane, rice, and sorghum.

The Function of RNAi in Plants and Yeast

C. Kidner, M. Ronemus, T. Volpe, R. Martienssen
[in collaboration with S. Grewal, and G. Hannon, Cold Spring Harbor Laboratory]

Double-stranded RNA (dsRNA) results in the silencing of cognate genes when introduced into cells. This process is known as RNA interference (RNAi), and it has been widely used to study hypomorphic phenotypes in systems from worms to mice. But neither the function nor the mechanism of RNAi is fully understood. Genetic and biochemical studies have identified a number of factors of known function involved in RNAi, including an RNA helicase, an RNase III, and an RNA-dependent RNA polymerase (RDRP). A family of related genes of unknown function—including *ARGONAUTE* (*AGO*) and *ZWILLE* of *Arabidopsis*, *Piwi* and *Sting* of *Drosophila*, and *RDE1* of *Caenorhabditis elegans*—have also been identified as modifers of RNAi.

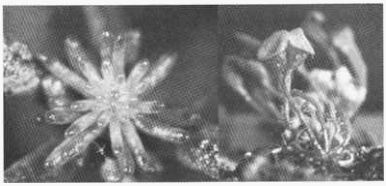


FIGURE 2 Strong (*left*) and weak (*right*) mutant alleles of the *argonaute* gene result in leaf polarity and stem cell defects in *Arabidopsis*.

In contrast to most modifiers of RNAi, which do not produce apparent phenotypic abnormalities when mutated, disruption in *AGO* function results in severe defects in stem cell maintenance and organ polarity in *Arabidopsis* (see Fig. 2). Using weak mutant alleles of *AGO*, we have been investigating the interaction of *AGO* with key developmental regulators. Through analysis of double mutants, we and others have shown that *SHOOT MERISTEMLESS* (*STM*) and *AGO* closely interact to control organ development and stem cell maintenance. These and other double-mutant phenotypes reveal a requirement for the RNAi mechanism in plant development. As mutation of the *AGO* homolog of *C. elegans*, *RDE1*, does not perturb development in the worm, these findings indicate that RNAi may have become specifically adapted to regulate the expression of endogenous genes in plants.

Many of the genes involved in RNAi are represented in *Schizosaccharomyces pombe*. These include a single *AGO* ortholog, as well as genes encoding an RDRP and an RNase III. We are taking advantage of the comparative ease of genetic and biochemical manipulation available in *S. pombe* to study these genes. Although RNAi has yet to be demonstrated in *S. pombe*, we have shown that the *AGO* ortholog of *S. pombe* is required for silencing at the centromere as well as proper chromosome segregation. Additional studies of RNAi in *S. pombe* will be performed to identify other components of the RNAi apparatus and to elucidate their functions.

We have also initiated studies to investigate further the function of *AGO* and RNAi in control of gene expression and genome stability in *Arabidopsis*. Using

microarrays, we are examining genome-wide expression in *ago* lines containing weak and strong mutations at multiple stages of development. Genes representing candidates for regulation by RNAi will then be characterized in depth by detailing their mutant phenotypes. DNA methylation, an agent of genome stability, is altered in transgenes that undergo RNAi in *AGO* mutant lines. We are extending these observations by assessing the methylation states of endogenous loci in *Arabidopsis*; preliminary results indicate that a subset of sites are specifically demethylated in *ago* lines.

PUBLICATIONS

The *Arabidopsis* Genome Initiative. 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* **408:** 796–815.

Byrne M.B., Barley R., Curtis M., Arroyo J.M., Dunham M., Hudson A., and Martienssen R. 2000. *Asymmetric leaves1* mediates leaf patterning and stem cell function in *Arabidopsis*. *Nature* **408:** 967–971.

Chory J., Ecker J.R., Briggs S., Caboche M., Coruzzi G.M., Cook D., Dangl J., Grant S., Guerinot M.L., Henikoff S., Martienssen R., Okada K., Raikhel N.V., Somerville C.R., and Weigel D. 2000. National Science Foundation-Sponsored Workshop Report: "The 2010 Project" functional genomics and the virtual plant. A blueprint for understanding how plants are built and how to improve them. *Plant Physiol.* **123(2):** 423–426.

The Cold Spring Harbor Laboratory, Washington University Genome Sequencing Center, and PE Applied Biosystems *Arabidopsis* Sequencing Consortium. 2000. The complete sequence of a heterochromatic island from a higher eukaryote. *Cell* **100:** 377–386.

Ferrandiz C., Gu Q., Martienssen R., and Yanofsky M. 2000. Redundant regulation of meristem identity and plant architechture by FRUITFULL, APETALA1, and CAULIFLOWER. *Development* **127(4):** 725–734.

Martienssen R. 2000. Weeding out the genes: *The Arabidopsis* Genome Project. *Functional and Integrative Genomics* **1:** 2–20.

Park S.H., Chin H.G., Cho M.J., Martienssen R.A., and Han Cd. 2000. Inhibitor of striate conditionally suppresses cell proliferation in variegated maize. *Genes Dev.* **14(8):** 1005–1016.

Petersen M., Brodersen P., Naested H., Andreasson E., Lindhart U., Johansen B., Nielsen H.B., Lacy M., Austin M.J., Parker J.E., Sharma S.B., Klessig D.F., Martienssen R., Mattsson O., Jensen A.B., and Mundy J. 2000. *Arabidopsis* map kinase 4 negatively regulates systemic acquired resistance. *Cell* **103:** 1111–1120.

Springer P.S., Holding D.R., Groover A., Yordan C., and Martienssen R.A. 2000. The essential Mcm7 protein PROLIFERA is localized to the nucleus of dividing cells during the G(1) phase and is required maternally for early *Arabidopsis* development. *Development* **127(9):** 1815–1822.

Tabata S., et al. (The Kazusa DNA Research Institute, The Cold Spring Harbor and Washington University in St Louis Sequencing Consortium, and The European Union Arabidopsis Genome Sequencing Consortium). 2000. Sequence and analysis of chromosome 5 of *Arabidopsis thaliana*. *Nature* **408:** 823–826.

In Press

Byrne M., Timmermans M., Kidner C., and Martienssen R. 2001. Development of leaf shape. *Curr. Opin. Plant. Biol.* (in press).

Martienssen R. 2001. Chipping away at chromatin. *Nat. Genet.* **27:** 240–241.

Singer T., Yordan C., and Martienssen R. 2001. Mutator-like transposons in *Arabidopsis thaliana* are regulated by the chromatin-remodeling gene Decrease in DNA Methylation (DDM1). *Genes Dev.* **15:** 591–602.

Settles A.M., Baron A., Barkan A., and Martienssen R.A. 2001. Duplication and suppression of chloroplast protein translocation genes in maize. *Genetics* **157:** 349–360.

DNA ARRAY TECHNOLOGY AND MOUSE MODELS OF HUMAN CANCER

V. Mittal K. Lavine

DNA microarray technology is a powerful tool for functional genomic research. The transcriptional profiling by microarray analysis holds the promise of yielding excellent molecular markers for the clinical classification of tumors. In particular, the patterns of genes expressed in a tumor can indicate important biological features such as responsiveness to therapy, predilection to invasion and metastasis, and the long-term survival of the patient. Several studies have recently shown how DNA arrays can be applied to the analysis and classification of cancers like leukemia and lymphoma.

We are developing and implementing the DNA array technology to understand (1) the molecular determinants of cancer pathways, using the mouse as a model system, by examining both patterns of gene expression and genetic lesions and (2) the molecular basis of drug sensitivity and resistance in tumors in response to chemotherapy. To do this, we have set up a reliable system for fabrication of high-density human and mouse expression arrays and informatics tools for quantitative analyses of the array data. We have assembled a collection of 15,000 mouse cDNAs and expressed sequence tags (ESTs) from the National Institute of Aging and 40,000 human cDNAs and ESTs from the Image Consortium. Kimberly Lavine and Jennifer Troge have processed these clones and have successfully printed, hybridized, and analyzed microarrays of greater than 10,000 cDNA probes.

UNDERSTANDING GENOME-WIDE CHANGES IN PRIMARY LYMPHOMAS IN RESPONSE TO CHEMOTHERAPEUTIC DRUGS

A major focus in cancer research is the search for molecular determinants of anticancer drug responses and resistance. These are likely to have an impact on the determination of clinical courses and treatment responses. In collaboration with Scott Lowe's lab here at the Laboratory, we have started to identify gene expression changes associated with drug response and resistance. These studies will help in identifying molecular pathways involved at the onset of drug treatment and how these pathways mature in the tumor to achieve drug resistance. We are using the Eμ-*myc* transgenic lymphoma mouse (Adams et al., *Nature 318:* 533 [1985]) as a tractable disease model for studying the therapy-induced response of lymphoma in vivo. The strengths of this model are as follows: (1) The genetics and histopathology of Eμ-*myc* lymphomas resemble human non-Hodgkin's lymphomas; (2) the tumor burden can be easily monitored by lymph node palpation or blood smears, or by monitoring green fluorescence protein (GFP) fluorescence in blood samples, a property that facilitates the examination of responses to therapy; (3) therapy is performed in immunocompetent mice; and (4) pure lymphoma cells can be transplanted into numerous syngeneic mice. Strikingly, the clinical and histopathological characteristics of a given lymphoma are virtually identical when portions of the same population are propagated in separate recipients, thus allowing enormous flexibility in conducting parallel studies.

The molecular signatures of tumor responses to various anticancer treatments alone and in combinations will be monitored in a time-dependent manner by high-density cDNA microarrays. Typical data from an array experiment are shown in Figure 1. A web-accessible database of reference gene expression profiles will be developed. To examine the molecular signatures of drug response, the data will be clustered based on positive and negative expression pattern correlation, similarity in promoter sequences, and functional annotations of cDNAs (an ongoing project in my laboratory). The drug response of tumors from human patients will be examined in parallel in collaborations with clinical experts. A comparison of the human and mouse gene expression patterns is essential not only to validate the mouse as a model for human cancer, but also to help us better understand treatment sensitivity of human cancers, facilitate the design of better anticancer agents or treatment regimens, and thus lead to more rational ways of designing combination therapies.

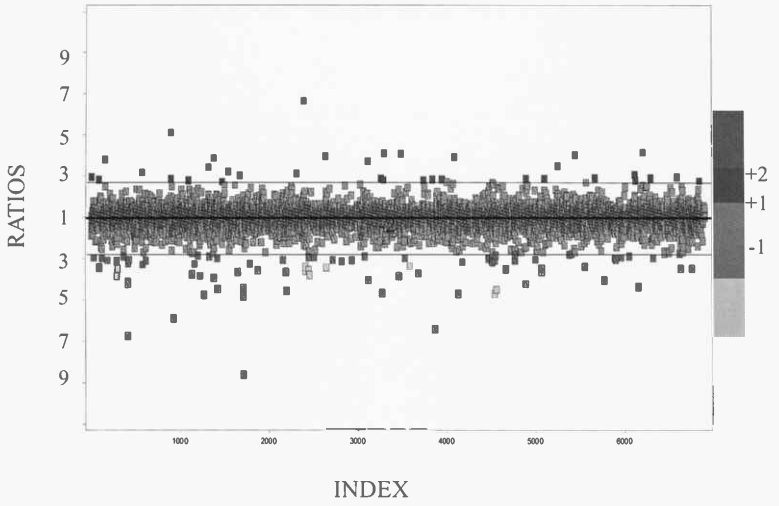


FIGURE 1 Scatterplot from a mouse cDNA microarray experiment. Microarrays were printed from a collection of 10,000 murine cDNA and EST clones. These were hyrbidized with mRNA derived from two different lymphoma samples (labeled with red and green fluorescent dye, respectively). The normalized ratios of red versus green channel is plotted for each spot. Each feature in the scatter plot has been assigned a standard deviation value from the mean (refer to the bar on the right). Genes whose ratios are within 1 standard deviation are assumed to be unchanged and genes with standard deviation above 1 or below 1 are used for further analysis. Overall, this scatter shows that more then 98% genes are unchanged (are within a twofold range).

IDENTIFICATION OF GENES INVOLVED IN Id SIGNALING IN ENDOTHELIAL CELLS

The Id family of proteins are essentially dominant negative helix-loop-helix transcription factors. Normal Id expression is required to support angiogenesis and vascularization of tumor xenografts. Blood vessels in Id knockout mice lack the ability to branch and sprout to support growth or metastasis of tumors, and any tumor growth present shows poor vascularization and extensive necrosis.

In collaboration with Robert Benezra and Marianna Russinova (Memorial Sloan-Kettering Cancer Center, New York), we are interested in understanding the molecular mechanisms by which Id exerts its effects on angiogenesis. We will use mouse cDNA arrays to examine differences in the expression of mRNAs in endothelial cells isolated from tumors grown in Id wild-type and knockout backgrounds. Benezra's group has previously shown that there is a dramatic difference in the growth of blood vessels into VEGF-impregnated matrigel plugs supplanted subcutaneously in Id knockout versus wild-type animals. We will also perform the array analysis on endothelial cells isolated from these plugs. This will allow us to determine which differences are inherent in the endothelial cells isolated from the wild-type and mutant mice and which may be attributed to tumor-specific alterations. Although it is difficult to predict the outcome of such experiments, our efforts after the identification of gene expression changes will be focused on molecules that might make good targets for anti-angiogenic drug design. Surface markers that are down-regulated, for example, in the absence of Id expression may mediate the effects of loss of Id. Antagonists of such markers would be sought for testing in the matrigel plug assays in an attempt to phenocopy the perturbed blood vessel development observed in the Id knockout backgrounds.

FUNCTIONAL ANALYSIS OF CANCER GENES

We have two collaborative projects with Mike Wigler's lab here at the Laboratory. One is to understand the mechanistic role of tumor suppressors and oncogenes in cancer by determining their specific cellular and physiological function. We have begun by examining the mechanistic role of the human tumor suppressor, PTEN. The PTEN gene product shares homology with protein tyrosine phosphatase family members and antagonizes phosphatidylinositol 3-kinase (PI3-kinase) by dephosphorylating its substrate PIP3. Because many of the PTEN cancer-related mutations have been mapped to its phosphatase catalytic domain, it has been suggested that the phosphatase activity of PTEN is required for its tumor suppressor function. We used an ecdyson-inducible mammalian expression system (developed in Mike Wigler's lab) and human cDNA microarrays (containing 15,000 elements) to survey changes in patterns of gene expression upon induction of either wild-type or mutant PTEN in the glioblastoma cell line U87MG (which lacks a functional PTEN). In parallel, we also compared changes upon induction of wild-type PTEN with changes caused by the compound LY294002, an inhibitor of PI3-kinase. The effects of wild-type PTEN induction on transcription shared common signatures with the effects of inhibition of PI3-kinase, consistent with the model positioning PTEN as an inhibitor of PI3-kinase signaling. In addition, comparison of wild-type and mutant PTEN array data identified PTEN-regulated genes, which were validated by sequencing, Northern blotting, and RNase protection assays. A majority of these genes were involved in cell proliferation, cytoskeleton restructuring, and signal transduction pathways. Strikingly, at least five genes in the cluster were involved in the sterol biosynthesis pathway. The role of this pathway in the pathophysiology of PTEN loss is a subject for future investigation. These results illustrate the power of a robust inducible expression system in conjunction with cDNA microarrays in understanding gene expression patterns induced by a tumor suppressor gene. Such an approach is directly applicable to the functional analysis of other cancer genes.

The other project is to measure gene copy number by using mirrored representation and cDNA microarrays. The main problem with most of the existing methodologies for measuring gene copy number on a genome-wide scale is inferior signal to noise, which is due to the high nucleotide complexity of the human genome. To solve this problem, a new strategy to detect amplifications and deletions in the cancer cell genome is currently under development. The principle of operation is to extract the nucleotide complexity from the genome of a cancer cell that matches (mirrors) the complexity of cDNA probes on the array by using a substractive hybridization method. The greatest strength of this approach will be much superior hybridization kinetics with a concomitant improvement in signal to noise. In addition to measuring gene copy number changes, these arrays also have the potential to detect the expression status of genes from the same tumor samples.

MAMMALIAN CELL GENETICS

M. Wigler J. Alexander, J. Brodsky, A. Buglino, K. Chang, J. Douglas, D. Esposito, E. Hatchwell, J. Healy, M. Riggs, A. Reiner, L. Rodgers, S. Rostan, L. Serina, J. Stolarov, E. Thomas, J. Troge, J. West, C. Yen

Cancer is caused by mutations in critical genes. Some of these mutations may be inherited, but much more frequently, they are acquired during the lifetime of the individual. The critical genes that are at risk are still largely unknown, both in the general sense (i.e., which gene targets ever contribute to cancer) and in the specific sense (i.e., which genes are targeted in an individual's cancer). Without this knowledge, it is likely that our attempts to control cancer will be incomplete. Therefore, work in our group is centered upon cancer genomics. We have developed several methods for detecting genetic alterations in cancers, identifying the genes that are the targets of these alterations, and exploring the functional analysis of these genes by a variety of means. PTEN, a tumor suppressor gene isolated by our methods, continues to be the subject of our most intensive study. We continue to work intensively on developing tools to find additional genes, and explore their function, using microarray and inducible gene expression. All aspects of our work have now been strongly influenced by the publication of a working draft of the human genome. Recently, we added a genome informatics component in order to exploit this new resource. We are also applying the methods that we develop to analyze the spontaneous mutations that underlie some human genetic diseases, especially those that cause sporadic disease in children.

Discovery of Loci and Genes Altered in Cancer

C. Yen, D. Esposito [in collaboration with R. Lucito, M. Hamaguchi, R. McCombie, and M. Zhang, Cold Spring Harbor Laboratory, and S. Powers, Tularik Corporation]

The main engine for gene discovery in our lab has been representational difference analysis (RDA), a subtractive hybridization technique that allows us to search for amplified loci and homozygously deleted regions in cancers (Lisitsyn et al., *Science 259:* 946 [1993]; Lisitsyn et al., *Proc. Natl. Acad. Sci. 92:* 151 [1995]). We have applied it to the study of breast cancer, analyzing a total of 18 pairs of tumor/normal pairs. This has resulted in the discovery of numerous amplified loci, many of them previously characterized, such as the loci for ErbB2, c-myc, and cyclin D, but the majority are uncharacterized. Of these, the 8q11 region has been analyzed by Scott Powers and colleagues at Tularik for further analysis. Probes from this region detect gene amplification in 6 out of 70 primary breast tumors, and they have narrowed the candidate region to two overlapping BACs (bacterial artificial chromosomes). We have also discovered with RDA nine regions of confirmed homozygous deletion. One of these was the region on 10q23 containing the PTEN tumor suppressor; one was the region containing the tumor suppressor p16(INK4), on chromosome 9p (Serrano et al., *Nature 366:* 704 [1993]; Kamb et al., *Science 264:* 436 [1994]); one was the region on chromosome 3p14 that contains the FHIT candidate tumor suppressor gene (Ohta, *Cell 84:* 587 [1996]); and one mapped near the p53 tumor suppressor. Five homozygously deleted regions remain that are not yet fully characterized. Two of these reside on chromosome 8q22, within 10 Mb of each other, one on chromosome 4p16, one on chromosome 21p11, and one on chromosome 20p11. All but one of these loci have been observed to be deleted in a number of clinical specimens and/or cell lines and have been confined to regions from a few megabases to a few hundred kilobases. In collaboration with the laboratories of Dick McCombie and Michael Zhang, the gene candidates from these regions are being identified using informatic tools.

In collaboration with Dr. Hamaguchi, seven transcripts have been identified from one candidate locus on 8q22. Further analysis, in collaboration with the

laboratory of Mary-Claire King at the University of Seattle, has helped us to focus on one candidate gene from this region, which encodes a heretofore unrecognized gene. See Dr. Hamaguchi's section for a more comprehensive report on this locus and its genetic content.

Development of Inducible Gene Expression Systems

J. Stolarov, K. Chang [in collaboration with V. Mittal, Cold Spring Harbor Laboratory]

In our early studies of PTEN, we used constitutively expressing retroviral vectors, and immediately encountered the problem of great variability in the phenotypes of the clones that emerged. Clonal variability, and lingering questions about the cellular adaption to the chronic expression of PTEN, prevented us from reaching clear conclusions. Thus, we sought to develop inducible expression systems in which, ideally, paired cultures could be compared for their acute responses to controlled levels of expression of the tumor suppressor.

In the ideal system, the inducer would be physiologically inert but would rapidly induce nearly 100% of modified cells to express close to endogenous levels of the target gene. Modified cells would not express this gene in the absence of inducer. Such a system would enable the observation of acute effects of expressing tumor suppressors in cells, both when growing in cell culture and when growing as a tumor in an animal. We chose a retroviral delivery system as it had the additional attraction that the expression system could be easily introduced into a variety of cell backgrounds. We chose the ecdysone system of Evans and co-workers (No et al., *Proc. Natl. Acad. Sci. 93:* 3346 [1996]) over the tetracycline system (Saez et al., *Curr. Opin. Biotechnol. 8:* 608 [1997]) because it has no background in the uninduced state and very swift kinetics of induction. In brief, we produce both amphotropic and ecotropic viruses using high-titer packaging lines that were derived by David Beach and Greg Hannon (Hannon et al., *Science 283:* 1129 [1999]) at Cold Spring Harbor Laboratory. Typically, we infect cells with the receptor viruses together, and the cells undergo double selection with puromycin and G418. Single-cell clones are selected, expanded, and tested for their ability to serve as a good "host," i.e., respond to inducer following infection and hygromycin selection with a third virus containing an inducible β-galactosidase gene.

Our first experiments were performed with U87MG, a human glioblastoma cell line known to have no functional PTEN gene. After double infection with receptor viruses, and double selection, five candidate host clones were selected. These were then infected with a target gene (*lacZ*) under the control of ecdysone. For all five hosts, virtually all cells in the colonies were inducible, and none showed detectable background expression in the absence of inducer.

We have now extended this system to other human and mouse host cells, and streamlined the selection of inducible hosts, and are exploring the inducibility of other genes in known signal transduction pathways.

Unfortunately, in our first attempts to test the induction system in transplantable tumors, we observed that the U87MG hosts expressed PTEN constitutively. We are currently exploring whether this is a defect in the expression system, a defect of the particular host, or an unavoidable consequence of the loosening of transcriptional controls in dying cells.

Analysis of PTEN Tumor Suppressor

J. Stolarov, K. Chang [in collaboration with V. Mittal and N. Tonks, Cold Spring Harbor Labortatory, and D. Durden, University of Indiana]

In previous years, we collaborated with Nick Tonks and Mike Myers on the study of PTEN in animal cells. This work led to the observation that PTEN, predicted and shown to be a protein phosphatase, was also a PIP3,4,5 phosphatase (Myers et al., *Proc. Natl. Acad. Sci. 94:* 9052 [1997]). Since PIP3,4,5 is an important second messenger that mediates such fundamental cellular processes as response to growth factors and inhibition of cell death, this lipid phosphatase activity appeared to be a logical explanation of PTEN's function as a tumor suppressor. This was confirmed by studies of mutant PTEN (Myers et al., *Proc. Natl. Acad. Sci. 94:* 9052 [1997]; Myers et al., *Proc. Natl. Acad. Sci. 23:* 13513 [1998]).

We have utilized the retroviral induction system described above for further studies on PTEN. Expression of PTEN was clearly controllable in

U87MG hosts with muristirone, an ecdysone analog, with induction levels up to 50-fold above a negligible background. At physiological levels of expression, induction of PTEN did not alter cellular growth rates. This result is in contrast to most reports in the literature, in which the levels of expression of PTEN were not so well controlled. Induction did cause a noticeably flattened cellular morphology, most clearly seen when cells were grown in low serum. An essentially identical change in morphology was induced by LY294002, a known small molecular inhibitor of PI3 kinase, the enzyme that produces PIP3,4,5. Even more striking results appeared following analysis of transcription patterns using cDNA microarrays. Of 6218 probes, 0.4% of transcripts appeared to be suppressed by greater than threefold upon PTEN induction, and 0.25% of transcripts appeared to be induced by greater than threefold upon PTEN induction. An essentially identical pattern of change in transcription was observed when U87MG cells were treated with LY294002. Enzymatically inactive mutants of PTEN produced essentially no transcriptional changes when induced. These results clearly confirm the hypothesis that PTEN action results from its perturbation of the PIP3,4,5 metabolism.

In collaboration with D. Durden, we have explored the effect of PTEN expression on tumorigenicity of the host-cell U87MG. PTEN expression suppresses the tumorigenicity of this cell line, reducing tumor growth and tumor vascularization. This happens without an apparent reduction of cell replication or increase in cell death within the tumor. Our present hypothesis is that the effect must be at the tumor margins, i.e., that PTEN retards invasiveness, and this is in keeping with the observation that loss of PTEN in glioblastomas is found most commonly in invasive tumors. The physiological mechanisms at play here are not at all clear, and warrant further investigation.

Microarray Analysis of Mutations in Tumors

J. West, A. Reiner, D. Esposito [in collaboration with R. Lucito and R. McCombie, Cold Spring Harbor Laboratory]

The search for tumor suppressors and oncogenes by RDA has been slow. The bottleneck has been the time and labor spent on "epicenter mapping." Once a locus has been identified, we need to demonstrate that the locus is affected in multiple cancers. Then, because these loci are large, we need to find the minimum region affected so as to facilitate identification of candidate genes. This process, which we refer to as epicenter mapping, has required more than 6000 individual quantitative polymerase chain reaction (Q-PCR) tests for the various loci we have identified. We therefore decided to develop a microarray-based method for gene-copy measurements. Our expectation is that once fully established, this method will accelerate the rate of gene discovery by a factor of perhaps 1000-fold, as well as provide a detailed way of categorizing clinical cancers.

Microarrays, although widely utilized for expression profiling, cannot be effectively used for measuring gene-copy numbers in a straightforward manner. Using the entire human genome for hybridization is inefficient due to its nucleotide complexity. To solve this problem, the microarray system we have developed is based on representations. A representation is a reproducible amplification of the genome with reduced complexity. In brief, a representation is produced by first cleaving the genome with a restriction enzyme, and adaptors are then ligated and used for a subsequent PCR amplification. The complexity of a representation based on *Bgl*II cleavage is approximately 5% of the genome, which is comparable to that of the complexity of transcripts within a cell. Single fragments from the representation are then cloned and arrayed on a slide. Representations from tumor and normal, prepared in the same manner as that of the array, are then hybridized to the array, and the results are analyzed to define regions that have undergone copy number changes such as deletion and amplification.

To determine the fidelity of our microarray format, we carried out several experiments. We made multiple *Bgl*II representations of the cell line SKBR-3 on different days. A representation derived from the cell line was labeled separately with Cy3 and Cy5 (two fluorescent dyes in common use) and compared. We found that there was very little deviation from a ratio of one. These experiments validate the extreme reproducibility of representations and suggest that making well-controlled parallel representations introduces no more noise than is inherent in the measurements made by the system as we practice it.

We also examined the reproducibility of our microarray measurements between multiple experi-

ments. Multiple representations of two different human breast cancer cell lines, SKBR-3 and MDA-MB-415, were compared by hybridization. The ratios obtained from one experiment were compared to those obtained from another parallel experiment. There was excellent concordance between independent microarray measurements and excellent agreement with measurements of gene copy number by Southern blot hybridization. These experiments again attest to the reproducibility of representations and also to the reproducibility of printing, labeling, and hybridization.

Due to the use of representations, we are able to use minute amounts of starting material. Thus, we are able to measure genomic changes in the sorted tumor and normal nuclei of a cancer biopsy. The results of the microarray hybridization completely corroborate the results obtained by other methods. A summary of this work has been published recently (Lucito et al. 2000).

The project is currently being scaled-up. Our collection of *Bgl*II representational fragments is about 12,000 and 8,000 have been sequenced. Greater than 80% of these fragments map to the "golden path," the publicly assembled human genome. Many do not map, indicating gaps in the genome data, and many map to multiple chromosomes, suggesting either miss-assembly of the genome or multiple regions of large chromosome duplications. Many of the probes contain repeat sequences, and we are presently determining which probes will be useful. To accomplish this, we are using partitions of BAC libraries, which we can use as quality control reagents for determining which probes can detect deletions and which can detect amplifications.

At present, we do not know what percentage of probes will be useful for arraying. To produce a useful tool, we will need microarrays with coverage of 1 probe every 100 kb, on average. This will require about 30,000 probes, and to achieve this, we may need to clone and characterize greater than 60,000 probes. This microarray will be used to scan the genomes of breast cancers to uncover regions that have undergone copy number changes. Our hope is that our scan will be done at such precise coverage, and against such a large number of tumors, that we will uncover new candidate loci for the location of tumor suppressors and oncogenes. Furthermore, by analyzing many tumors, we should also be able to limit the size of the region in which we need to search for genes. This method also has other potential uses, including measuring mutational load in cancers, monitoring DNA methylation patterns, genome-wide genetic typing, and detection of de novo mutations in humans.

Analysis of De Novo Mutation in the Human Germ Line

E. Hatchwell

Approximately 1–2% of all children are born with some degree of learning difficulty. The vast majority are likely due to genetic factors. In many cases, the intellectual problems are part of a more global syndrome, including various physical abnormalities. Conventional approaches to diagnosis include biochemical and cytogenetic analyses and, in specific circumstances, direct testing of suspect genes. When exhaustive testing has been performed, however, at least 50% of cases remain undiagnosed, either because the syndrome presentation is not specific enough or because the genetic abnormality is simply not detectable using conventional methods.

In recent years, a number of syndromes have been shown to result from submicroscopic genomic alterations (i.e., deletions, duplications, or inversions). In the vast majority, the knowledge gained depended heavily on serendipity. It is likely that many of the syndromes that are currently undiagnosed result from a similar general mechanism. Currently, no general method exists for detection of submicroscopic genomic alterations. This project aims to develop methods that will detect hemizygous loss, one of the known mechanisms by which sporadic mutations arise. Using the techniques of microarray analysis, one pilot study has shown the feasibility of detecting half-copy gene copy in children with William's syndrome. We are presently testing "unknowns."

Array-based Genomic Mapping

J. West, A. Reiner, L. Serina [in collaboration with R. Lucito and V. Mittal, Cold Spring Harbor Laboratory, and B. Mishra, Courant Institute, New York University]

Microarrays have potential uses in genome mapping. We have designed two major algorithms and run sim-

ulations of them to guide us in experimental design. Additionally, we have conducted experiments with BAC pools and a new form of representation, called reflective representations, with which we have demonstrated the successful assignment of arrayed probes to BACs. In principle, these tools can be applied to confirmation of the assembled map of the human genome, correction of errors in assembly, and the identification of probes and BACs from gaps in the assembled genome. The sequencing of these BACs could accclerate the completion of a correctly assembled genome.

Computational Genomics

J. Healy, A. Reiner, E. Thomas [in collaboration with R. Sachidanandam, Cold Spring Harbor Laboratory, and J. Schwartz, Courant Institute, New York University]

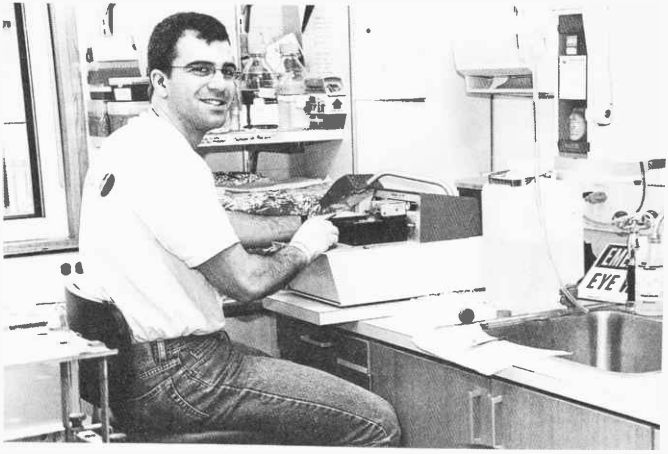
We have started a new informatics group within our lab group which is absolutely required for the tumor microarray project. However, we have begun to focus on other applications as well. One effort has been centered on probe characterization and the relation of probes to the human genome map and putative transcription units. The tools for this project have spun out other projects, including one to detect deletions/insertion alleles in the human genome, one to design a more efficient repeat masker, one to detect new repeats in the genome, and one to discover ancient segmental duplications.

PUBLICATIONS

Lucito R., West J., Reiner A., Alexander J., Esposito D., Mishra B., Powers S., Norton L., and Wigler M. 2000. Detecting gene copy number fluctuations in tumor cells by microarray analyis of genomic representations. *Genome Res.* **10:** 1726–1736.

In Press

Wen S., Stolarov J., Myers M.P., Su J.D., Wigler M.H., Tonks N.K., and Durden D.L. 2001. PTEN controls tumor-induced angiogenesis. *Proc. Natl. Acad. Sci.* (in press).



J. Trachtenberg

CELL BIOLOGY

This section comprises labs studying a diverse set of interests including signal transduction events that regulate gene expression and ultimately growth in normal and cancer cells.

- Shiv Grewal's laboratory studies the epigenetic control of gene expression.
- David Helfman's group is studying how specific actin assemblies are organized and regulated and how alterations in actin filament assembly contribute to the abnormal control of cell growth in cancer.
- Nouria Hernandez and her colleagues work on fundamental mechanisms of transcription using two model systems, the human snRNA genes and the HIV-1 LTR.
- Tatsuya Hirano's laboratory studies the molecular mechanisms that regulate higher-order chromosome dynamics in eukaryotic cells, with a focus on the roles of SMC (structural maintenance of chromosome) proteins in chromosome condensation and segregation.
- David Spector's laboratory studies the structural–functional organization of the mammalian cell nucleus, examining the nuclear organization of factors associated with pre-mRNA splicing and the RNA substrates with which these factors interact.
- Nick Tonks' laboratory takes a variety of approaches to the study of the structure, regulation, and function of the protein tyrosine phosphatase (PTP) family of signal transducing enzymes.
- Linda Van Aelst and her colleagues study the function of the Ras and Rho family of small GTPases in cell growth and development, together with the mechanisms by which they exert their effects.

EPIGENETIC CONTROL OF GENE EXPRESSION

S. Grewal J. Nakayama
K. Noma
C. Xiao

During cellular differentiation, the fate of individual cells is determined by the specification of unique patterns of gene expression. Once established, these expression patterns are faithfully maintained through multiple rounds of cell division and DNA replication to ensure proper development. It is believed that differentiated cells rely on an epigenetic cellular memory system, whereby nonmutational reversible alterations of the chromosomes promote stable transmission of determined states to progeny cells. Apart from controlling the expression of developmentally important genes, epigenetic modifications can also influence diverse processes ranging from dosage compensation to faithful transmission of chromosomes during cell division. Moreover, epigenetic changes are often suspected to be the main driving force for critical changes in gene expression and genome instability in cancer cells.

Among the diverse classes of epigenetic phenomena, some are regulated by reversible modifications of DNA, such as methylation, and some depend on post-transcriptional events, but a surprising majority seems to involve chromatin structure as an integral component of the epigenetic gene repression mechanism. Our laboratory is interested in understanding the role of chromatin and DNA replication proteins in the inheritance of epigenetic states. For this purpose, we are studying heterochromatin-mediated transcriptional silencing at the mating-type locus, centromeres, and telomeres of the fission yeast *Schizosaccharomyces pombe*. Previous studies have revealed that these domains share many regulatory features and that, together, they share many parallels with heterochromatin from higher eukaryotes. For example, silenced domains in the fission yeast and metazoan heterochromatin exert region-specific, rather than gene-specific, transcriptional repression. Second, silenced domains in yeast are associated with specific hypoacetylation of histones, which is also associated with heterochromatin in *Drosophila* and mammals. Third, similarities among the epigenetic silencing systems can be inferred from the homology of proteins involved. Interestingly, proteins affecting silencing in *S. pombe*, including Swi6 and Clr4, share homology with modifiers of position-effect variegation (PEV) in *Drosophila*, such as heterochromatin-specific protein HP-1 and *Su(var)3-9*, respectively. Likewise, fission yeast silencing factors Clr3 and Clr6 share structural and functional similarities to histone deacetylases from higher eukaryotes, including humans.

During the past year, we have made considerable progress toward understanding the mechanism of epigenetic inheritance. Our work suggests that the chromatin proteins contribute to the cellular memory, facilitating the inheritance of epigenetically determined states of gene expression; i.e., the unit of inheritance, the "gene," in some instances might comprise DNA plus the associated chromatin protein complexes. We also found that the DNA replication protein, Polα, might have a direct role in the recruitment and maintenance of chromatin proteins, such as Swi6, at the mating-type region and centromeres. These results might help us understand how chromosomal structures acting as epigenetic imprints are duplicated at the replication fork.

INHERITANCE OF EPIGENETIC STATES THROUGH CHROMATIN REPLICATION

Our previous studies have revealed that an epigenetic imprint marking the mating-type region contributes to the maintenance of a heterochromatin-like structure, which controls silencing throughout the *mat2/3* interval. In addition to the donor mating-type loci, *mat2* and *mat3*, silencing extends to an 11-kb interval between these loci called the *K*-region. Replacement of a part of the *K*-region, which shares homology with centromeric repeat sequences, with the *ura4*$^+$ marker gene (*KΔ::ura4*$^+$) results in variegation of *ura4*$^+$ expression and efficiency of mating-type switching. The Ura$^-$ efficiently switching (*ura4-off*) and the Ura$^+$ inefficiently switching (*ura4-on*) states are inherited in *cis* and controlled by an epigenetic mechanism. A significant component contributing to efficient silencing is the ability of the repressed state to promote its own reassembly; i.e., the persistence of macromolecular

protein complexes associated with the mating-type region presumably contributes to the propagation of the silenced state. Supporting this notion, we found that the Swi6 protein, a member of the highly conserved chromodomain family of proteins, is a dosage-critical component required for the establishment and maintenance of the imprint at the mating-type region. Chromatin immunoprecipitation analysis revealed that the Swi6 protein is differentially localized at the mating-type region of the *ura4-off* and *ura4-on* cells. Interestingly, *ura4-off* cells contain significantly higher levels of Swi6 at the *mat2/3* region when compared to *ura4-on* cells. Transient overexpression of Swi6 results in a heritable change from the *ura4-on* state to *ura4-off* state, and this change requires other *trans*-acting factors including the histone deacetylase, Clr3, as well as Clr4 protein. Moreover, the Swi6-induced establishment of the *ura4-off* state was tightly correlated with the increase in Swi6 levels at the *mat* locus. Once recruited, however, Swi6 was found to remain associated with the mating-type region throughout the cell cycle, presumably providing a molecular bookmark to clonally propagate a silenced chromatin state during cell division.

To further address the role of chromatin in epigenetic marking, we tested whether the differential Swi6 localization patterns at the mating-type region of *ura4-off* and *ura4-on* state cells are maintained even when both epialleles are present in the same nucleus. *ura4-on* and *ura4-off* parent strains were mated to construct a diploid. The diploid was allowed to grow for about 20 generations, sporulated, and then subjected to tetrad analysis. As shown previously, we found that *ura4-on* and *ura4-off* states are linked to the mating-type region as indicated by their cosegregation with respective alleles of *his2* (Fig.1), a marker closely linked to the *mat* locus. More importantly, chromatin immunoprecipitation (ChIP) analysis revealed that the *his2*$^-$ and *his2*$^+$ meiotic segregants, which inherited their *mat* locus from *ura4-off* and *ura4-on* parents, respectively, maintained their original Swi6 localization patterns; i.e., the mating-type region of His$^-$ segregants contains significantly higher levels of Swi6 than His$^+$ segregants (Fig. 1). This situation is analogous to X-chromosome inactivation in mammals, in which active and inactive chromosomes behave differently in the same nucleus, and strongly suggests that a self-templating chromatin structure might help promote inheritance of the silenced state at the mating-type region during both mitosis and meiosis. In other words, the "gene" at the *mat2/3* region comprises DNA plus the associated Swi6 protein complex.

ROLE OF DNA POLYMERASE α IN THE EPIGENETIC CONTROL OF TRANSCRIPTIONAL SILENCING

DNA replication may directly influence inheritance of epigenetically set chromatin complexes. In collaboration with Amar Klar's laboratory (National Cancer Institute), we showed that a mutation in *pol*α alters the epigenetic imprint and suppresses the variegation of

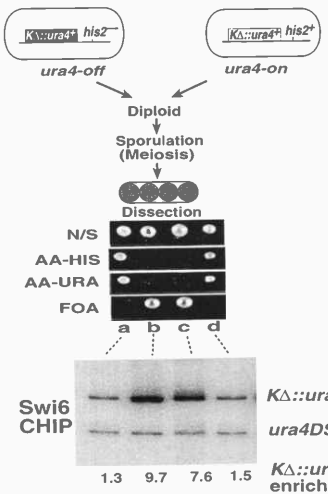


FIGURE 1 The unit of inheritance that is the "gene" at the mating-type locus comprises DNA plus the associated Swi6-containing protein complex. The *ura4-off* (*his2*$^-$) and *ura4-on* (*his2*$^+$) derivatives of *KΔ::ura4*$^+$ strains were crossed to construct a diploid. The diploid cells were sporulated and subjected to tetrad analysis. The four spores from each ascus were placed on nonselective (N/S) rich medium in a horizontal row by micromanipulation. After growth for 3 days, colonies formed by spores were replicated onto indicated medium. The medium lacking uracil (AA-URA) or histidine (AA-HIS) selects for Ura$^+$ or His$^+$ cells, respectively, whereas FOA counterselective medium selects for the growth of Ura$^-$ cells. The levels of Swi6 protein present at the *mat2/3* region of segregants were measured using the ChIP assay. The DNA recovered from anti-Swi6 immunoprecipitated chromatin fractions was analyzed using a competitive polymerase chain reaction (PCR) strategy, whereby one primer pair amplifies 694-bp and 426-bp size products from the *KΔ::ura4*$^+$ gene and the *ura4DS/E* minigene, containing a small deletion, at the endogenous location, respectively. PCR products were resolved on a polyacrylamide gel and then quantified using a phosphorimager. The relative enrichment of the *KΔ::ura4*$^-$ sequences is shown underneath each lane.

marker gene expression at the mating-type locus. Moreover, this mutation seems to globally affect silencing throughout the *mat2/3* interval, as indicated by the derepression of markers inserted at different locations in this region. In keeping with the fact that the mating-type region and centromeres in fission yeast share many features including *cis*-acting sequences and *trans*-acting factors, we found that Polα also affects centromeric silencing. ChIP analysis revealed that the *polα* mutation affects both recruitment and maintenance of Swi6 at the mating-type region; i.e., significantly lower levels of Swi6 were found at the *mat* locus of *polα* mutant cells, when compared with wild-type cells. Moreover, we found that *polα* mutant cells are defective in Swi6 localization at centromeres and telomeres. Biochemical analysis revealed that Polα binds directly to Swi6 protein in vitro. Significantly, the silencing-defective *polα* mutant displays reduced binding to Swi6 protein.

Whether the Polα requirement in silencing is coupled to its role in DNA replication remains to be explored and is the focus of our future work. It can be imagined that the silencing defects in the *polα* mutant might be through alteration in replication timing of the heterochromatic regions. In this model, Swi6 protein through its association with Polα might affect timing of DNA replication and hence epigenetic marking at centromeres and the mating-type region. Considering that Swi6 remains associated with chromatin throughout the cell cycle, its interaction with Polα might provide a mechanism for the maintenance of the stable chromosomal structures during cell division, ensuring the inheritance of a silenced state onto both chromatids.

CHARACTERIZATION OF HISTONE DEACETYLASE Clr6

Molecular analyses of three loci *clr3, clr6,* and *hda1*, which also affect silencing at the mating-type region and centromeres, have provided a link between genetically defined silencing proteins and a class of biochemically defined histone modifying enzymes known to have chromatin remodeling activity. Interestingly, factors encoded by these genes share homology with histone deacetylases. Clr6 and Hda1 share strong homology with *Saccharomyces cerevisiae* Rpd3 and human HDAC1, whereas Clr3 is similar to Hda1 from *S. cerevisiae* and HDAC4 and HDAC5 from humans. Interestingly, Clr6 acts in a partially redundant manner with Clr3, suggesting that silencing might be regulated by overlapping histone deacetylase activities. In addition to silencing, the *clr6* mutation also causes temperature as well as ultraviolet light sensitivity, suggesting a general chromatin assembly defect. Moreover, haploid segregants carrying the *clr6* deletion allele possessed a very short replicative life span of approximately eight generations.

We are now further characterizing Clr6 to understand its precise function in heterochromatin assembly and epigenetic control of gene expression. Immunofluorescence analysis revealed that Clr6 is localized to the nucleus on chromatin, forming a spotted pattern. Biochemical analysis revealed that Clr6 displays histone deacetylase activity. Moreover, a mutation in *clr6* causes overall strongly elevated acetylation levels of lysine residues at the histone H3 and H4 amino-terminal tails. This increase in acetylation of histones in mutant cells was correlated with silencing defects and increased missegregation of chromosomes. Purification of Clr6 revealed that it is part of a highly conserved mutiprotein complex. Future studies are aimed at characterizing the other members of the Clr6 protein complex and studying their effects on heterochromatin assembly at the silenced chromosomal domains.

PUBLICATIONS

Grewal S.I.S. 2000. Transcriptional silencing in fission yeast. *J. Cell. Physiol.* **184:** 311–318.

Nakayama J., Klar A.J.S., and Grewal S.I.S. 2000. A chromodomain protein, Swi6, performs imprinting functions in fission yeast during mitosis and meiosis. *Cell* **101:** 307–317.

THE CYTOSKELETON IN NORMAL AND TRANSFORMED CELLS

D.M. Helfman E. Araya S.-W. Lee
L. Connel G. Pawlak
E. Kim A. Rai

We are studying how alterations in actin filament assembly, a common characteristic of transformed cells, contribute to aberrant cell growth control and how specific actin structures are organized and regulated. The actin cytoskeleton is involved in a wide range of motile events such as cell movement, cell division, intracellular transport, phagocytosis, exocytosis, membrane ruffling, formation of microspikes and filopodia, growth cone formation, cell spreading, adhesion, and changes and maintenance of cell shape. In addition, the cytoskeleton has critical roles in the regulation of various cellular processes linked to transformation, including proliferation, contact inhibition, anchorage-independent cell growth, and programmed cell death (apoptosis). Below is a description of our studies during the past year to better understand the role of the cytoskeleton in normal and transformed cells.

Signaling Pathways Implicated in Actin Cytoskeleton Disruption during Ras Transformation

G. Pawlak

Oncogenic transformation by Ras is characterized not only by alterations in growth control, but also by pronounced morphological changes resulting from alterations in the organization of the actin cytoskeleton and adhesive interactions. Changes in the organization of actin filaments are highly correlated with both anchorage-independent growth and tumorigenicity, suggesting a fundamental role for actin filaments in cell growth control. Activation of multiple downstream signaling pathways is required to trigger the full spectrum of phenotypic traits associated with transformation by oncogenic Ras. We have used both normal and Ras-transformed rat fibroblasts (NRK and NRK/ras, respectively) to determine which of these pathways are important for disruption of the actin cytoskeleton.

NRK/ras cells have been treated with various inhibitors of signaling molecules, namely, LY294002 (an inhibitor of the phosphatidylinositol 3-kinase [PI3K] pathway), PD098059 (an inhibitor of mitogen-extracellular-regulated protein kinase kinase [MEK]) or UO126 (an inhibitor of MEK kinase). Whereas inhibition of the PI3K pathway has no effect on the cell morphology, inhibition of MEK with either compound leads to a striking restoration of actin stress fibers in a time- and dose-dependent manner (Fig. 1). This is accompanied by assembly of focal adhesions, in which structural (vinculin) and signaling molecules (FAK, paxillin) are recruited.

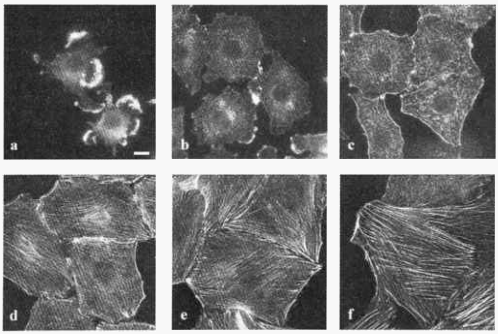


FIGURE 1 Reorganization of the actin cytoskeleton in Ras-transformed NRK cells after treatment with a MEK inhibitor. NRK/ras cells were left untreated (*a*) or treated with 50 μM PD098059 for 10 (*b*), 24 (*c*), 48 (*d*) or 72 (*e*) hr, and then actin was visualized with oregon-green-conjugated phalloidin. Untransformed NRK cells are shown as a control (*f*). Bar, 20 μm.

To determine whether activation of the Ras/mitogen-activated protein kinase (MAPK) pathway alone is sufficient to disrupt actin cytoskeleton and focal contacts, untransformed NRK cells have been transfected with the Ras effector loop mutants RasV12S35 (activates the Raf pathway), RasV12C40 (PI3K pathway), and RasV12G37 (RalGDS pathway). The RasV12S35-transfected cells exhibited a spindle-shaped morphology and grew in multiple layers. Indirect immunofluorescence analysis shows that like NRK/ras cells, RasV12S35-transfected cells exhibit disrupted actin stress fibers and focal contacts. In contrast, both RasV12C40- and RasV12G37-transfected cells retain organized stress fibers and abundant focal contacts characteristic of NRK cells.

Collectively, these studies show that a MEK-dependent pathway is involved in the disruption of the actin cytoskeleton during Ras transformation of NRK cells. The mechanism is currently under investigation, and preliminary studies implicate inactivation of Rho-kinase (ROCK), an effector molecule for the small GTPase Rho, whose activity has been shown to be critical for the formation of stress fibers.

Expression of Tropomyosin Isoforms in Normal and Transitional Cell Carcinoma of the Bladder

G. Pawlak [in collaboration with T. McGarvey and B. Malkowicz, University of Pennsylvania, Philadelphia]

Various studies have shown that specific tropomyosin (TM) isoforms are down-regulated in oncogene-transformed fibroblasts and human breast carcinoma cell lines. However, an analysis of expression of tropomyosin isoforms in patient tumor tissue is limited. We have examined the expression of the tropomyosin isoforms at the protein level in normal bladder mucosa and transitional cell carcinoma of the bladder. Protein was extracted from normal bladder mucosa ($n = 5$), and transitional cell carcinoma (a total $n = 32$, superficial) samples and Western blots were performed with antibodies specific for TM4, TM5, and TM1,2,3 isoforms. Protein levels were quantitated by densitometry. We found that normal bladder mucosa expresses the TM1 and TM2 isoforms, whereas TM3 and TM5 isoforms are not expressed. On the other hand, TM5 is expressed in 91% (29/32) and TM3 is present in 72% (23/32) of tumor specimens. TM1 protein levels were reduced in 27/32 (84%) tumors. TM1 was reduced by greater than 60% in 19/32 (59%) tumors and by 20–60% in 8/32 (25%) tumors. TM2 protein levels were reduced in 26/32 (81%) tumors. TM2 was reduced by greater than 60% in 16/32 (50%) tumors and by 20–60% in 10/32 (31%) tumors. There appeared to be no difference in tropomyosin isoform expression patterns between muscle invasive and superficial bladder tumors. In contrast, TM4 is expressed in normal human bladder mucosa and all translational cell carcinoma samples. In conclusion, we have demonstrated alterations in tropomyosin isoforms in bladder carcinoma, which may provide further insight into malignant transformation in transitional cell carcinoma.

The Cytoskeleton and Apoptosis

L. Connell

Disruption of the interaction of normal epithelial cells with the extracellular matrix (ECM) results in induction of apoptosis, a phenomenon termed "anoikis." This process is believed to have a critical role in preventing the growth of cells outside their local environment, i.e., metastasis. Conversely, inhibition of anoikis in tumor cells allows them to invade surrounding tissues. Several molecules and signaling pathways induced by interaction of cells with the ECM have been implicated in the regulation of anoikis, among them focal adhesion kinase (FAK), PI3K, Akt, nuclear factor (NF)-κB, and MAP kinases. Activation of FAK is a critical component as it prevents anoikis. The downstream effectors remain to be established, but recent studies indicate that FAK can suppress p53-mediated programmed cell death. In addition, p53 serves to monitor survival signals from ECM/FAK, since apoptosis is suppressed by dominant negative p53. In addition to FAK, a second component of focal adhesions, namely, CAS, has been implicated in regulation of apoptosis. The CAS proteins (p130Cas, HEF1/Cas-L and Efs/Sin) are a family of docking proteins that contain multiple interaction domains, and they are important components of integrin receptor signaling. Thus, cell-matrix interactions have an essential role in preventing apoptosis in normal cells, and this property is abrogated in transformed cells. It

still remains to be determined how these pathways interact to regulate cell growth and survival and what role cytoskeletal elements have in their function. The generation of signals induced by the interaction of cells with the ECM requires the maintenance of an intact cytoskeleton. We are studying the signaling pathways involving cell attachment to the ECM and the actin cytoskeleton, and the role of the cytoskeleton in the regulation of apoptosis in normal and transformed cells.

Function of Caldesmon in the Regulation of Actomyosin Contractility and Oncogenic Transformation in Nonmuscle Cells

S.-W. Lee

Regulation of actomyosin contractility has been shown to be critical in the control of focal-adhesion-dependent cell growth. Caldesmon is a cytoskeletal protein containing actin-, myosin-, and tropomyosin-binding domains. Caldesmon is thought to inhibit actomyosin ATPase activity by blocking interaction between actin and myosin. Recent studies have provided important information about the role of caldesmon in contractility and transformation. First, we showed for the first time that overexpression of caldesmon in fibroblast cells inhibits cell contractility, and thereby interferes with contractility-dependent formation of focal adhesions (Helfman et al., *Mol. Biol. Cell. 10:* 3097 [1999]). Second, we found that MAP kinase (ERK) targets nonmuscle caldesmon in vivo when cellular contractility is stimulated by an external growth factor such as lysophosphatidic acid (LPA). Nonmuscle caldesmon has also been found to be hyperphosphorylated in Ha-RasV12-transformed mouse fibroblast cells (NIH-3T3) in which the ERK pathway is constitutively active. We hypothesize that transformation-sensitive changes including hyperphosphorylation of caldesmon by ERK might contribute to aberrant growth control in Ras-transformed cells by inhibiting the ability of caldesmon to negatively regulate actomyosin contractility. Consequently, this results in constitutive activation of downstream signaling pathways dependent on actomyosin contractility. Work is in progress to determine the physiological role of caldesmon phosphorylation in the control of contractility-dependent growth.

Characterization of the Metastasis-associated Protein, S100A4

E. Kim

Distinct changes occur to propel a tumor from benign to malignant. Increased motility, invasiveness, and survival in foreign environments are some of the changes that confer a metastatic phenotype. One potentially key protein involved in generating these changes is S100A4, a member of the S100 family of small calcium-binding proteins. Although the proteins in this family share relatively high homology, their expression, distribution, protein partners, and putative roles are quite diverse. Elevation of A4 expression 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 A4 and three cytoskeletal proteins: actin, tropomyosin, and myosin. We have confirmed a calcium-dependent interaction between A4 and the heavy chain of nonmuscle myosin in vivo, and experiments are currently in progress to identify additional protein partners. Future experiments will utilize mutational analysis to further characterize these interactions.

Molecular Organization and Regulation of Actin Filaments

E. Araya [in collaboration with C. Berthier, University of Lyon, France]

In nonmuscle cells, the formation of actin filaments and their assembly into various structures, e.g., stress fibers, contractile ring, filopodia, 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 been studying the dynamic localization of tropomyosin isoforms using green fluorescent protein (GFP)-tagged constructs. Tropomyosins are a family of actin-binding proteins that bind to both grooves of filamentous actin. Although they are expressed in all eukaryotic cells, different forms are characteristic of

specific cell types. In fibroblasts, tropomyosins exist as high-molecular-weight isoforms (HMW) containing 284 amino acids (TM1, TM2, and TM3) or low-molecular-weight isoforms (LMW) containing 248 amino acids (TM4, TM5[NM-1], TM5a, and TM5b). The multiple tropomyosins found in fibroblasts are an integral part of the actin filaments, although how the different isoforms contribute to actin filament assembly and function is poorly understood. In addition, in fibroblasts, HMW tropomyosins exist as homodimers, whereas LMW tropomyosins can exist as heterodimers. How these coiled-coiled interactions are regulated and how they effect the interaction of tropomyosins with actin filaments and their subsequent cellular functions are under study. We have found that specific tropomyosin isoforms are involved in different subsets of actin filaments. For example, we have found that during cytokinesis, only a subset of tropomyosin isoforms is found in the contractile ring. The mechanism(s) involved in isoform-specific sorting is currently under study. We anticipate that elucidating the molecular bases for the differential localization of the specific tropomyosin isoforms during various cellular events involving reorganization of the actin-cytoskeleton will provide important new insights into the regulation of distinct actin structures.

Snapin, a Ubiquitously Expressed Protein Involved in Intracellular Vesicular Transport, Is Phosphorylated in a Cell-cycle-dependent Manner

A. Rai

Snapin was first identified as a brain-specific component of the SNARE complex involved in calcium-sensitive regulation of synaptic vesicle fusion (Ilardi et al., *Nat. Neurosci. 2:* 119 [1999]). We have found that human snapin is encoded by a single-copy gene which, in contrast to previous reports, is expressed ubiquitously in a wide variety of tissues and cell types. Furthermore, snapin homologs are found in *Caenorhabditis elegans, Drosophila melanogaster*, zebrafish, and rodents. Consistent with its proposed function in secretion, snapin was found to be localized to a cytoplasmic vesicular compartment with a strong juxtanuclear concentration. Overexpression of an epitope-tagged full-length snapin results in a tubulation phenotype, consistent with perturbation of membrane traffic through the secretory pathway, whereas expression of truncated forms of snapin suggests that different domains have distinct roles in its transport through the secretory pathway. Snapin was found to be phosphorylated on Ser-133, which lies within a Cdc2 kinase consensus site. Phospho-snapin is enriched during the G_2/M phase of the cell cycle, when Cdc2 kinase–cyclin B activity is high, suggesting that snapin is a Cdc2 substrate. In vitro kinase assays using recombinant proteins demonstrate that Cdc2–cyclin B can phosphorylate GST-snapin in vitro. Collectively, these data demonstrate that snapin functions in a wide variety of metazoan organisms, having a role in both neuronal and nonneuronal membrane fusion processes, and is phosphorylated in a cell-cycle-dependent manner during the G_2/M transition on Ser-133.

PUBLICATIONS

Sung L.A., Gao K.M., Yee L.J., Temm-Grove C.J., Helfman D.M., Lin J.J., and Mehrpouryan M. 2000. Tropomyosin™ isoform 5b is expressed in human erythrocytes: Implications of tropomodulin-TM5 or tropomodulin-TM5b complexes in the protofilament and hexagonal organization of membrane skeletons. *Blood* **95:** 1473–1480.

In Press

Pawlak G. and Helfman D.M. 2001. Cytoskeletal changes in cell transformation and tumorigenesis. *Curr. Opin. Genet. Dev.* **11:** 41–47.

INITIATION AND TERMINATION OF TRANSCRIPTION IN HUMAN snRNA GENES AND HIV-1

N. Hernandez F. Emran, P. Hu, B. Ma, P.S. Pendergrast, A. Saxena, L.M. Schramm, S. Sepehri, M. Shanmugam, Y. Sun, X. Zhao, A. Zia

We use the human small nuclear RNA (snRNA) genes and the human immunodeficiency virus type-1 (HIV-1) long terminal repeat to study fundamental mechanisms of transcription. The human snRNA genes form a family of genes, of which some members are transcribed by RNA polymerase II and others are transcribed by RNA polymerase III. The promoters of the RNA polymerase II and III snRNA genes are, however, very similar in structure. In both cases, the enhancer region (called the distal sequence element or DSE) contains an octamer sequence that recruits the transcription factor Oct-1, and the core region contains a proximal sequence element (PSE). In the RNA polymerase II snRNA promoters, the PSE is sufficient to recruit, in vitro, RNA polymerase II and direct basal transcription. In the RNA polymerase III snRNA promoters, the core region contains a second element, a TATA box, which functions in concert with the PSE to recruit RNA polymerase III and direct basal transcription.

We use this model system to study how transcription complexes are assembled on core promoters and then recruit the correct RNA polymerase, and how enhancers mediate activation of transcription. In the last year, we have concentrated on the characterization of novel transcription factors required for RNA polymerase III transcription of snRNA genes. As described in more detail below, this has resulted in the identification of two new members of the RNA polymerase III initiation complexes formed on snRNA promoters. We have also pursued our studies on the characterization of the snRNA activating protein complex ($SNAP_c$), which binds to the PSE of both the RNA polymerase II and III snRNA promoters, and completed a map of the subunit-subunit interactions within the complex.

The HIV-1 promoter gives rise, in a regulated manner, to two types of transcripts: short prematurely terminated transcripts and full-length transcripts. We previously characterized FBI-1, a protein that binds to an element located downstream from the HIV-1 transcription start site called the inducer of short transcripts or IST. IST is a DNA element that specifically activates the formation of short transcripts. We have continued our characterization of the function of this protein.

Factors Required for snRNA Transcription by RNA Polymerase III

L. Schramm, P.S. Pendergrast, Y. Sun, N. Hernandez

The U6 snRNA promoter is recognized by RNA polymerase III, and its core region contains a PSE and a TATA box. We have known for a long time that the PSE recruits $SNAP_c$ and the TATA box recruits the TATA-box-binding protein (TBP). Typical gene-internal RNA polymerase III promoters, such as the promoters of the tRNA gene or the 5S RNA gene, also depend on TBP for transcription, but, in this case, TBP is recruited as part of a complex called TFIIIB. The TFIIIB complex in yeast is completely defined and consists of two subunits besides TBP: the TFIIB-related factor BRF and a polypeptide called the B´´ polypeptide.

Like yeast TFIIIB, mammalian TFIIIB contains TBP and a homolog of the yeast BRF, human BRF (hBRF), which associates strongly with TBP. However, it was unclear whether a homolog of yeast B´´ existed. Furthermore, we had shown that hBRF, although required for transcription of RNA polymerase III genes with gene-internal promoters, was not required for transcription of the human U6 gene. Thus, two main questions remained concerning mammalian TFIIIB. First, it was unclear whether a mammalian homolog of yeast B´´ existed and whether it was required for RNA polymerase III transcription of both genes with internal promoters, such as the VAI

gene, and genes with external promoters that recruit $SNAP_c$, such as the human U6 gene. Second, the apparent lack of requirement for hBRF in U6 transcription raised the possibility that the mammalian snRNA-type RNA polymerase III promoters use a factor different from, but related to, hBRF.

We searched the human and mouse EST databases and identified a mouse EST encoding a short polypeptide with strong similarity to part of the yeast B″ protein. The similarity mapped to a region of yeast B″ that had been shown to be important for function. We used the mouse EST to design oligonucleotides and screen human cDNA libraries by polymerase chain reaction (PCR). Through a combination of PCR screening and database searches, we were able to isolate a cDNA encoding a full-length protein that is a functional homolog of yeast B″. Human B″ is required for RNA polymerase III transcription from both gene-internal and U6-type RNA polymerase III promoters. The protein shows similarity to the yeast protein over an internal region and diverges from it at both the amino and carboxyl termini. The carboxy-terminal region of human B″ is striking because it consists of a series of repeats that are completely absent from the yeast protein and that contain a number of potential phosphorylation sites, reminiscent of the carboxy-terminal domain of the largest subunit of RNA polymerase II. This region is not required for transcription in vitro, suggesting that it may serve a regulatory role.

We also searched the database for proteins related to hBRF that might be involved in U6 transcription. The search identified TFIIB as well as another protein with no known function. This other protein, which we call BRFU, is equally similar to both TFIIB and BRF. Very strikingly, when a transcription extract is depleted of both BRF and BRFU with antibodies that recognize both proteins, transcription from gene-internal RNA polymerase III promoters is restored by addition of BRF but not by addition of BRFU. Conversely, transcription from the U6 gene is restored by addition of BRFU but not by addition of BRF. Thus, BRFU is specifically required for RNA polymerase III transcription from U6-type promoters.

Together, these results show that there are different forms of the basal RNA polymerase III transcription factor IIIB in mammalian cells. They also identify the first transcription factors uniquely required for transcription of RNA polymerase III, but not RNA polymerase II, snRNA promoters. Together with our previous results indicating that TFIIB is required for transcription of the human RNA polymerase II snRNA genes, they suggest that the key event in the determination of RNA polymerase specificity in the human snRNA promoters is the recruitment of hBRFU versus TFIIB.

Protein-Protein Interactions among $SNAP_c$ Subunits

B. Ma, N. Hernandez

$SNAP_c$ is a complex containing five different types of subunits. From previous experiments, we knew that

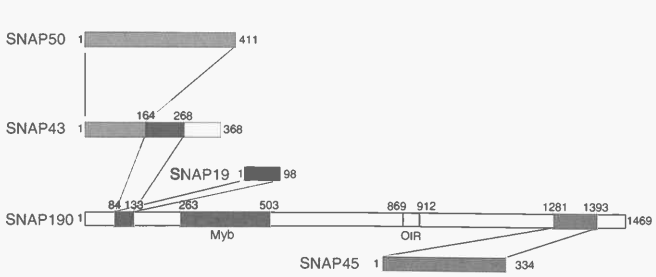


FIGURE 1 Subunit-subunit interactions within $SNAP_c$. The various subunits are symbolized by boxes, and the regions sufficient for association with other subunits are indicated by thin lines. The numbers above the boxes indicate amino acid numbering. The Myb and Oct-1 interacting domains (OIR) in SNAP190 are also indicated.

SNAP19 and SNAP45 can each associate with SNAP190 and that SNAP43 can associate with SNAP50. SNAP43 can also associate with SNAP190, but in coimmunoprecipitations of in-vitro-translated proteins, this association is not detectable unless SNAP19 is present. This suggests that SNAP43 has weak contacts with both SNAP190 and SNAP19, only the sum of which is measurable by the stringent coimmunoprecipitation assay.

To map more precisely the protein-protein contacts within $SNAP_c$, we generated truncated or mutated versions of the various $SNAP_c$ subunits by translation in vitro, mixed them in various combinations, and determined their ability to coimmunoprecipitate with each other. In this way, we were able to generate the map shown in Figure 1. Within SNAP190, amino acids 84–133 were sufficient for association with SNAP19 alone and with SNAP43 together with SNAP19. This SNAP190 region and the amino-terminal part of SNAP19 are likely to form α helices and may be involved in a coiled-coil type of interaction with each other. Indeed, SNAP19 contains five leucines spaced by six amino acids, and mutations of these leucines had a strong negative effect, whereas mutation of a leucine out of register had little effect. Similarly, SNAP190 contains six leucines and glutamines which are separated by six amino acids and are therefore predicted to reside on the same face of an α-helix. Mutations that change subsets of these amino acids debilitated association with SNAP19 and SNAP43 together with SNAP19, whereas a double mutation that changed two amino acids predicted to reside on another face of the helix, one of which is a leucine, had a much weaker negative effect on these associations. Interestingly, changing in register glutamines to leucines (mutations Q94L and Q115L) was as debilitating as changing them to alanines. Thus, although this SNAP190 region is likely to form an α-helix involved in a coiled-coil type of interaction, it does not correspond to a classical leucine zipper. In SNAP43, amino acids 164–268 were sufficient for association with SNAP190 and SNAP19, and amino acids 1–163 were sufficient for association with SNAP50. Thus, these two association domains in SNAP43 are completely separable.

Carboxy-terminal to the SNAP19/SNAP43 association domain, SNAP190 contains an unusual Myb domain extending from amino acid 263 to 503, with four and one half Myb repeats designated the Rh (for R half), Ra, Rb, Rc, and Rd repeats. The last two Myb repeats (Rc and Rd), but not the first two and one half (Rh, Ra, and Rb), are required for binding to the PSE. Within the carboxy-terminal half of SNAP190 are two additional regions involved in protein-protein contacts: the Oct-1 interaction domain (OIR) lies between amino acids 869 and 912 and the region required for interaction with SNAP45 lies between amino acids 1281 and 1393. Thus, the SNAP190 regions required for association with SNAP19/SNAP43 and with SNAP45 lie at opposite ends of the linear molecule.

We then tested whether the assumption that protein-protein contacts between isolated subunits reflected protein-protein contacts within $SNAP_c$ was likely to be true by assembling partial SNAP complexes from truncated subunits containing little more than the regions mapped as required for subunit-subunit interactions. Such complexes could indeed be assembled and shown to bind specifically to the PSE. Together, these results provide a detailed map of a basal transcription complex.

Mechanism of $SNAP_c$ Auto-down-regulation of DNA binding

A. Saxena, N. Hernandez

$SNAP_c$ binds relatively poorly to DNA, but a subcomplex of $SNAP_c$ called mini-$SNAP_c$ and consisting of the amino-terminal third of SNAP190, SNAP43, and SNAP50 binds very efficiently to the PSE. The presence or absence of SNAP19 has no effect on DNA binding, suggesting that it is the carboxy-terminal two thirds of SNAP190 and/or SNAP45 that are responsible for the down-regulation of DNA binding. We are studying the mechanism involved.

Identification of $SNAP_c$-associated Factors

F. Emran, N. Hernandez

Some of the transcription factors required for RNA polymerase II and III snRNA gene transcription are likely to associate with $SNAP_c$. We are developing a yeast screen as well as cell lines to identify such $SNAP_c$-associated factors.

Characterization of a Human RNA Polymerase III Holoenzyme

S. Sepehri, P. Hu, N. Hernandez

We are purifying an RNA polymerase III complex that, together with TBP and a $SNAP_c$ fraction, is sufficient for U6 transcription. The goal is to determine the composition of this complex and thus identify new factors required for U6 transcription.

Transcription from Chromatin Templates

X. Zhao, N. Hernandez

The goal of this project is to study the transcription of snRNA genes wrapped into chromatin templates and to determine the role played by cooperative interactions between the Oct-1 POU domain and $SNAP_c$, and $SNAP_c$ and TBP, for transcription from such templates.

Protein Transduction

M. Shanmugam, N. Hernandez

Proteins fused to a small peptide derived from the HIV-1 Tat protein have been shown by other investigators to have the capacity to enter cells. We are testing the ability of various proteins fused to the Tat peptide to enter cells and elicit a biological response.

Function of FBI-1

P.S. Pendergrast, P. Stavropoulos, N. Hernandez

Through a combination of transfection and co-immunoprecipitation experiments, we are deciphering the function of FBI-1 in HIV-1 transcription and localizing it in the cell.

PUBLICATIONS

Schramm L., Pendergrast P.S., Sun Y., and Hernandez N. 2000. Different human TFIIIB activities direct RNA polymerase III transcription from TATA-containing and TATA-less promoters. *Genes Dev.* **14:** 2650–2663.

In Press

Ma B. and Hernandez N. 2001. A map of protein-protein contacts within the snRNA activating protein complex $SNAP_c$. *J. Biol. Chem.* **276:** 5027–5035.

Sepehri Chong S., Hu P., and Hernandez N. 2001. Reconstitution of transcription from the human U6 snRNA promoter with eight recombinant polypeptides and a partially purified RNA polymerase III complex. *J. Biol. Chem.* (in press).

Zhao X., Pendergrast P.S., and Hernandez N. 2001. A positioned nucleosome on the human U6 promoter allows recruitment of $SNAP_c$ by the Oct-1 POU domain. *Mol. Cell* (in press).

HIGHER-ORDER CHROMOSOME DYNAMICS

T. Hirano O. Cuvier A. Losada
M. Hirano D. MacCallum
K. Kimura

Our laboratory is interested in understanding the molecular mechanisms that regulate higher-order chromosome dynamics in eukaryotic cells. Our previous efforts, primarily using a cell-free extract derived from *Xenopus laevis* eggs, have identified two protein complexes, 13S condensin and 14S cohesin, that have central roles in chromosome condensation and sister chromatid cohesion, respectively. Each of the complexes has a different pair of structural maintenance of chromosome (SMC) subunits, implicating a mechanistic connection between condensation and cohesion. The long-term goal in our laboratory is to understand how the two eukaryotic SMC protein complexes work at a mechanistic level and how they interact with other proteins to execute their essential functions in the cell. We also use a bacterial SMC homodimer as a model system to dissect the basic mechanisms of SMC actions.

Identification of Two Different Cohesin Complexes in *Xenopus* and Human Cells

A. Losada, T. Yokochi, T. Hirano [in collaboration with R. Kobayashi, Cold Spring Harbor Laboratory]

We showed previously that the cohesin complex is required for the establishment and maintenance of sister chromatid cohesion in *Xenopus* egg extracts. During the past year, we have found that two distinct cohesin complexes exist in the extracts. A 14S complex (termed cohesin^{SA1}) contains XSMC1, XSMC3, XRAD21, and a newly identified subunit, XSA1. In a second 12.5S complex (cohesin^{SA2}), XSMC1, XSMC3, and XRAD21 associate with a different subunit, XSA2. Both XSA1 and XSA2 belong to the SA family of mammalian proteins and exhibit similarity to the yeast cohesin subunit Scc3p. In *Xenopus* egg extracts, cohesin^{SA1} is predominant, whereas cohesin^{SA2} constitutes only a very minor population. We also found that human cells have a similar pair of cohesin complexes, but the SA2 type is the dominant form in somatic tissue culture cells.

Cell Cycle Regulation of Sister Chromatid Cohesion

A. Losada, T. Hirano

Immunolocalization experiments suggest that the association of cohesin^{SA1} and cohesin^{SA2} with interphase chromatin may be differentially regulated at the level of nuclear transport in the early embryonic cell cycle. In somatic cells, however, the two cohesin complexes exhibit no major differences in their kinetics of chromatin association and dissociation. Most cohesins (up to 95%) dissociate from chromatin at the onset of mitosis, both in vitro (in the cell-free extracts) and in vivo (in tissue culture cells). A very small population of cohesin (~5%), however, can be found on the metaphase chromosomes by immunostaining. This population is likely to have a role in holding the sister chromatids until the onset of anaphase. These results suggest that the linkage between sister chromatids is resolved by a two-step mechanism in vertebrate cells. The first step takes place during prophase when tight cohesion between interphase chromatids is partially released, allowing each chromatid to condense. The second step occurs at the metaphase-anaphase transition and results in complete separation of the sister chromatids. This is in striking contrast to the regulation of cohesion in *Saccharomyces cerevisiae*. In this organism, most of cohesin stays bound to chromatin until the metaphase-anaphase transition when their dissociation, triggered by cleavage of the Scc1/ RAD21 subunit, leads to a single-step dissolution of cohesion.

We have investigated the mechanisms that drive the prophase dissociation of cohesins in vertebrate cells. At least in the cell-free extracts, none of the cohesin subunits are cleaved upon entry into mitosis. Instead, release of cohesins from chromatin correlates with phosphorylation of XSA1. Purified Cdc2-cyclin B can phosphorylate XSA1 in vitro and reduce the ability of x-cohesin^{SA1} to bind to DNA and chromatin. Evidence from other laboratories indicates that the RAD21 subunit of vertebrate cohesin is cleaved coincidentally at the onset of anaphase in human cells. We therefore propose that two different mechanisms regu-

late cohesin's dissociation from chromatin at two different stages of mitosis in vertebrates. The major release, which takes place at prophase, is likely to involve phosphorylation of the SA subunit. The final release of the minor mitotic population is probably promoted by separin-mediated cleavage of Scc1/RAD21, as has been shown in *S. cerevisiae*.

Structural and Functional Dissection of *Xenopus* Condensin

K. Kimura, T. Hirano

Condensin reconfigures DNA structure in an ATP-dependent manner in vitro and has a central role in mitotic chromosome condensation in *Xenopus* egg-cell-free extracts. The *Xenopus* 13S condensin complex (13SC) is composed of two subcomplexes: an 8S core subcomplex (8SC) consisting of two SMC subunits (XCAP-C and -E) and an 11S regulatory subcomplex (11SR) containing three non-SMC subunits (XCAP-D2, -G, and -H). During the past year, we have performed a biochemical and functional dissection of this chromosome condensation machinery. Although both 8SC and 13SC can bind to DNA in vitro and contain the SMC ATPase subunits, only 13SC is active as a DNA-stimulated ATPase and supports ATP-dependent supercoiling activity. In the cell-free extracts, 13SC is the active form that binds to chromosomes and induces their condensation. Neither 11SR nor 8SC alone is able to bind to chromatin. Our results suggest that the non-SMC subunits have dual roles in the regulation of condensin functions: One is to activate SMC ATPases and the other is to allow the holocomplex to associate with chromatin in a mitosis-specific manner.

Identification of HEAT Repeats in Condensin Subunits

T. Hirano [in collaboration with A. Neuwald, Cold Spring Harbor Laboratory]

HEAT repeats are highly degenerated repeating motifs that have been found in a wide variety of proteins with diverse functions. They form tandemly arranged bihelical structures that may serve as flexible scaffolding on which other proteins can assemble. In collaboration with Andy Neuwald here at the Laboratory, we have found that two of the non-SMC subunits, XCAP-D2 and XCAP-G, share HEAT repeats. The former and the latter have at least 12 and 9 repeats, respectively, which distribute along the entire length of the polypeptides. Intriguingly, we also found that two other proteins genetically implicated in sister chromatid cohesion (Scc2/Mis4 and BimD/Pds5) have HEAT repeats. Although neither of the cohesion factors are stoichiometric components of cohesin, they may work closely with the complex in establishing sister chromatid cohesion. These results further emphasize the structural similarity between the condensation and cohesion machinery. The HEAT proteins could function as regulators of SMC ATPases or could have an architectural role in SMC functions.

Human Condensin Complex

K. Kimura, O. Cuvier, T. Hirano

A long-term goal in our lab is to understand how the condensin complex works at a mechanistic level. To determine the functional contribution of individual subunits and of structural motifs conserved in each subunit, we wish to reconstitute a whole condensin complex from recombinant polypeptides. For a number of technical reasons, we decided to use human cDNAs and their protein products as starting materials for such reconstitution. Since the existence of the condensin complex in mammalian cells had not yet been fully established, it was important to demonstrate first that human cells have a complex that is equivalent to *Xenopus* 13S condensin. Using a peptide antibody raised against one of the putative subunits of human condensin, we affinity-purified a 13S complex from HeLa cell nuclear extracts. We found that the human complex has exactly the same size and subunit composition as its *Xenopus* counterpart, being composed of two SMC (hCAP-C and hCAP-E) and three non-SMC (hCAP-D2, hCAP-G, and hCAP-H) subunits. Human condensin purified from asynchronous HeLa cell cultures fails to reconfigure DNA structure in vitro. When phosphorylated by purified Cdc2-cyclin B, however, it gains the ability to introduce positive supercoils into DNA in the presence of ATP and topoisomerase I. Strikingly, human condensin can induce chromosome condensation when added back into a *Xenopus* egg extract that has been immunodepleted of endogenous condensin. Thus, the structure and function of the condensin complex are highly conserved between *Xenopus* and humans, fully justifying our plan to reconstitute the condensin complex starting from human cDNAs.

Chromatin Remodeling Complexes in *Xenopus* Egg Extracts

D. MacCallum, T. Hirano [in collaboration with R. Kobayashi, Cold Spring Harbor Laboratory]

We previously characterized major protein components of chromosomes assembled in *Xenopus* egg extracts and collectively referred to them as *Xenopus* chromosome-associated polypeptides (XCAPs). They included five subunits of the condensin complex (XCAP-C, -E, -D2, -G, and -H) and topoisomerase II α (XCAP-B), all of which have been shown to be essential for chromosome condensation. In an effort to identify novel proteins required for chromosome condensation, we have isolated XCAP-F, a 135-kD polypeptide, and identified it as the *Xenopus* homolog of the *Drosophila* ISWI protein (termed XISWI). ISWI is known to act as the core ATPase subunit of different chromatin remodeling complexes, such as NURF, CHRAC, and ACF in *Drosophila*.

We have cloned a cDNA encoding XISWI and raised antibodies against the carboxy-terminal peptide. Immunoprecipitation experiments show that XISWI exists in two different heterodimeric complexes. In the first complex, XISWI associates with a 190-kD polypeptide that is homologous to *Drosophila* ACF1. The second one is a novel complex that contains the *Xenopus* homolog of the human Williams syndrome transcription factor (WSTF), a gene deleted in the developmental disorder Williams syndrome. We have cloned cDNAs of these partner proteins (termed XACF1 and XWSTF) and raised antibodies against them. We have shown by immunodepletion that XISWI is not required for bulk assembly of nucleosomes on sperm chromatin or naked DNA templates, but it is required for regular spacing of the nucleosomes. This regular nucleosome spacing is not apparently necessary for chromosome condensation because immunodepletion of XISWI from the extracts has little effect on their ability to condense chromosomes. We have also analyzed the effect of immunodepletion on other chromatin-related activities, including DNA replication and transcription, and found no essential role for these XISWI-containing complexes. Thus, our initial expectations of a role for XISWI in higher-order chromatin structure may not be the case. Nevertheless, XISWI is clearly required for regular spacing of nucleosomes, and it is likely that a lack of nucleosome spacing has important consequences on chromatin function. One possibility is that each of the two XISWI-containing complexes may possess its unique function and have antagonistic roles in chromatin remodeling. To test this hypothesis, we are currently studying the specific role of each complex.

Bimodal Regulation of BsSMC ATPase

M. Hirano, T. Hirano

SMC proteins are conserved not only among eukaryotes, but also in bacterial and archaeal species. We use a bacterial SMC homodimer from *Bacillus subtilis* (BsSMC) as a model system for understanding the basic mechanisms of SMC actions. A previous electron microscopy study showed that BsSMC is composed of two antiparallel coiled-coil arms, each having an ATP-binding domain in its distal end. Although this structural model suggests an intriguing model of the action of SMC proteins as ATP-modulated DNA cross-linkers, no direct biochemical evidence for this model has been available. During the past year, we have constructed a number of BsSMC mutant derivatives including "single-armed" BsSMC and have provided the first set of biochemical evidence to support the antiparallel dimer model. We have shown further that the two-armed structure connected by a flexible hinge has a crucial role in modulating intra- and intermolecular interactions of BsSMC and is responsible for bimodal regulation of its ATPase cycle. This novel mode of ATPase modulation is likely to provide fundamental implications for our understanding of the actions of eukaryotic SMC protein complexes.

PUBLICATIONS

Hirano T. 2000. Chromosome cohesion, condensation and separation. *Annu. Rev. Biochem.* **69:** 115–144.

Kimura K. and Hirano T. 2000. Dual roles of the 11S regulatory subcomplex in condensin functions. *Proc. Natl. Acad. Sci.* **97:** 11972–11977.

Losada A. and Hirano T. 2000. New light on sticky sisters. *Curr. Biol.* **10:** R615.

Losada A., Yokochi T., Kobayashi R., and Hirano T. 2000. Identification and characterization of SA/Scc3p subunits in the *Xenopus* and human cohesin complexes. *J. Cell Biol.* **150:** 405–416.

Neuwald A.F. and Hirano T. 2000. HEAT repeats associated with condensins, cohesins, and other complexes involved in chromosome-related functions. *Genome Res.* **10:** 1445–1452.

In Press

Kimura K., Cuvier O., and Hirano T. 2001. Chromosome condensation by a human condensin complex in *Xenopus* egg extracts. *J. Biol. Chem.* (in press).

Losada A. and Hirano T. 2001. Intermolecular DNA interactions stimulated by the cohesin complex in vitro: Implications for sister chromatid cohesion. *Curr. Biol.* (in press).

CELL BIOLOGY OF THE NUCLEUS

D.L. Spector S. Janicki T. Howard
P. Sacco-Bubulya J. Herbst
N. Saitoh

Studies in our laboratory are focused on the structural-functional organization of the mammalian cell nucleus. Our research program evolves around understanding the nuclear organization of factors associated with pre-mRNA splicing and the RNA substrates with which these factors interact. The microscopy core facility has been used extensively during the past year, and numerous collaborations were pursued with the excellent technical expertise of Tamara Howard.

Organization of Interchromatin Granule Clusters

P. Sacco-Bubulya

Pre-mRNA splicing factors are localized in a speckled pattern 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, the position of nuclear speckles within the nucleus is maintained for many hours. We would like to understand how interchromatin granule clusters (IGCs) are organized within the nucleus and elucidate their relationship to specific aspects of gene expression. Many splicing factors contain an RS domain that when hyperphosphorylated results in the release of these factors from IGCs and may be a crucial step for recruitment to sites of pre-mRNA synthesis. The release of SR proteins from IGCs is regulated by SR protein kinases. Overexpression of SR protein kinases in cultured cells alters the organization of nuclear speckles and can ultimately cause complete redistribution of SR proteins from their typical speckled nuclear localization to a diffuse nuclear localization.

We are using one of these SR protein kinases, *c*dc2-*l*ike *k*inase (Clk/STY), as a tool to disassemble nuclear speckles in vivo to test the hypothesis that IGCs may be held together by a specific underlying framework and to assess the function of IGCs in living cells. We speculated that a structural protein would maintain its position upon SR protein hyperphosphorylation, whereas RNA processing factors would be dispersed. Alternatively, if a specific framework is not present, SR proteins would simply be released and we would see no underlying structure.

Overexpression of wild-type Clk/STY in A-431 cells causes a dramatic reorganization of the SR protein SC35 from its typical nuclear speckled localization to a diffuse nuclear localization (Fig. 1A,B). All nuclear speckle constituents tested, including SR proteins, small nuclear ribonucleoprotein (snRNP) proteins, stable poly(A)$^+$ RNA, and other novel IGC constituents, behaved identically. Redistribution of nuclear speckle constituents to a diffuse nuclear localization upon hyperphosphorylation of SR proteins is therefore a general response. However, overexpression of a kinase-dead mutant Clk/STY (Fig. 1C,D) does not result in a reorganization of splicing factors.

At the ultrastructural level, the IGCs of untransfected nuclei appear as large clusters of electron-dense granules that contain SR proteins when labeled using immunogold-conjugated antibodies. In cells that overexpress wild-type Clk/STY, there are no intact IGCs, and immunogold labeling for SR proteins is distributed evenly throughout the nucleus. Furthermore, upon overexpression of Clk/STY, we did not detect any underlying filaments or specific regions in the transfected cell nuclei that may serve as a scaffold to specifically cluster interchromatin granules. On the

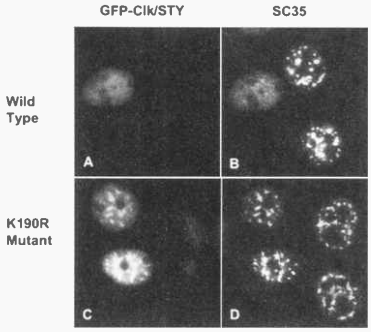


FIGURE 1 A-431 cells transiently transfected with wild-type GFP-Clk/STY (*A* and *B*) or GFP-Clk/STY K190R (*C* and *D*) were fixed in 2% formaldehyde and processed for fluorescence microscopy using anti-SC35 antibody followed by Texas Red-coupled secondary antibodies. Whereas catalytically dead mutant Clk/STY K190R colocalizes with SC35 in nuclear speckles (compare *C* and *D*), overexpression of wild-type Clk/STY induces a reorganization of SC35 from its typical nuclear speckle localization to a diffuse nuclear localization (*B*).

basis of these data, we conclude that individual interchromatin granules may be held within the cluster through RS-RS interactions that are disrupted upon hyperphosphorylation of SR proteins. We are currently investigating the importance of the integrity of IGCs for supplying factors involved in RNA metabolism to active genes. For example, splicing factors may arrive at transcription sites in preassembled complexes that are completely functional only if they originate from IGCs. Interestingly, transcription is not affected in cells that have disrupted IGCs for up to 16 hours. Studies are currently under way to evaluate how IGC disassembly affects splicing activity in vivo.

Proteome Analysis of Interchromatin Granule Clusters: Identification of IGC Components by Mass Spectrometry

N. Saitoh [in collaboration with S. Patterson and C. Spahr, Amgen, Inc., and A. Neuwald, Cold Spring Harbor Laboratory]

To address the molecular mechanism of the assembly and maintenance of IGCs in mammalian cell nuclei and the dynamic properties of IGCs, especially the process of recruitment of splicing factors to the site of active transcription, it is crucial to identify the protein complement of this nuclear compartment. We have purified approximately 1 mg of IGCs from mouse liver nuclei and applied the final fraction to proteome analysis using liquid chromatography and tandem mass spectrometry (LC-MS/MS) combined with automated database searching. Ionization patterns were compared to protein sequences in databases by automated searches. As a result, 2214 peptide sequences were obtained. From these sequences, we have identified 138 proteins that are known to be localized in the IGCs, or whose functions are associated with the IGCs. As expected, numerous splicing factors as well as 5′ and 3′ pre-mRNA processing factors, some transcription factors, and several subunits of RNA polymerase II were identified. Most interestingly, 22 new proteins (sequences have been deposited in databases, but no biological characterization has been reported) and 25 EST clones have been identified. To obtain insight into the function of these proteins, each of the identified proteins were analyzed for known sequence motifs (Fig. 2). Many of them have RNA-binding/recognition motifs, suggesting a possibility that they are novel RNA processing factors located in IGCs. In addition, the presence of clones containing motifs that are thought to have chaperone-like functions (AAA ATPase and cyclophilin domains) suggests a possibility that these are good candidate proteins responsible for assembly of the IGCs or individual IGC particles.

Currently, investigations are under way to directly compare IGC protein composition from normal mouse liver cells versus cells that are treated with α-amanitin. In the drug-treated cells, transcription by RNA polymerase II is turned off, and the morphology of the IGCs is dramatically changed as these structures are reorganized into fewer (4–6) and larger rounded-up structures as compared to the large number (20–30) of irregularly shaped clusters observed in actively transcribing cells. We have begun to use a recently developed technology to perform quantitative proteome analysis using a class of chemical reagents called isotope-coded affinity tags (ICATs) in combination with tandem mass spectrometry. We have prepared identical amounts of IGC proteins from both the α-amanitin-treated cells and nontreated cells. Preliminary evaluation by immunoblot analysis of these fractions has shown that a hyperphosphorylated form of RNA polymerase II in IGCs is decreased

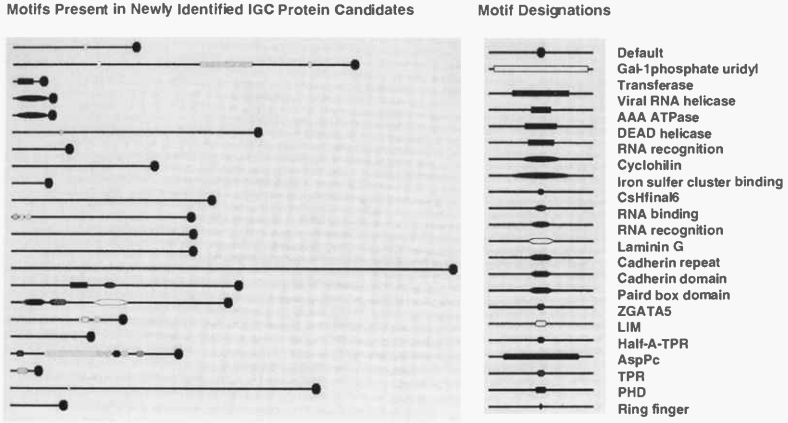


FIGURE 2 Domains present in newly identified IGC proteins.

upon treatment with α-amanitin, although the amount of the splicing factor SF2/ASF is unchanged upon treatment with α-amanitin. We are currently surveying changes in all of the IGC proteins by quantitative proteome analysis. Using this approach, we anticipate identifying important proteins having a role in transcriptionally active IGCs, or in the formation and maintenance of IGCs.

Role of SCAF10 in IGC Organization and in Coordinating the Recruitment of the Transcription and Pre-mRNA Processing Machinery

S. Janicki

The role that IGCs have in organizing the large number of proteins required for gene expression is being investigated by studying the function of SCAF10, an IGC component that may link transcription and pre-RNA processing. SCAF10 localizes to both nuclear speckles and the nucleoplasm and was identified in yeast two-hybrid screens for proteins that interact with the CTD of RNA polymerase II (Bourquin et al., *Nucleic Acids Res. 52:* 2055 [1997]) and Clk/STY kinase (Nestel et al., *Gene 180:* 151 [1996]). SCAF10 is a 106-kD protein with an amino-terminal cyclophilin domain and a carboxy-terminal serine/arginine-rich RS domain. Cyclophilins have been reported to act as chaperones in protein trafficking and macromolecular assembly, and they contain peptidyl-prolyl *cis-trans* isomerase (PPIase) domains which catalyze *cis-trans* isomerizations about Xaa-Pro peptide bonds. The peptide folding activity of this protein may regulate interactions between RNA processing factors and the CTD or have a role in the assembly and disassembly of the macromolecular complexes involved in splicing.

SCAF10 dynamics are being studied in a cell line (clone 2), developed in our laboratory, in which a regulatable genetic locus can be visualized in living cells (Tsukamoto et al. 2000). BHK cells were selected for the integration of a construct composed of a tetracycline-responsive promoter which drives the expression of an intron-containing cyan fluorescent protein (CFP) gene preceded by a series of *lac* operator repeats.

When a yellow fluorescent protein (YFP) Lac repressor fusion protein is expressed in these cells, the genetic locus can be visualized directly in living cells. When pTet-On is expressed and doxycycline

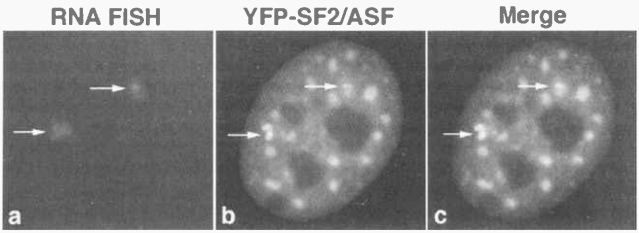


FIGURE 3 YFP-SF2/ASF is recruited to the active transcription site in clone 2 cells (*a–c*) and colocalizes with the transcribed RNA as detected by RNA fluorescence in situ hybridization (RNA FISH). When the locus is actively transcribing, RNA FISH or YFP-*lac* repressor can be used to localize the transcription site. The presence of two transcription sites in this cell is due to polypoidy.

added to the culture medium, transcription is initiated and the CFP protein which is fused to a peroxisomal targeting signal can be visualized in peroxisomes within the cytoplasm. We have been able to demonstrate recruitment of the splicing factor SF2/ASF to the genetic locus in living cells (Fig. 3), and we are presently investigating the dynamics of SCAF10 in this system. Additionally, polyclonal antibodies to the carboxyl terminus and monoclonal antibodies to the amino terminus of SCAF10 have been generated, and immunoprecipitation combined with mass spectrometry will be used to identify SCAF10 interacting factors. The combination of live-cell microscopy and biochemical studies will advance our understanding of how the processes involved in gene expression are coordinated within the structural framework of the nucleus.

PUBLICATIONS

Eils R., Gerlich D., Tvaruskó W., Spector D.L., and Misteli T. 2000. Quantitative imaging of pre-mRNA splicing factors in living cells. *Mol. Biol. Cell* **11:** 413–418.

Lorenz P., Misteli T., Baker B.F., Bennett C.F., and Spector D.L. 2000. Nucleocytoplasmic shuttling: A novel *in vivo* property of antisense phosphorothioate oligodeoxynucleotides. *Nucleic Acids Res.* **28:** 582–592.

Mintz P.J. and Spector D.L. 2000. Compartmentalization of RNA processing factors within nuclear speckles. *J. Struct. Biol.* **129:** 241–251.

Tsukamoto T., Hashiguchi N., Janicki S.M., Tumbar T., Belmont A.S., and Spector D.L. 2000. Visualization of gene activity in living cells. *Nat. Cell Biol.* **2:** 871–878.

PROTEIN TYROSINE PHOSPHATASES AND THE CONTROL OF CELLULAR SIGNALING RESPONSES

N.K. Tonks J.N. Andersen, H.H. Chen, R.L. Del Vecchio, T. Fukada, T.C. Meng, M.P. Myers, H.L. Palka, K.A. Pennino, Y. Shen, L. Zhang

The phosphorylation of tyrosyl residues in proteins is a key component of the regulation of signaling pathways that control many fundamental physiological processes including cell proliferation and differentiation. Protein phosphorylation is a reversible, dynamic process in which the net level of phosphate observed in a target substrate reflects not only the activity of the kinases that phosphorylate it, but also the competing action of 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 cytosolic species and represent a major family of signaling enzymes. The structures of the PTPs indicate important roles in the control of key cellular functions. We are utilizing a variety of strategies to characterize the normal physiological function of several members of the PTP family. Disruption of normal patterns of tyrosine phosphorylation has been implicated as a contributor to several human diseases. Therefore, insights into the mechanisms involved in modulating PTP function may ultimately yield important information to help counter such diseases.

During the last year, we were joined by Jannik Andersen, a visiting graduate student who is completing the academic component of his Industrial Ph.D. Educational Program in our lab, sponsored by the Danish Academy of Technical Sciences.

PTP1B AND THE REGULATION OF SIGNAL TRANSDUCTION IN HUMAN DISEASE

Targeted disruption of the *PTP1B* gene was reported to yield apparently normal mice that displayed enhanced sensitivity to insulin and resistance to weight gain when fed a high-fat diet (Elchelby et al., *Science 283:* 1544 [1999]; Klaman et al., *Mol. Cell. Biol. 20:* 5479 [2000]). These effects were reported to coincide with changes in the phosphorylation status of the insulin receptor (IR), suggesting that the IR may be a physiological substrate of PTP1B. We have explored this possibility further by characterizing the recognition of synthetic peptides modeled on the sequence of the activation loop of the IR as substrates by PTP1B. These studies were performed in collaboration with David Barford (Institute of Cancer Research, Charles Beatty Labs, London, U.K.). Our approach, involving crystallographic, kinetic, and binding analyses, has revealed at the molecular level the mechanism underlying the specificity of the interaction between PTP1B and the IR. Furthermore, many elements of the PTP1B-IR interaction are unique to PTP1B, suggesting that it may be possible to generate specific, small molecule inhibitors of this interaction. Such inhibitors would offer a novel strategy for therapeutic intervention in diabetes and obesity.

The activation loop of the IR contains three sites of autophosphorylation, pTyr-1158, pTyr-1162, and pTyr-1163. In the inactive state of the IR, the activation loop impedes access to the catalytic center, with pTyr-1162 occupying the position of the tyrosyl side chain of a potential substrate. Following hormone binding and autophosphorylation, the activation loop moves out of the active site and is then accessible for dephosphorylation. When the activity of PTP1B toward synthetic peptides modeled on the activation loop of the IR was examined, it was found that the phosphatase displays the lowest K_m for peptides in which both Tyr-1162 and Tyr-1163 are phosphorylated. Furthermore, PTP1B substrate-trapping mutants preferentially recognize forms of the peptide that are doubly phosphorylated on these adjacent sites. Crystal structures of complexes between the PTP1B C>A mutant and phosphorylated forms of the IR activation loop peptide reveal how selectivity is achieved. The pTyr-1162 residue binds to the active site of PTP1B in a manner identical to that which we described previously for the pTyr residue in the PTP1B-EGF receptor peptide complex. Specificity for pTyr-1162, the critical autophosphorylation site for regulation of the activity of the IR, is determined by extensive and specific interactions between residues on the surface of PTP1B and on both the amino- and carboxy-terminal

sides of this phosphorylation site in the IR activation loop. Most striking is the fact that the adjacent phosphotyrosyl residue, pTyr-1163, is located within a shallow groove on the surface of PTP1B, which is connected to the catalytic site by a channel. The interactions of pTyr-1163 in this "second pocket" are dominated by salt bridges between its phosphate group and the side chains of Arg-24 and Arg-254 in PTP1B. The positioning of these arginyl residues also confers specificity for pTyr to the pocket. Interestingly, among PTP superfamily members, Arg-24 is unique to PTP1B and TC-PTP. Moreover, access to the second pocket is possible because of the presence of a glycine at position 259 in PTP1B. Nearly all other PTPs have a bulky hydrophobic residue at the cognate position.

In addition to the tandem pTyr residues, there are further interactions between PTP1B and the IR activation loop. The guanidinium side chain of Arg-47 of PTP1B, a residue that is poorly conserved within the PTP family, hydrogen-bonds to the carboxylate group of Asp-1161 on the amino-terminal side (P-1) of the pTyr-1162 substrate site. Similar interactions were noted previously with other substrates and explain the preference of PTP1B for acidic residues at the P-1 and P-2 positions. The side chain of Arg-47 is flexible and can accommodate various combinations of acidic residues amino-terminal to the substrate pTyr site. There are further interactions between PTP1B and residues carboxy-terminal to the phosphorylation site. In particular, the guanidinium side chain of Arg-1164 at the P+2 position in the substrate forms a classical π-cation interaction with Phe-182 in the closed conformation of the WPD loop, highlighting the concerted nature of the PTP-substrate interaction.

These observations provide definition at the molecular level of the recognition of substrate and determination of specificity for PTP1B. Interestingly, the third pTyr residue in the IR activation loop, pTyr-1158, is not recognized by PTP1B in any of the structures determined to date. Presumably, other PTPs dephosphorylate this site. The critical role of the Asp/Glu-pTyr-pTyr-Arg/Lys motif for optimal substrate recognition by PTP1B may suggest additional potential physiological substrates. Several interesting candidates contain this motif, including the receptor PTKs Trk, FGFR, and Axl. This motif also is found in the JAK family of PTKs, which have critical roles in transmitting signals from cytokine receptors. This raises the intriguing possibility that the effects of PTP1B on obesity may be mediated via effects on JAK2, which is associated with the receptor for the satiety hormone leptin. We are currently investigating this possibility.

Changes in the levels of PTP1B have been noted in several human diseases, particularly those associated with disruption of normal patterns of tyrosine phosphorylation. Previous work in our lab showed that the expression of PTP1B is induced specifically by the p210 bcr-abl oncoprotein, a PTK that is directly responsible for the initial manifestations of chronic myelogenous leukemia (CML). We have also observed that PTP1B, but not TC-PTP, its closest relative, suppresses p210 bcr-abl-mediated signaling and transformation. Therefore, PTP1B may function as an antagonist of the p210 bcr-abl oncoprotein PTK in vivo. We have observed that the effects of p210 bcr-abl on expression of the *PTP1B* gene are manifested at the transcriptional level. To define the mechanism by which *PTP1B* gene expression is regulated, we cloned the 5′ flanking region of the human gene. We found two elements that are important for expression from the human *PTP1B* promoter. A sequence motif that possesses features of a site of interaction with GATA-binding proteins was identified at −167 to −151 bp from the transcription start site. Disruption of this site inhibited promoter activity in the presence of p210 bcr-abl, but responsiveness to the oncoprotein PTK was maintained. However, a p210 bcr-abl-stimulated element, termed PSE, which was important for stimulation of activity in response to the PTK, was identified at −49 to −37 bp from the transcription start site. The PSE is contained in a sequence that displays features of a stress response element (STRE), a feature originally identified in *Saccharomyces cerevisiae* and which functions as a binding site for C_2H_2 zinc finger proteins. In our study, we have shown that three mammalian C_2H_2 zinc finger proteins, Egr-1, Sp1, and Sp3, bind to PSE. Of these, both Sp1 and Sp3 function as positive regulators, whereas Egr-1 represses Sp3-mediated *trans*-activation of the *PTP1B* gene. Furthermore, expression of p210 bcr-abl results in down-regulation of the levels of Egr-1. Our data illustrate that the reciprocal actions of the Sp1/Sp3 and Egr-1 C_2H_2 zinc finger transcription factors are an important aspect of the regulation of PTP1B expression in response to the p210 bcr-abl oncoprotein.

CHARACTERIZATION OF DUAL SPECIFICITY PHOSPHATASES

By searching the human expressed sequence tag (EST) database for sequences that have homology with the signature motif of members of the PTP family, 11 novel *d*ual *s*pecificity *p*hosphatases (DSPs) have been identified and cloned. These are currently being

expressed and characterized in a collaborative project with Ralf Luche and Bo Wei at CEPTYR Inc. (Bothell, Washington). A variety of properties, including structural features, expression pattern, and chromosomal localization, are being used to prioritize these DSPs for future analysis.

Among these novel enzymes is JSP-3, which comprises 184 residues and is expressed broadly. In light of the fact that members of the DSP family have been implicated in the regulation of the MAP kinase family of signal transducing enzymes, including previous work from our own lab on the characterization of the DSP MKP-1, we tested whether JSP-3 affected the activation of MAP kinases. The result was striking. Whereas DSPs, such as MKP-1, have been implicated previously in down-regulating the activity of MAP kinases, we found that co-overexpression of JSP-3 with either the JNK, p38, or Erk-1 MAP kinases led to specific up-regulation of JNK and did not effect the activity of p38 or Erk-1 (hence the name *J*NK *s*timulatory *p*hosphatase-1). The inactive C-S mutant form of JSP-3 did not activate JNK, suggesting that this effect required the enzymatic activity of the phosphatase. In addition, our data indicate that JSP-3 induced activation of MKK4, the MAP kinase kinase immediately upstream of JNK in the signaling pathway. Furthermore, we have preliminary data to suggest that JSP-3 may be required for full activation of JNK in response to Rho family GTPases and stresses such as anisomycin, heat shock, and sorbitol. Currently, we are trying to identify the critical substrates of JSP-3 that mediate these effects.

FURTHER STUDIES OF THE PTEN TUMOR SUPPRESSOR

PI3-kinase produces phosphoinositide second messengers, phosphorylated at the D3 position of the inositol sugar ring, that regulate many of the pathways involved in tumor progression, including cell cycle and apoptosis. In previous years, in collaboration with Dr. Mike Wigler's lab here at the Laboratory, we have shown that PTEN antagonizes the actions of PI3 kinase by dephosphorylating the D3 position in inositol phospholipids. We have generated a series of glioblastoma cell lines in which PTEN expression was reconstituted, and we saw no effects on cell cycle or apoptosis. However, PTEN reconstituted cell lines did not grow as tumors when injected into immunodeficient mice, suggesting that another pathway important for tumor progression has been altered by PTEN expression. In collaboration with Dr. Don Durden and his colleagues at the Indiana School of Medicine in Indianapolis, we have found that reconstitution of PTEN inhibits the ability of these cells to induce the formation of new blood vessels, effectively limiting tumor volume. Significantly, the clinical prognosis of glioblastoma is strongly affected by the ability of these tumors to induce the production of new blood vessels, suggesting that intervention in this pathway will be important for therapeutic intervention in the disease.

SUBSTRATE-TRAPPING MUTANT PTPs–STYX

We have developed a method by which we can examine PTP substrate specificity in a cellular context, by the production of mutant PTPs that maintain a high affinity for substrate but do not catalyze dephosphorylation effectively. We refer to these mutants as "substrate traps." Following expression, the mutant PTP binds to its physiological substrates in the cell, but, because it is unable to dephosphorylate the target efficiently, the mutant and substrate form a complex. Upon isolation of this complex, the substrates can be identified, yielding important insights into the function of the PTP. Progress has been made in defining the substrate specificity of several members of the PTP family using this approach.

The PTP superfamily is characterized by the presence of a conserved signature motif [I/V]HCX-AGXXR[S/T]G, containing many residues that are required for catalysis. Interestingly, proteins have been identified that look similar to the PTPs, but bear alterations in the signature motif that would preclude catalytic activity. The first example of such a "dead" phosphatase was characterized by Jack Dixon and his colleagues (Ann Arbor, Michigan) and termed STYX. In STYX, the nucleophilic cysteine from the signature motif is replaced by glycine. Site-directed mutagenesis of STYX to produce a cysteine residue in the signature motif created a catalytically competent enzyme. This led to the hypothesis that STYX may represent a novel phosphotyrosine-binding protein with the potential to function on a naturally occurring "substrate trap." Now, additional STYX-like proteins have been identified, including some that have been implicated in human disease. We have identified two potential STYX proteins from database searches. One of these, termed STYX2, shows significant homology with MKP1 and may have a role in regulating the MAP kinase pathway. Importantly, we have found that changing two residues restores phosphatase activity to STYX2. Restoration of activity also changes the pattern of coimmunoprecipitating proteins, which may indicate that these proteins

are STYX2 "substrates." We are currently trying to identify these proteins and verify their interaction with STYX2 so as to characterize the physiological function of this interesting protein.

PROTEOMIC STRATEGIES FOR THE ANALYSIS OF PTP FUNCTION

The presence of the signature motif both confers unique properties to the PTP family of enzymes and facilitates their identification within the sequence of the human genome. In this postgenomic era, we have embarked on a proteomics-based approach to defining the complement of PTPs/DSPs from various cellular sources or subcellular locations. Such analyses not only will provide insights into function, but also will allow us to examine changes in the levels of PTP proteins in various pathological conditions. The strategy involves the use of nonspecific PTP/DSP inhibitors, targeted to the active site, as affinity reagents to purify the complement of PTPs present in a particular sample. This is combined with mass spectrometric sequence analyses to identify the PTPs present in the sample. We hope that this technology can be adapted further to the identification of PTPs based on their substrate specificity and to explore the selectivity of various PTP inhibitors, particularly in the context of their development as therapeutics.

A striking feature of the cysteine residue at the active site of members of the PTP family is its unusually low pK_{α}. Although this enhances the nucleophilic properties of the cysteine residue, it also renders the PTPs susceptible to oxidation with concomitant inhibition of activity. A variety of studies, particularly from the laboratory of Sue Goo Rhee, have illustrated that certain PTPs are susceptible to inactivation through oxidation of this invariant cysteine. We have examined further the potential role of such a modification as a mechanism for the control of PTP function in vivo. We have shown that various PTPs could be oxidized quickly by treatment of Rat-1 cells with H_2O_2. Those PTPs were visualized by a modified "in-gel" PTP activity assay. Our results demonstrated that the oxidation of PTPs in the cell was a reversible process and that the reduction/reactivation of PTPs was dependent in part on the level of intracellular glutathione. Our data also suggested that the oxidation/inactivation of PTPs was a key step in the activation of tyrosine phosphorylation-dependent signaling in response to H_2O_2. We examined further whether the receptor PTK-induced production of reactive oxygen species (ROS) leads to oxidation of PTPs. By utilizing the modified "in-gel" PTP activity assay, we showed that platelet-derived growth factor (PDGF) induced a rapid and selective oxidation of one major PTP of 70 kD. Immunodepletion experiments identified PTP as the SH2 domain-containing phosphatase, SHP-2. The transient oxidation of SHP-2 was concomitant with autophosphorylation of the PDGF receptor (PDGFR). Furthermore, when PDGF-induced oxidation of SHP-2 was inhibited by an antioxidant, *N*-acetyl cysteine, the ligand-induced tyrosine phosphorylation of PDGFR and the activation of MAP kinases were suppressed. Therefore, our data suggest that the PDGFR-induced production of ROS leads to a burst of PTK activity, augmented by a temporary inactivation of PTPs, that promotes a signaling response. We propose that this situation may apply to the regulation of tyrosine-phosphorylation-dependent signaling in general and are currently developing methods for the identification of PTPs that become transiently inactivated through oxidation in response to various mitogenic signals.

PUBLICATIONS

Lavoie C., Chevet E., Roy L., Tonks N.K., Fazel A., Posner B.I., Paiement J., and Bergeron J.J.M. 2000. Tyrosine phosphorylation of p97 regulates transitional endoplasmic reticulum assembly *in vitro*. *Proc. Natl. Acad. Sci.* **97:** 13637–13642.

Salmeen A., Andersen J.N., Myers M.P., Tonks N.K., and Barford D. 2000. Molecular basis for the dephosphorylation of the activation segment of the insulin receptor by protein tyrosine phosphatase 1B. *Mol. Cell* **6:** 1401–1412.

In Press

Cohen J., Klingmuller U., Tonks N.K., and Neumann D. 2001. The erythropoietin receptor is Tyr-phosphorylated when coexpressed with a "substrate trapping" mutant of protein tyrosine phosphatase 1B. In *Structure and function of interacting protein domains in signal and energy transduction* (ed. L.M.G. Heilmayer). NATO/FEBS Advanced Study. (In press.)

Tonks N.K. and Neel B.G. 2001. Combinatorial control of the specificity of protein tyrosine phosphatases. *Curr. Opin. Cell Biol.* (in press).

Wen S., Stolarov J., Myers M.P., Su J.D., Wigler M.H., Tonks N.K., and Durden D.L. 2001. PTEN controls tumor-induced angiogenesis. *Proc. Natl. Acad. Sci.* (in press).

Yart A., Laffargue M., Mayeux P., Chrétien Peres C., Tonks N.K., Roche S., Payrastre B., Chap H., and Raynal P. 2001. A critical role for phosphoinositide 3-kinase upstream of Gab1 and SHP2 in the activation of Ras and mitogen-activated protein kinases by epidermal growth factor. *J. Biol. Chem.* (in press).

SIGNAL TRANSDUCTION

L. Van Aelst B. Boettner, E.-E. Govek, M. McDonough, A. Schmitz, M. Zhao, R. Packer, Y. Qin

Research in our laboratory is focused on the role of the Ras and Rho family members of small GTPases in signal transduction and the mechanisms by which they exert their effects. These proteins are signal transducing GTPases that cycle between GDP-bound inactive states and GTP-bound active states, relaying signals from various membrane receptors to the nucleus to mediate cellular activities such as cell growth control and morphogenesis. The ratio of the two states 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. In particular, our research efforts during the past year have been focused on the following topics: (1) further analysis of a protein, AF-6, which we had identified as a novel Ras-binding protein; (2) 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; (3) identification and characterization of Rac effectors mediating Rac's effects on the cytoskeleton and invasion; and (4) assessment of the role of the Rho GTPases and their regulators on neuronal development.

RAS GTPases

Members of the Ras GTPases include the Ha-Ras, Ki-Ras, N-Ras, and Rap proteins. The importance of Ha-Ras, Ki-Ras, and N-Ras genes in the etiology of human cancers is made evident by the frequent findings of activating mutations in these *ras* genes in a wide variety of cancers. In addition to deregulated proliferation, these cancer cells, and oncogenic Ras-transformed cell lines, are characterized by changes in morphology and cell-cell adhesion. Previous studies have shown that oncogenic Ras proteins signal through the well-established Raf/MEK/MAPK, RalGDS, and PI3-kinase pathways and that all of these pathways participate in the cell-transforming activity of oncogenic Ras. In addition to the above effector molecules, we had identified AF-6 as a Ras-binding protein and found that AF-6 accumulates at cell/cell contact sites. More recently, we showed that AF-6 binds with even higher affinity to the Rap1 GTPase. Although the Rap GTPase, which contains a virtually identical effector loop region as the above Ras proteins, was originally identified in a screen for revertants of the morphology exerted by Ki-Ras-transformed cells, more recent studies support fundamental differences between Ras- and Rap1-controlled signaling pathways. The functions of Rap proteins seem to be primarily related to morphological and differentiative events, rather than to those that govern proliferation and cell-fate specification, phenomena that often require signaling via conventional Ras proteins. Thus, we have initiated studies to define whether AF-6 is a bona fide effector of Ras and/or Rap GTPases in specific cellular events.

FUNCTIONAL CHARACTERIZATION OF THE AF-6/CANOE PROTEIN

The *AF-6* gene was originally found as a fusion partner of the *ALL-1* gene in a subset of acute lymphoblastic leukemias caused by chromosomal (t6;11) translocation events; hence, the name *A*LL-1 *f*used gene on chromosome *6*. Subsequent database analysis led to the prediction of an array of motifs, such as two amino-terminal Ras-binding domains (RBDs), U104 and DIL motifs that were initially described in microtubule and actin-based motor proteins, respectively, and a PDZ domain followed by an extended carboxy-terminal tail interspersed with proline-enriched patches. Various subcellular localization experiments performed in polarized epithelial cells and tissue sections of intestinal epithelia suggest its distinct residency in cell-cell junctional complexes. We observed that in MDCK and MCF7 cells, AF-6 colocalizes with zona

occludens (ZO-1), an integral component of tight junctional complexes. Furthermore, we isolated profilin as an AF-6-associated protein. Profilin has a critical role in actin polymerization events, and hence, the AF-6/profilin interaction might provide a dynamic link between junctional complexes and the actin cytoskeleton.

To examine whether Ras and/or Rap GTPases use AF-6 as a bona fide effector in specific cellular events, we turned to *Drosophila* as a model system (in collaboration with Ulrike Gaul at The Rockefeller University, New York). A *Drosophila* homolog of AF-6, known as Canoe, was first identified by virtue of its severe rough eye phenotype, and it has genetically been placed in the Notch signaling pathway, a pathway that determines various cell fates in a multitude of developmental processes. Furthermore, Canoe is involved in a process called dorsal closure, the dorsalward movement of the lateral and ventral epidermis to enclose the embryo. We first confirmed that Canoe interacts with the *Drosophila* homologs of Ras and Rap1 (DRas1 and DRap1) and then investigated the effects of the *Drosophila* GTPase on dorsal closure. Through the use of dominant negative and active mutant forms of *Drosophila* Ras and Rap1 GTPases, we established that DRap1, but not DRas1, has a crucial role in dorsal closure. This phenotypic similarity between DRap1 and Canoe strongly supports the idea that Canoe is a true effector of DRap1. To further manifest this, we are presently performing genetic epistasis analysis between DRap1 and Canoe genes.

FUNCTIONAL CHARACTERIZATION OF P62^{DOK}

p62^{dok} has been identified as a substrate of many oncogenic tyrosine kinases such as the CML chimeric p210$^{bcr\text{-}abl}$ oncoprotein, as well as of hematopoietic and nonhematopoietic growth factor receptors. Upon phosphorylation, p62^{dok} associates with p120 Ras GTPase-activating protein (Ras GAP), and it is by virtue of its ability to bind Ras GAP that p62^{dok} was isolated. The protein contains a PH and a PTB domain at its amino terminus, and it harbors 15 tyrosines, 9 proline-rich domains, and numerous serine/threonine residues at its carboxy-terminal tail. To address the role of p62^{dok}, we initiated a collaboration with P.P. Pandolfi and colleagues (Memorial Sloan-Kettering Cancer Center, New York), who generated a null mutation in the *dok* gene. We obtained evidence that p62^{dok} acts as a negative regulator of growth-factor-induced cell proliferation, since p62^{dok}-deficient cells possess a higher proliferation rate in response to growth factors. Furthermore, p62^{dok} inactivation causes a significant shortening of the latency of the fatal myeloproliferative disease induced by retrovirus-mediated transduction of p210$^{bcr\text{-}abl}$ in bone marrow cells. In addition, we obtained evidence that p62^{dok} exerts its negative effect on growth-factor-induced cell proliferation, at least in part, by negatively influencing the Ras/MAPK pathway. We further demonstrated that recruitment of p62^{dok} to the membrane is essential for its function as a negative regulator of the platelet-derived growth factor receptor (PDGFR)/MAPK pathway. Moreover, we found that the PDGF-triggered translocation of p62^{dok} to the plasma membrane involves the activation of phosphatidylinositol-3 kinase (PI3-kinase) and binding of its PH domain to 3′-phosphorylated phosphoinositides.

RHO GTPASES

Members of the Rho family, namely, RhoA, Rac, and Cdc42, are best known as regulators of the actin cytoskeleton. It is well established that alterations in the actin cytoskeleton triggered by the Rho proteins contribute to their effects observed on adhesion, invasion, transformation, and, more recently, neuronal development. In addition to their role in controlling the actin cytoskeleton, the Rho GTPases are also known to regulate gene expression, which contributes to their effects on the above activities. To date, most of the identified Rho GTPases' effector molecules are proteins mediating their effects on actin cytoskeleton organization. Among them, we had identified POR1 as a Rac1-interacting protein. To identify genes whose transcription is regulated by Rac, we made use of representational difference analysis (cDNA-RDA), in combination with the microarray technique to identify genes whose expression is altered as a consequence of activated Rac expression. With regard to the role of the Rho GTPases on neuronal development, it is interesting to note that of the eight genes thus far identified to be involved in nonspecific X-linked mental retardation (MRX), three of them are regulators or effectors of the Rho GTPases. These regulators and effectors are oligophrenin-1, which is a putative Rho GAP, PAK3, which is a downstream serine/threonine target of Rac and Cdc42, and a novel exchange factor for Rac, called PIX. Of particular interest to our lab is

how a lack of oligophrenin-1, a potential Rho GAP and regulator of GTPases, results in a phenotype of mental retardation on a molecular and morphological level.

POR1, A RAC-INTERACTING PROTEIN INVOLVED IN ACTIN REMODELING

In a search for Rac-interacting proteins, we had identified POR1 in a yeast two-hybrid screen and demonstrated a role for POR1 in cytoskeletal organization. Further studies revealed that POR1 also interacts with ARF6, a GTPase shown to regulate endocytic traffic at the cell periphery. In addition to its role in membrane trafficking, ARF6 also mediates cytoskeletal organization. We obtained data showing that POR1 is localized at the perinuclear region and is translocated toward the plasma membrane in response to various growth factors known to activate Rac, as well as in the presence of activated Rac. The above observations hint at a potential role for POR1 in membrane trafficking. In light of this, in collaboration with C. D'Souza-Schorey (Notre Dame, Indiana) we found that the Gq-coupled agonist, bombesin, triggers the redistribution of vesicle-associated ARF6 and Rac1 to the plasma membrane, resulting in peripheral actin rearrangements. This bombesin-induced translocation of vesicle-associated ARF6 and Rac1 to the cell surface is regulated by ARF6 activation. These findings support a role for vesicle transport in cortical actin remodeling at the cell periphery.

IDENTIFICATION OF RAC TARGET GENES USING cDNA-RDA

To identify specific genes whose expression is regulated by Rac, we performed cDNA-RDA. In this technique, one cDNA population (called the driver) is hybridized in excess against a second population (the tester) to remove common hybridizing sequences, thereby enriching target sequences unique to the tester. To confirm the differential expression of the isolated clones obtained after two rounds of polymerase chain reaction (PCR), we made use of microarray technology (in collaboration with R. Lucito and M. Wigler here at the Laboratory). The PCR-amplified inserts were printed on a glass slide and then driver and tester cDNAs, each labeled with a different fluorophore, were hybridized simultaneously to this chip. The fluorescence intensities of each of the two labels were quantified for each clone and as such we were able to discern which clones were up-regulated or down-regulated as a consequence of RacV12 expression. At present, 100 clones have been sequenced, resulting in 36 independent gene fragments, of which 14 are novel. We found a total of 22 apparently down-regulated and 14 apparently up-regulated candidate genes. We have been taking two approaches to prioritize genes for further analysis. (1) We started to explore the effects of various drugs known to activate or to interfere with previously characterized downstream signaling pathways of Rac on the transcription of these genes using microarray. Numerous growth factors, including PDGF, epidermal growth factor (EGF), and hepatocyte growth factor (HGF) are known to stimulate Rac's activity, and activated Rac has been shown to activate the JNK, p38, and ERK MAPK pathways. (2) To assure that the differences we observed when comparing two representations of cDNAs reflect true differences in mRNA abundance, we performed Northern blot analysis for approximately 20 clones. These analyses confirmed the differential expression observed in the cDNA-RDA and microarray experiments.

ROLE OF RHO GTPases AND THEIR REGULATORS IN NEURONAL DEVELOPMENT

Recent studies have demonstrated that Rho GTPases are important at different stages of neuronal development, including neuroblast migration, axon pathfinding, and growth cone morphology and function. There is also in vivo evidence that perturbing the activity of the Rho GTPases causes abnormal dendritic arbor development. In collaboration with Z. Li and H. Cline here at the Laboratory, we investigated whether Rho GTPases regulate branch dynamics and branch extensions in optic tectal neurons in live *Xenopus* tadpoles. These studies indicated that the three members of the Rho GTPases have distinct effects on dendritic arbor development, namely, Rac and Cdc42 regulate branch additions and retractions, whereas RhoA regulates the elongation of existing branches. The importance of the fine-tuned regulation of the neuronal dendritic arbor is emphasized by reports suggesting that children with mental retardation have reduced dendritic arbors. We

have initiated studies on the functional characterization of oligophrenin-1. Oligophrenin-1 contains a domain which is typical of Rho-GTPase-activating proteins (Rho GAP), and this oligophrenin-1 GAP domain acts as a GAP for Rac, Rho, and Cdc42 in vitro, thus down-regulating the activity of these molecules. Oligophrenin-1 also contains a conserved amino-terminal domain of unknown function, a PH domain, and a unique carboxy-terminal domain. We recently performed in situ hybridization experiments which indicate that oligophrenin-1 is expressed in all tissues of the developing mouse brain, including the hippocampus. The hippocampus is the part of the cerebral cortex involved in the formation of new declarative memories. We initiated experiments to determine what effect oligophrenin-1 has on cellular morphology in developing neurons by doing live-cell imaging of biolistically transfected pyramidal cells in hippocampal slices over time, using confocal and two-photon microscopy. We have already established the effects of the Rho GTPases on dendritic length in pyramidal cells. Furthermore, we performed a yeast two-hybrid screen using oligophrenin-1 as bait to identify interacting proteins. This screen resulted in the identification of two potentially interesting oligophrenin-binding proteins. We believe that a detailed analysis of oligophrenin-1 will contribute to a better understanding of neuronal plasticity and how modulation of molecules involved in Rho signaling may result in MRX.

PUBLICATIONS

Boettner B., Govek E.E., Cross J., and Van Aelst L. 2000. The junctional multidomain protein AF-6 is a binding partner of the Rap1A GTPase and associates with the actin cytoskeletal regulator profilin. *Proc. Natl. Acad. Sci.* **97:** 9064–9069.

Boshans R.L., Szanto S., Van Aelst L., and D'Souza-Schorey C. 2000. ADP-ribosylation factor 6 regulates actin cytoskeleton remodeling in coordination with Rac1 and RhoA. *Mol. Cell. Biol.* **20:** 3685–3694.

Chen R.A., Michaeli T., Van Aelst L., and Ballester R. 2000. A role for the non-catalytic N terminus in the function of Cdc25, a *Saccharomyces cerevisiae* Ras-guanine nucleotide exchange factor. *Genetics* **154:** 1473–1484.

Schmitz A.A., Govek E.E., Bottner B., and Van Aelst L. 2000. Rho GTPases: Signaling, migration, and invasion. *Exp. Cell Res.* **261:** 1–12.

Zheng L., Van Aelst L., and Cline H.T. 2000. Rho GTPases regulate distinct aspects of dendritic arbor growth in *Xenopus* central neurons in vivo. *Nat. Neurosci.* **3:** 217–225.

In Press

Boettner B., Herrmann C., and Van Aelst L. 2001. Ras and Rap interaction with the AF-6 effector target. *Methods Enzymol.* (in press).

Di Cristofano A., Niki M., Zhao M., Karnell F.G., Clarkson B., Pear W.S., Van Aelst L., and Pandolfi P.P. 2001. p62^{dok} is a negative regulator of MAP-kinase signaling and cell proliferation. *J. Exp. Med.* (in press).

Schmitz A., Lucito R., and Van Aelst L. 2001. Identification of Rac-regulated genes using cDNA-RDA and microarraying. *Methods Mol. Biol.* (in press).

Zhao M., Schmitz A., Qin Y., Di Cristofano A., Pandolfi P.P., and Van Aelst L. 2001. PI3-kinase dependent membrane recruitment of p62^{dok} is essential for its negative effect on MAP-kinase activation. *J. Exp. Med.* (in press).

STRUCTURE AND GENOMICS

Major strides have been made in the past year in the structure and genomics area. These advances have been both in determining the structure of biologically important macromolecules and in further improving the technology by which we do such work. The former enhances our knowledge of biology while the latter ensures our future progress. The structure and genomics group has determined the structure of both protein and DNA structures as well as structures relating to the interaction of the two.

- In the classical wet laboratory setting, the Joshua-Tor lab has continued their studies of bleomycin hydrolase. They have identified several potential specific inhibitors of the activity of this protein. They have also expanded their studies of protein DNA interactions, determining the structures of the DNA-binding domain of the papillomavirus initiator protein E1 as well as using mutagenesis to determine the role of the DNA repair enzyme MutY.

- The lab of Rui-Ming Xu has determined the structure of an evolutionarily conserved silent information regulator protein (SIR). These proteins are involved in the transcriptional silencing of genes in certain genomic regions. This structural information is being used to determine the molecular basis of the silencing phenomenon.

- The Kobayashi lab has set up a new high-sensitivity mass spectrometer to enable rapid identification of small protein samples.

- The McCombie lab, working as part of an international consortium, completed the determination of the primary structure of the genome of *Arabidopsis thaliana*. This is the first plant genome to be completed and is now being used to determine the function of all of the roughly 25,000 genes identified as well as in studies of centromere structure and function. They are also actively working to determine the sequences of the rice and mouse genomes.

- On a strictly computational front, the Neuwald lab has used a combination of computational techniques to identify repeat-containing proteins. They have also developed a new approach to find protein structure components more accurately and rapidly.

- The Zhang lab has focused a significant amount of their efforts on the detection of promoters and exon selection variation. They have also carried out comparative analyses on the cadherin family and have worked to identify new cell cycle genes using computational means.

- The Stein lab has continued their efforts to integrate and make accessible the wealth of genome information being developed worldwide. They have developed a database comprising the first high-density maps of human sequence polymorphisms. This map contains on average one polymorphism every 2.3 kb. They have also made significant enhancements to the *Caenorhabditis elegans* community database, WormBase, and significant progress in developing ATIDB, a database of insertional mutants for functional genomics in *Arabidopsis*. They are extending their work in plants by beginning the Gramene database, which will serve as a database for the grasses, particularly rice, which is currently being sequenced.

STRUCTURAL BIOLOGY OF REGULATION OF PROTEOLYSIS AND DNA REGULATORY MOLECULES

L. Joshua-Tor A. Auster, E. Enemark, T. Messick, P.R. Kumar, P. O'Farrell, N. Tolia, D. Vaughn, H. Zhou, D. Weinstein

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 in collaborative efforts to study protein function.

Our current efforts center around two distinct themes. The first 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. The second theme in the lab involves structural studies of protein complexes involved in DNA regulatory processes.

The DNA-binding Domain of the Papillomavirus Initiator Protein E1

E. Enemark, G. Chen, 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 (HPVs) 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. Although the progression to malignancy represents a low-frequency event, due to the high frequency of infection, this disease affects a large number of individuals. Bovine papillomavirus (BPV) has served as a prototype for this group, especially regarding viral DNA replication. 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 and also have other activities related to DNA replication, including a DNA distortion and a DNA helicase activity, the latter activity generated by a hexameric form of the protein. Furthermore, these proteins interact with cellular replication proteins such as DNA polymerase α and replication protein A (RPA). Thus, this group of proteins is intimately involved with initiation of DNA replication. In collaboration with Arne Stenlund's group here at the Laboratory, we embarked on structural studies to provide high-resolution structural information about E1 and its DNA-binding activity. These studies 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.

This year, we determined the crystal structure of the DNA-binding domain of E1 to 1.9 Å resolution (Fig. 1). Residues critical for DNA binding are located on an extended loop and on an α-helix. The DNA-binding loop adopts a rather extended path, and although it does not possess any secondary structure, it is well defined in the three-dimensional structure. We identified the E1 dimerization surface by selective mutations at an E1/E1 interface observed in the crystal. On the basis of these observations, we propose a model for the $(E1)_2$-DNA complex (Fig. 2), important for initial *ori* recognition. However, recognizing the origin is not its sole function. The E1 DNA-binding domain has an important role in double-stranded DNA

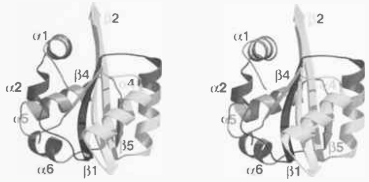


FIGURE 1 Stereo ribbon diagram of the three-dimensional structure of E1-DBD.

melting and formation of the hexameric replicative helicase. These and other observations suggest how the E1 DNA-binding domain might orchestrate the assembly of the hexameric helicase on the *ori*. Further insight will be gained with the elucidation of structures of higher-order complexes.

Role of the Fe-S Cluster in the DNA Repair Enzyme MutY

T. Messick [in collaboration with N. Chmiel and S. David, University of Utah]

DNA is subject to a variety of chemical modifications resulting from hydrolytic reactions, the action of alkylating agents, radiation, and oxidative stress. Such modifications can cause disruptions in DNA synthesis and produce permanent alterations in the genome. Fortunately, elaborate repair pathways exist in all organisms to protect them from the potential deleterious and mutagenic effects of DNA damage and mismatches. An increasing number of examples show that such pathways may be involved in the prevention of diseases such as cancer.

The base excision repair (BER) pathway is primarily responsible for the repair of damage to heterocyclic DNA bases. In this pathway, the marquee players are the DNA glycosylases that recognize mismatched or damaged bases and catalyze *N*-glycosidic bond cleavage. The subsequent actions of apurinic/apyrimidinic (AP) endonucleases and phosphodiesterases provide the appropriate 3′ hydroxyl and 5′ phosphate ends for restoration of the DNA by DNA polymerase and DNA ligase. The oxidation of 2′-deoxyguanosine (G) to 7,8-dihydro-8-oxo-2′-deoxyguanosine (OG) is one of the most common types of damage to DNA in cells. When an OG is present in DNA, it can mispair with an adenine (A) during a replication event and introduce a permanent G-to-T transversion mutation. In *Escherichia coli*, the "GO" repair pathway is dedicated to the prevention of mutations caused by oxidative damage to guanine. This pathway relies on the action of three proteins: MutT, MutM, and MutY. The MutT protein catalyzes the hydrolysis of d(OGTP) to remove it from the dNTP pool and prevents its incorporation in DNA. Both MutM and MutY are DNA glycosylases; MutM (also called FPG protein) removes OG from DNA, whereas MutY removes A when mispaired with an OG. Although OG:A is the preferred substrate, MutY is able to remove A mispaired with G or C.

An interesting feature of MutY is that it contains a [4Fe-4S]$^{++}$ cluster that has been suggested to have an important role in substrate recognition. The presence of a [4Fe-4S]$^{++}$ cluster in several DNA repair enzymes is unusual since iron-sulfur clusters are most commonly found in proteins involved in electron transfer

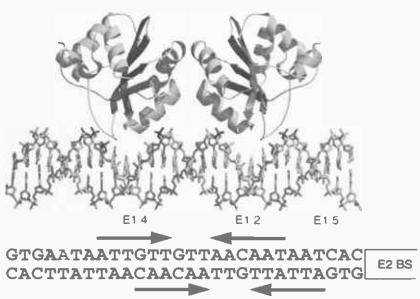


FIGURE 2 Model for DNA binding and dimerization by the E1 DNA-binding domain. A crystallographic interaction between E1 monomers reveals a potential model for E1/E1 interaction in which the distance between the two DNA-binding surfaces corresponds to the separation between two major grooves of DNA. The resulting E1 dimer was docked onto a DNA double helix containing the *ori* sequence. The E1-binding sites occupied in the initial complex are sites E1-2 and 4. The DNA-binding loop and the amino-terminal portion of the DNA-binding helix of each monomer fit nicely into the major groove. Monomer-monomer contacts are made primarily through helix α3. Shown below is the sequence of the *ori* with the E1- and E2-binding sites indicated. Sites E1-5 and 6 are putative E1-binding sites.

reactions. However, there are many nonredox roles for Fe-S clusters that have been uncovered, suggesting that they are versatile cofactors. A relatively new role for Fe-S clusters is the participation in sensing of small molecules for regulation of gene expression or enzymatic activity. We are studying the role of the Fe-S cluster in this system by examining the functional and structural consequences of mutating residues that are involved in cluster ligation, solvent accessibility of the cluster, and other features surrounding the Fe-S cluster. The structures of three such mutants were solved, and their correlation with the activity of the enzyme is shedding light on the role of the Fe-S cluster in MutY.

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 is thought to be a major cause of tumor cell resistance to bleomycin therapy. It was shown that, both in yeast and in 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. There are homologs 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.

Previously, we 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 BH/Gal6's unique ability 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.

PUBLICATIONS

Enemark E.J., Chen G.Y., Vaughn D.E., Stenlund A., and Joshua-Tor L. 2000. Crystal structure of the DNA-binding domain of the replication initiation protein E1 from papillomavirus. *Mol. Cell* **6:** 149–158.

van Aalten D.M.F., Chong C.C., and Joshua-Tor L. 2000. Crystal structure of carboxypeptidase A complexed with D-cysteine at 1.75 Å: Inhibitor-induced conformational changes. *Biochemistry* **39:** 10082–10089.

van Aalten D.M.F., Crielaard W., Hellingwerf K.J., and Joshua-Tor L. 2000. Conformational substates in different crystal forms of the photoactive yellow protein: Correlation with theoretical and experimental flexibility. *Protein Sci.* **9:** 64–72.

PROTEIN CHEMISTRY

R. Kobayashi A. Ahmed S. Lopez
L. Hurton D. Wurtz

David Sargent and Andrew Lin left our lab this year, with David moving to a pharmaceutical company in Manhattan and Andrew returning to the private sector. Professor Daniel Bogenhagen returned to Stony Brook after a 6-month sabbatical in our lab. Aysha Ahmed and Lenka Hurton joined the lab in the last year.

Our lab has been making an effort to elucidate the primary structure of novel proteins and posttranslational modification. We hope that characterization of these proteins will contribute to our knowledge of normal cellular pathways and how they are modified during disease development. The analysis of posttranslational modifications may provide novel insights into the regulation of protein activities in normal and cancer cells.

IMPROVEMENT IN PROTEIN IDENTIFICATION

The three general strategies we use for protein identification are shown in Figure 1; these strategies are used either individually or in combination to study protein chemistry. In 1999, the ion-trap electrospray ionization mass spectrometer (ion-trap ESI MS) was introduced to our lab and has provided unparalleled sensitivity for the identification of peptides in complex mixtures. We have integrated the ion-trap ESI MS with reversed-phase high-performance liquid chromatography (HPLC) to perform LC-MS/MS analysis. This set up allows us to analyze as little as 100–200 fmoles of protein; this is at least one order of magnitude more sensitive than traditional Edman-degradation-based strategies. Although we routinely use this set up to analyze peptides generated by in-gel digestion, this system is capable of analyzing mixtures of polypeptides without prior gel separation. This approach has been used successfully in collaboration with Dr. Stillman's lab to identify ORC interacting proteins.

PROTEOMICS STUDY OF HUMAN MITOCONDRIAL PROTEINS

Mitochondria are highly conserved organelles in eukaryotic cells required for oxidative phosphorylation, the TCA cycle, heme biosynthesis, and metabolism of amino acids and lipids. Mitochondria have a significant role in oxidative damage and apoptosis. It is estimated that in humans, one half to two thirds of mitochondrial proteins have not yet been identified. Prof. Bogenhagen at SUNY Stony Brook spent 6 months on sabbatical in our lab and studied human mitochondrial proteomics. We separated mitochondri-

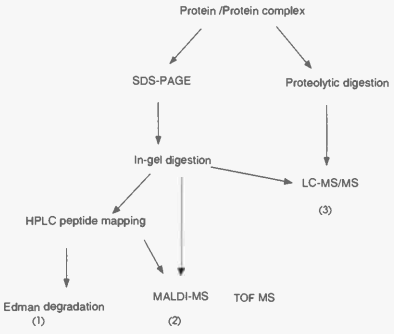


FIGURE 1 Three general strategies for protein identification. (*1*) Direct sequence determination. Proteins are first resolved by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and digested "in-gel." Fragments are separated by HPLC and directly sequenced by Edman degradation. This technique requires at least 2 pmoles of protein. (*2*) MALDI-TOF mass spectrometry. Proteins are separated by SDS-PAGE, and digested in-gel to liberate peptide fragments. Molecular weights of peptides are determined by MALDI-TOF mass spectrometry either in a mixture or following HPLC purification. Proteins are identified by matching the molecular weights of the peptides to those predicted from DNA databases. This technique requires 500–2000 fmoles of protein. (*3*) LC-tandem mass spectrometry. This method is conceptually similar to the MALDI-TOF method, but it utilizes the ability of the ion-trap electrospray ionizing mass spectrometer (ion-trap ESI MS) to directly break down and analyze peptides from a primary proteolytic digestion (LC-MS/MS). This technique requires as little as 100–200 fmoles of protein.

al proteins by two-dimensional gel electrophoresis and identified each protein spot by mass spectrometry. We are continuing our collaboration with Dr. Bogenhagen's lab to study mitochondrial protein in apoptosis.

TYROSINE PHOSPHORYLATION OF P62^{DOK} IN CHRONIC MYELOGENOUS LEUKEMIA

The Philadelphia chromosome translocation is found in almost all patients with chronic myelogenous leukemia (CML). Hematopoietic progenitors isolated from CML patients in the chronic phase contain constitutively tyrosine-phosphorylated p62^{dok} protein. We investigated the role of tyrosine phosphorylation of p62^{dok} in CML. We demonstrated that p62^{dok} is directly tyrosine-phosphorylated by p210$^{Bcr\text{-}Abl}$ and associates with the Ras GTPase-activating protein (RasGAP) but only when p62^{dok} is tyrosine-phosphorylated. By using MALDI-TOF mass spectrometry, truncation constructs, and mutants of p62^{dok}, we identified five tyrosine residues that are involved in in vitro RasGAP binding. We also found that tyrosine-phosphorylated p62^{dok} inhibits RasGAP activity. We are currently studying other proteins that have site-specific interaction with tyrosine-phosphorylated p62^{dok} by Bcr-Abl.

BCR-ABL SIGNALING IN PHILADELPHIA CHROMOSOME-POSITIVE LEUKEMIA

In addition to p210$^{Bcr\text{-}Abl}$, which causes CML, Philadelphia chromosome translocation generates two additional forms of fusion proteins: p190$^{Bcr\text{-}Abl}$ and p230$^{Bcr\text{-}Abl}$. Interestingly, p190$^{Bcr\text{-}Abl}$ is usually associated with acute lymphoblastic leukemia (ALL) and is only rarely associated with CML, acute myelogenous leukemia (AML), or other diseases such as multiple myeloma or B-cell lymphoma; p230$^{Bcr\text{-}Abl}$ has been found in chronic neutrophilic leukemia (CNL). The mechanism by which these three different Bcr-Abl fusion proteins contribute to CML, ALL, and CNL is not well understood. We hypothesize that the tyrosine kinases of the different Bcr-Abl fusions have shared and distinct signals and that these signaling molecules may contribute to the differences in the development of leukemia. Identifying the substrate(s) of the three types of Bcr-Abl fusion proteins may provide important insights into the protein molecules that are responsible for the progression of leukemia. We are interested in studying how these Bcr-Abl protein signal transduction pathways are related to disease phenotypes.

PUBLICATIONS

Dallas P.B., Pacchione S., Wilsker D., Bowrin V., Kobayashi R., and Moran E. 2000. The human SWI-SNF complex protein p270 is an ARID family member with non-sequence-specific DNA binding activity. *Mol. Cell. Biol.* **20:** 3137–3146.

Kashige N., Carpino N., and Kobayashi R. 2000. Tyrosine phosphorylation of p62^{dok} by p210$^{bcr\text{-}abl}$ inhibits RasGAP activity. *Proc. Natl. Acad. Sci.* **97:** 2093–2098.

Losada A., Yokochi T., Kobayashi R., and Hirano T. 2000. Identification and characterization of SA/Scc3p subunits in the *Xenopus* and human cohesin complexes. *J. Cell Biol.* **150:** 405–416.

Willy P.J., Kobayashi R., and Kadonaga J.T. 2000. A basal transcription factor that activates or represses transcription. *Science* **290:** 982–984.

SEQUENCE-BASED ANALYSIS OF COMPLEX GENOMES

W.R. McCombie M. de la Bastide, M. Bell, L. King, A. O'Shaughnessy, L. See, M. Vil, A. Bahrat, D. Cunnius, K. Kirchoff, R. Preston, R. Shah, T. Zutavern, J. Baker, B. Dedhia, K. Kuit, M. Rodriguez, M. Sheker, H. Bal, E. Huang, B. Miller, S. Rodriguez, L. Spiegel, V. Balija, M. Katari, L. Nascimento, L. Santos, K. Toth

During the last several years, our lab has evolved from largely developing tools and infrastructure to carry out genome analysis to using those capabilities to carry out sequence analysis in biologically important organisms or to address biologically important problems. At the same time, our efforts to improve our technology and infrastructure are ongoing. This year, we reached important milestones in those efforts. The first sequence of a higher plant was completed in the past year by a consortium in which we had a major role. Our group, in combination with the Washington University Genome Sequencing Center, completed about 10% of the *Arabidopsis thaliana* genome and two of the five *Arabidopsis* centromeres.

Sequencing the *A. thaliana* Genome

M. de la Bastide, E. Huang, L. Spiegel, L. Gnoj, A. O'Shaughnessy, R. Preston, K. Habermann, M. Bell, N. Dedhia, L. Parnell, R. Shah, M. Rodriguez, L. Hoon See, M. Vil, J. Baker, K. Kirchoff, K. Toth, L. King, A. Bahret, B. Miller, W.R. McCombie
[in collaboration with the Washington University Genome Sequencing Center and The Cold Spring Harbor Laboratory Plant Biology Group]

We began sequencing the *Arabidopsis* genome on a large scale in 1996. This year, we finished our assigned region on chromosome V as well as an additional region that was not completed by one of our collaborators. All together, our consortium sequenced 4,153,198 bases in the past year. Of this amount, 2,202,098 was sequenced here at the Laboratory, with the reminder being sequenced by our collaborator Washington University.

This region included much of the centromere of chromosome V. Figure 1 shows a diagram of this region. These clones were extremely difficult to sequence. While this region was being sequenced, our collaborators in the *Arabidopsis* Genome Initiative were completing the remainder of the genome. The *Arabidopsis* genome is the first plant genome to be completed (The *Arabidopsis* Genome Initiative 2000). It is also one of the most complete and highly accurate genomes completed to date. There were a number of significant findings from this work. In general terms, two of the most noteworthy were the large number of genes contained in the *Arabidopsis* genome, nearly 25,000, and the large degree of duplication in the genome (The *Arabidopsis* Genome Initiative 2000). Approximately 60% of the *Arabidopsis* genome is represented in large duplications (The *Arabidopsis* Genome Initiative 2000). Another point of importance uncovered by the completion of the sequence is the large percentage of *Arabidopsis* genes that are in relatively large multigene families when compared to animal genomes (The *Arabidopsis* Genome Initiative 2000). From an even broader perspective, the sequence revealed that although *Arabidopsis* shares the genes involved in some biological processes with mammals, including humans, other genes thought to be crucial to animals appear not to be present in the *Arabidopsis* genome (The *Arabidopsis* Genome Initiative 2000).

The large number of genes in *Arabidopsis* relative to humans raises fundamental issues of how each organism generates complexity in its proteome. *Arabidopsis* likely generates this diversity with its large number of genes, many of which appear to be specific transcription factors that regulate gene expression (The *Arabidopsis* Genome Initiative 2000). A large amount of the diversity in humans is likely generated by alternative splicing.

As the first plant genome sequenced, it will allow comparison between the complete gene content of plants and animals. This has already led to the discov-

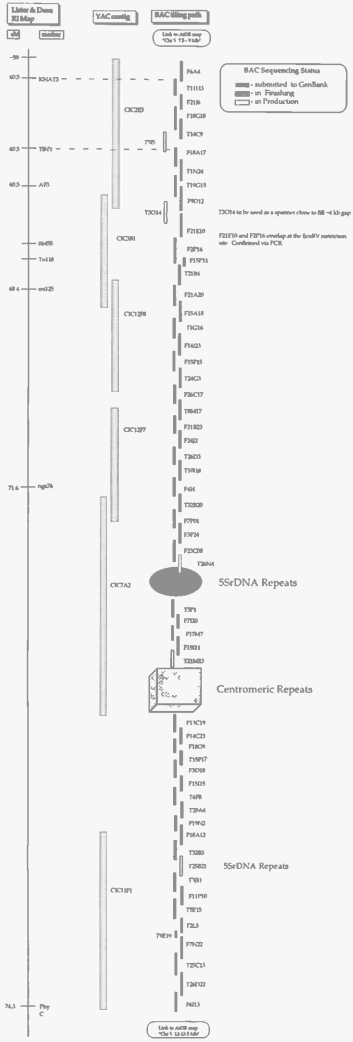


FIGURE 1 Diagram showing much of the region of the centromere of chromosome V.

ery of surprising differences between plants and animals (The *Arabidopsis* Genome Initiative 2000). Second is the impact of the sequence of the *Arabidopsis* genome on plant biology. The availability of this sequence will allow the subsequent dissection of the function of each gene in the organism and their interactions with one another.

It is obvious, although nonetheless important, to reiterate that these observations and the future efforts to determine the function of all *Arabidopsis* genes would not be possible without the complete, finished sequence of the *Arabidopsis* genome.

Rice Genome Sequencing

M. de la Bastide, L. Gnoj, L. Spiegel, M. Bell, V. Balija, N. Dedhia, A. O'Shaughnessy, H. Bal, E. Huang, K. Kirchoff, L. King, R. Preston, R. Shah, A. Bahret, J. Baker, M. Vil, M. Rodriguez, L. Hoon See, T. Zutavern, L. Santos, B. Miller, S. Rodriguez, D.-M. Cunnius, K. Kuit, W.R. McCombie [in collaboration with the Clemson University Genome Institute and the Washington University Genome Sequencing Center]

Rice is the major staple food crop in the world. It also has the smallest size genome of any monocot plant (*Arabidopsis* is a dicot). This makes it an ideal target for concerted genomics efforts. We are part of a consortium that began sequencing the genome of rice in late 1999. To date, we have sequenced about 2,933,074 bases in rough form and have finished sequencing 1,451,243 bases. We have found, as have other groups in the International Rice Genome Sequencing Program, that the rice genome offers particular challenges for finishing sequence to higher accuracy. We are working with our collaborators at Clemson University and Washington University to address these challenges. Our consortium and The Institute for Genomic Research are scheduled to complete chromosome 10 of rice in 2001. Initial analysis of the rice genome indicates that it has a relatively high gene density (9.4 kb per gene) which means that the genome may have significantly more genes than does the *Arabidopsis* or even the human genome.

PUBLICATIONS

The *Arabidopsis* Genome Initiative. 2000. The analysis of the genome of the flowering plant *Arabidopsis thaliana*. *Nature* **408:** 796–815.

The Cold Spring Harbor Laboratory, Washington University Genome Sequencing Center, and PE Biosystems *Arabidopsis* Sequencing Consortium. 2000. The complete sequence of a heterochromatic island from a higher eukaryote. *Cell* **100:** 377–386.

Tabata et al. (The Kazusa DNA Research Institute, The Cold Spring Harbor Laboratory and Washington University in St. Louis Sequencing Consortium, and The European Union *Arabidopsis* Genome Sequencing Consortium). 2000. Sequence and analysis of chromosome 5 of the plant *Arabidopsis thaliana*. *Nature* **408:** 823–826.

In Press

Soengas M.S., Capodieci P., Polsky D., Mora J., Esteller M., Opitz-Araya X., McCombie W.R., Herman J.G., Gerald W.L., Lazebnik Y.A., Cordón-Cardó C., and Lowe S.W. 2001. Inactivation of the apoptosis effector *Apaf-1* in malignant melanoma. *Nature* **409:** 207–211.

Wines M.E., Lee L., Katari M.S., Zhang L., DeRossi C., Shi Y., Perkins S., Feldman M., McCombie R., and Holdener B.C. 2001. Identification of mesoderm development (mesd) candidate genes by comparative mapping and genome sequence analysis. *Genomics* **72:** 88–98.

SEQUENCE-BASED PREDICTION OF PROTEIN STRUCTURE AND FUNCTION

A.F. Neuwald A. Poleksic N. Kannan
N. Hata F. Tan

Our goal is to improve sequence-based prediction of protein structure and function and thereby enhance our understanding of cellular processes mediated by their associated proteins. Advancement toward this goal follows a certain logical progression. First, a better understanding of the relationship between sequence and protein structure and function is obtained. This understanding then guides the improvement of statistical and algorithmic methods for modeling proteins. Next, these methods are incorporated into data analysis tools, which are then applied to the study of specific protein families. Moreover, as biological analysis enhances our understanding, this often leads to further improvements in the statistical and algorithmic methods that, in turn, lead to even better analytical tools. Indeed, to ensure consistent progress, it is helpful to iterate this entire process, and, as a result, our strategy is to work concurrently and synergistically on all four areas: understanding basic principles, developing new models, implementing new tools, and applying these and existing tools to biological discovery. Of course, the ultimate goal, namely, a better understanding of the cellular processes mediated by the proteins under investigation, is what drives and guides our research—not the development of new methods as such. This year's focus has been the continued characterization of sequence motifs corresponding to protein structural repeats, the development of a fast method for searching a database of hidden Markov models, and the development and application of a new approach to functional prediction called CHAIN analysis.

PSI-BLAST SEARCHES USING HIDDEN MARKOV MODELS OF STRUCTURAL REPEATS

We have made substantial progress on the modeling of protein families characterized by subtly conserved repeats, an important yet underdeveloped area of computational biology important to our understanding of many cellular processes. Focusing on short elements keeps the alignment models simple and therefore easier to understand in terms of basic structural principles. Our hope is that this will lead not only to more accurate alignments and more sensitive searches, but also to useful insights into principles of protein folding.

The modeling approach we used combined hidden Markov models (HMMs), Gibbs sampling, and PSI-BLAST. (An HMM is a standard statistical model used to represent protein families within a computer program. Gibbs sampling is a mathematically rigorous procedure to optimize the parameters of a HMM. PSI-BLAST, developed at the National Center for Biotechnology Information, is a very fast iterative multiple alignment and database search procedure that has proved to be considerably more effective than pairwise search methods at detecting distant relationships between proteins.) More specifically, we have developed HMMs with insertion and deletion (indel) penalties based on secondary structure predictions derived from multiple alignment data. Basing these penalties on secondary structure predictions indirectly provides an estimate of the actual likelihood of insertions and deletions because gaps are inherently more likely in loops than in helices or strands. This yielded significant improvements in sequence alignments as judged from comparison to structural alignments. Alignment of short repeats was achieved using a new HMM-based Gibbs sampling procedure. This procedure works by iteratively sampling alignments of individual sequences against the HMM, where sampling is based on the degree of statistical uncertainty associated with the residue match scores of the HMM. Finally, for database searches, we used PSI-BLAST initialized with "checkpoint-recovered" profiles derived from simulated repeat-containing sequences generated by the (optimized) HMM. These searches detected distant structural relationships with, in some cases, substantially greater sensitivity than a normal PSI-BLAST search.

Our recent discovery and analysis of repeat-containing proteins illustrates the biological and medical significance of these new methods. These include sev-

eral discoveries that involve DNA- and RNA-associated proteins with structurally conserved repeats. In particular, an unusual putative sliding DNA clamp protein was detected in the thermophilic bacterium *Thermotoga maritima*. This protein appears to have arisen by way of a duplicated β-clamp gene that then acquired features of a proliferating cell nuclear antigen (PCNA)-like clamp—perhaps to accommodate the substantial archaeal DNA present in this organism. (This archaeal DNA was presumably acquired through lateral gene transfer.) In another example, β-propeller domains were predicted in the large subunit of UV-damaged DNA-binding protein and in related proteins including the large subunit of cleavage-polyadenylation specificity factor, the yeast Rse1p and human SAP130 pre-mRNA splicing factors, and the fission yeast Rik1p gene silencing protein. UV-damaged DNA-binding protein is associated with the hereditary disease xeroderma pigmentosa (XP) group E, which is characterized by sensitivity to UV light and a disposition to skin cancer. XP cells are defective in nucleotide excision repair. For details, see our article referenced below.

FAST AND SENSITIVE SEARCHES OF A DATABASE OF PROTEIN DOMAIN HIDDEN MARKOV MODELS

A key development coming out of our efforts to merge PSI-BLAST, HMM, and other technologies are fast search procedures involving HMMs. An important component of the PSI-BLAST program in this regard is the gapxdrop routine developed by Webb Miller's group at Pennsylvania State University. It works by extending a gapped alignment from the center of a short ungapped aligned region detected by the BLAST heuristic. Within PSI-BLAST, these short ungapped regions correspond to an alignment of a sequence against a profile. We have generalized this profile version of the gapxdrop routine to allow alignment of a sequence against an HMM with position-specific gap penalties. This HMM version of gapxdrop has been incorporated into a new program for searches of a query sequence against a database of HMMs, which is analogous to Sean Eddy's hmmpfam program in the HMMer software package. In particular, our program, which also incorporates other heuristic procedures used in BLAST and PSI-BLAST, allows fast searches of the PFAM and SMART databases because it adopts the HMMer format for HMMs. For this program, we have fit the HMM scores to extreme value distributions, which will provide measures of statistical significance comparable to those used in PSI-BLAST and HMMer. In addition, this HMM version of gapxdrop will be generally useful in other future applications.

IDENTIFYING DETERMINANTS OF PROTEIN STRUCTURE AND FUNCTION THROUGH CHAIN ANALYSIS

An alignment of distantly related protein sequences reveals patterns of conserved residues reflecting the structural and functional constraints acting on these proteins during evolution. Deciphering the biological significance of these patterns, however, is a formidable task, especially when a set of related sequences contains hundreds or thousands of functionally diverse proteins. Moreover, when particular protein subfamilies within this set manifest a high degree of cross-phylum or cross-kingdom sequence conservation, the interpretation of conserved patterns becomes even more complicated. To address these issues, we have devised a strategy called CHAIN (Comparative

FIGURE 1 CHAIN analysis of subfamily 1 of the G-protein α-subunit. The hierarchical alignment shown, which our program automatically generated, corresponds to the switch regions of seven subfamily 1 G_α subunits (from rat, starfish, snail, hydra, lobster, roundworm, and sponge, respectively). Below the top alignment is a series of telescoping alignments corresponding to increasingly larger sets of related sequences at higher levels in the GTPase functional hierarchy. (For each alignment, only those sequences present in the top alignment are shown. The second through fifth alignments contain additional GTPases that are represented by consensus residues below each of these alignments.) The top alignment highlights conserved residues in G_α subfamily 1 using a standard background model, which ignores the evolutionary and functional implications of more distantly related GTPases. Below this, the series of five alignments are highlighted to reveal those residues specifically conserved (respectively) within the G_α subfamily 1 (6 proteins), the G_α family (263 proteins), the G_α/Arf/Sar family (437 proteins), the Ras-like GTPases (1532 proteins), and P-loop GTPases as a whole (3185 proteins). The histograms above these alignments correspond to the degree of functional category-specific sequence conservation (i.e., log-binomial tail probabilities) at each position. Conserved residues within each functional category often correspond to key structural features within that category. For example, the most conserved residues in the bottom alignment correspond to residues contacting the bound GTP or GDP. The most conserved residue among Ras-like GTPases (Q203 in 1ARGA of alignment 4) is known to work in conjunction with GTPase-activating proteins (GAPs). Similarly, a conserved residue among members of the G_α family (R177 in 1ARGA of alignment 3) undergoes an ADP-ribosyl modification, a characteristic of this family.

FIGURE 1 (*See facing page for legend.*)

*H*ierarchical *A*lignment with *I*nterpretive *N*otation) analysis, which quantitatively visualizes conserved patterns characteristic of specific functional categories within a (typically large) set of related protein sequences. The acronym "CHAIN" also alludes to the presumed evolutionary process examined through this approach, namely, a series or chain of increasingly specialized functional adaptations leading to the divergence of related sequences into superfamilies, superfamilies into families, families into subfamilies, and so forth. CHAIN analysis relies on a "hierarchical alignment" and on corresponding three-dimensional structural images to quantitatively highlight functional-category-specific conserved patterns and their corresponding structural features, respectively. More precisely, a hierarchical alignment consists of a series of telescoping multiple sequence alignments, each of which corresponds to the next higher category in the functional hierarchy (and, thus, to a superset of the set of sequences in the previous category).

We have performed CHAIN analyses on various proteins leading to specific functional predictions. Figure 1, for example, shows a hierarchical alignment for subfamily 1 of the G protein α-subunit. The legend to this figure explains aspects of this analysis in more detail. Notably, the programs we have developed allow such an analysis to be performed quickly and require little computational expertise to use. In another example, we constructed a hierarchical alignment using as query the NSF D2 ATPase module—a domain of known structure that is involved in vesicle membrane fusion. This revealed conserved patterns specific to the NSF D2 ATPase module that map to structural regions located at the interface between adjacent subunits, which is consistent with the role of this module in hexamer formation. When structural coordinates for a particular family are unavailable, our procedures can construct a homology-based structural model. This allowed us to perform, for example, an analysis of the NSF D1 module (a second ATPase domain present in NSF and related to the D2 module) leading to the identification of subfamily-specific conserved residues presumably important for NSF's chaperone function. CHAIN analyses were also performed on other domains and on domains that interact with these domains, thereby revealing additional functional aspects of various proteins.

PUBLICATIONS

Neuwald A.F. and Hirano T. 2000. HEAT repeats associated with condensins, cohesins, and other complexes involved in chromosome-related functions. *Genome Res.* **10:** 1445–1452.

Neuwald A.F. and Poleksic A. 2000. PSI-BLAST searches using hidden Markov models of structural repeats: Prediction of an unusual sliding DNA clamp and of β-propellers in UV-damaged DNA binding protein. *Nucleic Acids Res.* **28:** 3570–3580.

Wilbur W.J. and Neuwald A.F. 2000. A theory of information with special application to search problems. *Comput. Chem.* **24:** 33–42.

STRUCTURAL BIOLOGY

R.-M. Xu M. Hayashi H. Shi
J. Jiang J. Vitali
J. Min Y. Zhang
O. Merkel J. Hu

During 2000, Jiangzhong Jiang left to take a job at Morgan Stanley Dean Witter & Co. in Manhattan, and Mariko Hayashi went to the Massachusetts Institute of Technology as a research associate. Two new postdocs, Jingrong Min and Olaf Merkel, and a visiting scientist, Jacqueline Vitali, joined us last year.

RNA Splicing

J. Jiang, H. Shi, Y. Zhang

In the past year, heterogeneous nuclear ribonucleoprotein (hnRNP) A1, SR proteins, and U2AF remained the focus of our structural studies of pre-mRNA splicing. In collaboration with Adrian Krainer here at the Laboratory, we previously solved two crystal structures of the two-RRM (RNA-recognition motif) domain of hnRNP A1 alone and in complex with single-stranded telomeric DNA. Structure and function studies are being pursued of hnRNP A1 concerning (1) protein-RNA interaction, (2) protein-protein interactions in hnRNP A1 multimers, and (3) the structure of the carboxy-terminal domain of hnRNP A1. SR proteins are among the most extensively characterized metazoan splicing factors. They contain either one or two RNA recognition motifs at the amino terminus and a variable number of arginine-serine repeats at the carboxyl terminus. SR proteins are important in constitutive splicing, as well as in enhancer-dependent splicing. The structural basis of protein-RNA interaction and protein-protein interaction governing the function of SR proteins is not well understood. We have made significant progress in crystallizing an SR protein, and this should lead to crystal structure determination in the near future. We are also pursuing crystallographic studies of U2AF, an essential splicing factor in splice site recognition and spliceosome assembly.

Transcriptional Silencing

M. Hayashi, J. Min, O. Merkel, Y. Zhang

Genes located in certain regions of eukaryotic chromosomes are permanently repressed. This heritable, transcriptionally silent state is caused by an altered chromatin structure that can be propagated from one generation to the next. In *Saccharomyces cerevisiae*, silencing is observed for at least three genomic loci: the silent mating-type loci, *HML* and *HMR*, telomeres, and the ribosomal DNA region. Several protein complexes are known to be critical for the establishment and maintenance of transcriptional silencing in yeast, including the *s*ilent *i*nformation *r*egulator (SIR) proteins, Sir1p, Sir2p, Sir3p, and Sir4p, and several sequence-specific DNA-binding proteins, the *o*rigin *r*ecognition complex (ORC), Rap1p, and Abf1p.

During 2000, we crystallized and determined the crystal structure of an archaeal homolog of Sir2p and the amino-terminal domain of Orc1p. Sir2p is unique among the SIR proteins because it is required for silencing at all three known silenced genomic loci, and it is the only SIR protein that has been evolutionarily conserved. Recently, Sir2p and homologous proteins have been shown to be nicotinamide-adenine dinucleotide (NAD)-dependent protein deacetylases. Significantly, they can deacetylate lysine residues at the amino termini of histones that are known to be critical for silencing. In collaboration with Rolf Sternglanz at SUNY, Stony Brook, we have determined two crystal structures of a SIR2 homolog from *Archaeglobus fulgidus* (SIR2-Af1) complexed with NAD, one at 2.1 Å and the other at 2.4 Å resolution. The structures reveal that the protein consists of a large domain having a Rossmann fold and a small domain containing a three-stranded zinc ribbon motif. NAD is bound in a pocket between the two domains (Fig. 1). A distinct mode of NAD binding

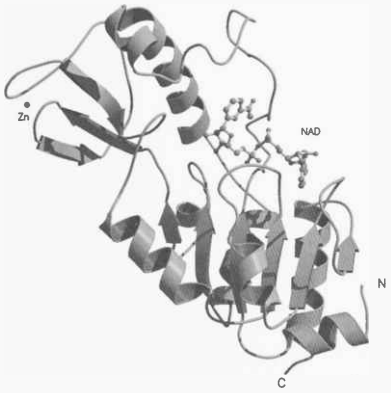


FIGURE 1 Overall view of the structure of *A. fulgidus* SIR2-Af1 complexed with NAD. The protein is shown as a ribbon representation and the NAD molecule is shown in a ball-and-stick model. A zinc ion, part of the zinc ribbon motif, is shown as a ball.

and an unusual configuration of the zinc ribbon motif are observed. The structures also provide important insights into the catalytic mechanism of NAD-dependent protein deacetylation by this family of enzymes.

The amino-terminal domain of Orc1p, the largest subunit of ORC, functions specifically in transcriptional silencing. This domain shares high sequence homology with the amino-terminal domain of Sir3p and contains the recently identified *b*romo-*a*djacent *h*omology (BAH) structural motif found in many chromatin-associated proteins. In collaboration with Bruce Stillman here at the Laboratory, we have determined the crystal structure of the BAH domain of Orc1p to 2.0 Å resolution. The structure of the BAH domain has a novel fold. The structural implication of the Orc1p domain in transcriptional silencing is currently being tested.

DNA Replication

M. Hayashi, J. Vitali, Y. Zhang

In collaboration with James Chong in Bruce Stillman's lab, we had previously shown that an archaeal minichromosome maintenance (MCM) protein forms a double hexamer. It binds to DNA in an ATP-independent manner, and it has a DNA-stimulated ATPase activity and a helicase activity. The archaeal MCM protein serves as a simplified system for understanding the structure and function of the more complicated eukaryotic MCM proteins. Crystallographic study of the archaeal MCM protein complex is currently on-going.

PUBLICATIONS

Chong J.P.J., Hayashi M.K., Simon M.N., Xu R.-M., and Stillman B. 2000. A double-hexamer archaeal minichromosome maintenance protein is an ATP-dependent DNA helicase. *Proc. Natl. Acad. Sci.* **97:** 1530–1535.

Jiang J., Horowitz D.S., and Xu R.-M. 2000. Crystal structure of the functional domain of the splicing factor Prp18. *Proc. Natl. Acad. Sci.* **97:** 3022–3027.

COMMUNITY ACCESS TO GENOME RESOURCES

L.D. Stein T. Harris, J. Kakol, H. Liu, M. Mangone, N. Pavy, D. Priimak, R. Sachidanandam, S. Schmidt, P. Van Buren, D. Weissman, G. Wu

During the calendar year 2000, our lab reached several important milestones:

A HIGH-DENSITY MAP OF HUMAN SINGLE NUCLEOTIDE POLYMORPHISMS

Single nucleotide polymorphisms, or SNPs, are the most common source of genetic diversity in mankind, accounting for more than 90% of differences between individuals' genomes. These single-base-pair changes occur approximately once every 1300 nucleotides and are usually silent. However, SNPs are of keen medical and research interest because they provide a fast and convenient way of mapping and identifying the genes responsible for genetically linked diseases.

The ability of geneticists to identify the genes responsible for human disease is directly proportional to the density of available genetic maps. The current generation of human genetic maps are based on the older technologies of restriction-fragment-length polymorphisms (RFLPs) and simple sequence repeats (SSRs). These maps typically contain several thousand mapped markers, providing a resolution of at most 1 Mb. This means that even after establishing tight genetic linkage between a trait and a map marker, the researcher still must search through a million or more bases of DNA sequence data in order to identify candidate genes that might be responsible for the trait.

In contrast, by virtue of the abundance of SNPs, an SNP-based map can contain hundreds of thousands or

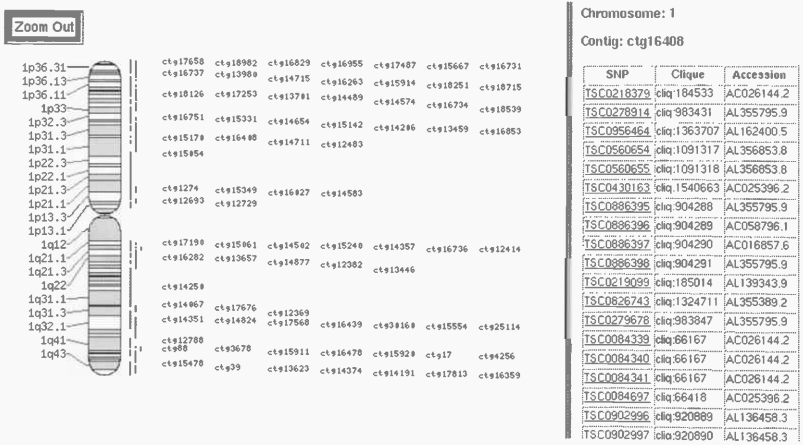


FIGURE 1 A searchable view of SNPs contained on chromosome 1.

millions of markers, giving the map a theoretical resolution of a few thousand bases. This means that researchers can skip the tedious search for candidate genes and immediately hone in on the gene of interest. Furthermore, a highly dense genetic map allows researchers to design studies that take advantage of linkage desequilibrium in isolated populations. This technique obviates the need to collect large detailed family pedigrees and instead allows geneticists to search for association between an SNP and a genetic trait in the general population.

Other uses for SNPs include the design and development of diagnostic tests for genetically linked disorders and the use as a potent tool for studying the evolution and migration of human populations.

Under a grant from The SNP Consortium, our lab is coordinating a large multicenter study to identify SNPs in human sequences. The identified SNPs are verified, archived, and mapped to the human genome using the emerging working draft sequence. After mapping, the data are released into the public domain and to GenBank and other major sequence databases.

To date, we have identified and mapped more than 900,000 SNPs (for example, see Fig. 1). These mapped SNPs have been combined with data from other publicly financed SNP-discovery efforts and with gene prediction data from the human working draft, to yield an integrated map containing more than 1,430,000 SNPs. This map contains 1 SNP every 2.3 kb of DNA on average and represents an increase of two orders of magnitude in resolution over previous maps of the genome. This work resulted in a major publication in the journal *Nature*, "A High-density SNP Map of the Human Genome."

An unexpected feature of the SNP map is that the SNPs are not distributed evenly in the genome, but show occasional areas of high and low density. We are

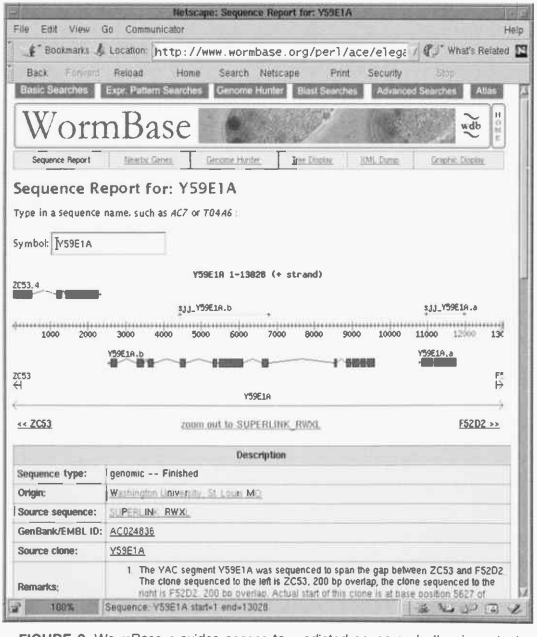


FIGURE 2. WormBase provides access to predicted genes and other important features of the *C. elegans* genome.

currently working with our collaborators to interpret this finding, which may indicate the existence of long-range structure in the genome.

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

During the past year, our lab received a major grant to develop WormBase (Fig. 2), an online 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. Last year, we added the following key features to Wormbase:

- *A cell pedigree browser*, which allows researchers to quickly determine the developmental derivation of any cell, cell group, or tissue in the organism.
- *A genome browser*, which provides researchers with the ability to select a region of the genome and retrieve the DNA sequence and annotations in a variety of formats.
- *Expression pattern pages*, which provide researchers with searchable access to the results of gene-tagging experiments.
- *RNA interference pages*, which provide access to the results of recent large-scale studies in which *C. elegans* genes are selectively disabled.

The WormBase project resulted in a publication, "Wormbase: Network Access to the Genome and Biology of *C. elegans*," which appeared in the January issue of the journal *Nucleic Acids Research*.

ATIDB: A DATABASE FOR INSERTIONAL MUTAGENESIS EXPERIMENTS

It is increasingly common in genomics to probe the function of genes by systematically knocking them out using transposon-mediated mutagenesis, or similar technology. For example, both *Arabidopsis thaliana* and *Zea mays* have been probed this way. After generating a large collection of mutant strains, the transposon insertion sites are sequenced to determine the gene that has been knocked out, and the plant is studied for morphologic or physiologic consequences of the knockout. A closely related technology are gene-tagging studies, such as the "GeneTrap" technique pioneered by Robert Martienssen here at the Laboratory. In this technique, genes are not knocked out, but are replaced by a visible tag, which can then be used to study the gene's spatial and temporal expression pattern.

In 1999, our lab designed and deployed MTMDb, a database for a specific maize insertional mutagenesis project. On the basis of this experiment, we designed and implemented a portable, generic system for managing this type of research protocol. The system includes an Open Source database, a Web server, a user interface system, and a series of software modules for loading and maintaining the system. With this package, a researcher can rapidly determine the genomic location of an insertion site, find the gene or genes affected by the insertion, manage the pedigree of the manipulated strains, and store phenotypes associated with each individual. The system is Web-accessible and can be used to publish the results of insertional mutagenesis studies and to make correlations between genes and phenotypes.

The database has been used successfully to curate GeneTrap data from the Martienssen lab and to manage a large-scale insertional mutagenesis project in the lab of our collaborator, Michael Bevan. We plan to release the software to the public under an Open Source license in late January or February and are preparing a manuscript to describe it.

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 the error-prone

and time-consuming reformatting currently required. Although the project is still under way, we passed important milestones during 2000.

- *The release of Geodesic, a graphical DAS client.* This software is a genome browser. With it, researchers can retrieve genomic data from multiple on-line annotation databases and view the data as an integrated whole. Heretofore, it was difficult or impossible to integrate data from disparate annotation data sources.

- *The adoption of DAS as the output format by several key data sources.* DAS has been adopted as the data exchange format by Ensembl, the Joint Genomics Initiative at Oak Ridge and the University of California at Santa Cruz, three major sources of human genome annotation data; by FlyBase and WormBase, two model organism databases; and by Incyte, a commercial venture. These adoptions give the DAS standard significant momentum.

We continue to develop other key components of the DAS system, including a lightweight annotation server that will allow small laboratories to publish comments on the genome with minimal investment in hardware and software.

GRAMENE: A RESOURCE FOR COMPARATIVE MAPPING IN RICE AND OTHER GRAINS

In October, our lab was awarded a highly competitive USDA IFAFS grant to develop a comprehensive resource for comparative mapping in rice and other monocots. This resource will contain the emerging rice genomic sequence and associated annotations, plus a series of comparative genetic and physical maps comprising the major monocot crops, maize, barley, oats, millet, sorghum, and wheat. By following these evolutionarily conserved segments, researchers will be able to identify and clone candidate genes in monocots that have not been sequenced by analogy with corresponding sequenced rice genes. We have set up an initial Web Site, www.gramene.org, and are proceeding with the design of the database and other software.

PUBLICATIONS

Piano F., Schetterdagger A.J., Mangone M., Stein L., and Kemphues K.J. 2000. RNAi analysis of genes expressed in the ovary of *Caenorhabditis elegans*. *Curr. Biol.* **10:** 1619–1622.

Stein L., Sternberg P., Durbin R., Thierry-Mieg J., and Spieth J. 2001. WormBase: Network access to the genome and biology of *Caenorhabditis elegans*. *Nucleic Acids Res.* **29:** 82–86.

In Press

The International SNP Map Working Group. 2001. A SNP map of the human genome at kilobase resolution. *Nature* (in press).

COMPUTATIONAL GENOMICS

M.Q. Zhang R.V. Davuluri, G. Chen, Z. Xuan, A. Kel, T. Zhang, N. Banerjee, N. Pavy, M. Vichnevskaia, J. Hizver, J. Zhu

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 entered its large-scale sequencing phase, 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 characteristics 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 more difficult problems of finding regulatory *cis*-elements in noncoding regions. This year, we continue to focus on splicing enhancers, promoters, and transcriptional start site and polyadenylation site regions in order to develop new computational methods for identification of these functional elements. To facilitate large-scale gene expression data mining, we have also developed several computational tools for clustering and promoter analysis.

We have also worked jointly with other investigators here at the Laboratory. In collaboration with the Krainer lab, we have studied the mechanism of exon skipping caused by a nonsense mutation in the *BRCA1* gene; and in collaboration with the Wigler and McCombie labs, we have been developing computational methods and searching for tumor suppressor genes. For a more detailed description of these studies, please see the Krainer, Wigler, and McCombie sections of this Annual Report.

CART Classification of Human 5′ UTR Sequences

R. Davuluri, M.Q. Zhang [in collaboration with S. Sugano, University of Tokyo]

A nonredundant database of 2312 full-length human 5′-untranslated regions (5′UTRs) was carefully prepared using state-of-art experimental and computational technologies. A comprehensive computational analysis of these data was conducted for characterizing the 5′UTR features. Classification and regression tree (CART) analysis was used to classify the data into three distinct classes. Class I consists of mRNAs that are believed to be poorly translated with long 5′UTRs filled with potential inhibitory features. Class II consists of TOP (terminal oligopyrimidine tract) mRNAs that are regulated in a growth-dependent manner. Class III consists of mRNAs with favorable 5′UTR features that may help efficient translation. The most accurate tree that we found has 92.5% classification accuracy as estimated by cross-validation. The classification model included the presence of TOP, secondary structure, 5′UTR length, and the presence of upstream AUGs (uAUGs) as the most relevant variables. The present classification and characterization of the 5′UTRs provide precious information for better understanding the translational regulation of human mRNAs. Furthermore, this database and classification can help investigators build better computational models for predicting the 5′-terminal exon and separating the 5′UTR from the coding region.

JTEF: A novel 3′-terminal Exon Recognition Algorithm

J. Tabaska, R. Davuluri, M.Q. Zhang

JTEF, a new program for finding 3′-terminal exons in human DNA sequences, is based on quadratic discriminant analysis, a standard nonlinear statistical pattern-recognition method. The quadratic discriminant functions used for building the algorithm were trained on a set of 3′-terminal exons of type *3tuexon* (those containing the true STOP codon). We showed that the average predictive accuracy of JTEF is higher than the best presently available programs (GenScan and Genemark.hmm) based on a test set of 65 human DNA sequences with 121 genes. In particular, JTEF per-

forms well with larger genomic contigs containing multiple genes and significant amounts of intergenic DNA. It will become a valuable tool for genome annotation and gene functional studies.

A Database and Statistical Analysis of Alternative Exons

J. Zhu, M.Q. Zhang [in collaboration with S. Stamm, Max-Planck Institute, and K. Nakai, University of Kyoto]

We compiled a comprehensive database of alternative exons from the literature and analyzed them statistically. Most alternative exons are cassette exons and are expressed in more than two tissues. From all exons whose expression was specific for a certain tissue, the majority was expressed in the brain. On average, alternative exons are shorter than constitutive exons, and their splice sites deviate more from the consensus, where their 3′ splice sites are characterized by a higher purine content in the polypyrimidine stretch and their 5′ splice sites deviate mostly at the +4 and +5 positions from the consensus sequence. Furthermore, exons expressed in a single tissue use more adenosine at the –3 position of the 3′ splice site. In addition to the known adenosine/cytosine-rich and purine-rich exonic sequence elements, sequence comparison using a Gibbs algorithm identifies several motifs in exons surrounded by weak splice sites and in tissue-specific exons. Together, these data indicate a combinatorial effect of weak splice sites, short exon length, atypical nucleotide usage at certain positions, and supplementary enhancers as a cause of alternative exon usage.

Computational Characterization of Liver-specific Promoters

T. Zhang, M.Q. Zhang

This work focuses on developing computational tools to detect regulatory regions (i.e., promoters) that confer tissue-specific gene expression. We demonstrate our approach using liver-specific gene promoters, mainly because of the recent advances in the studies of liver-specific transcription factors.

Our approach consists of the following steps. First, we have collected all liver-specific transcription-factor-binding sites that are experimentally verified in literature. Second, we have constructed PWMs (positional weighted matrices) that summarize the binding sites in our collection. Third, we have applied linear regression analysis to combine matches to multiple binding sites into a single model. For various test sets, our models have achieved state-of-the-art performance.

We are currently investigating ways to enhance the performance of our models. The most promising direction is to incorporate additional regulatory elements into these models. Such elements could bind to transcription factors other than the four factors already examined in building the existing models, or they could be related to secondary structures. To identify such elements, we have developed new algorithms searching for motifs that are significantly over/under-represented in the given promoter sequences. Different from the previous motif-finding methods, we allow both mismatches and gaps in our motifs, which reflect more realistically the actual DNA and protein interaction. Another approach to discover putative motifs is by comparing promoters from distantly related species. In general, functional regions are more likely to be conserved over evolution than nonfunctional regions. By carefully selecting the organisms under examination, we can identify conserved regions, which are the putative functional regions.

Comparative DNA Sequence Analysis of Mouse and Human Protocadherin Gene Clusters

T. Zhang, M.Q. Zhang [in collaboration with T. Maniatis, Harvard University, and R.M. Myers, Stanford University]

The genomic organization of the human protocadherin α, β, and γ gene clusters (designated *Pcdh*α, *Pcdh*β, and *Pcdh*γ) is remarkably similar to that of immunoglobulin and T-cell receptor genes. The extracellular and transmembrane domains of each protocadherin protein are encoded by an unusually large "variable" region exon, whereas the intracellular domains are encoded by three small "constant" region exons located downstream from a tandem array of variable region exons. Here, we report the results of a comparative DNA sequence analysis of the orthologous human (750 kb) and mouse (900 kb) protocadherin gene clusters. The organization of *Pcdh*α and *Pcdh*γ gene clusters in the two species is virtually identical; the mouse *Pcdh*β gene cluster is larger and contains more genes than the human *Pcdh*β gene clus-

ter. We identified conserved DNA sequences upstream of the variable region exons and found that these sequences are more conserved between orthologs than between paralogs. Within this region, there is a highly conserved DNA sequence motif located at about the same position upstream of the translation start codon of each variable region exon. In addition, the variable region of each gene cluster contains a rich array of CpG islands, whose location corresponds to the position of each variable region exon. These observations are consistent with the proposal that the expression of each variable region exon is regulated by a distinct promoter, which is highly conserved between orthologous variable region exons in mice and humans.

Microarray Study of Mouse ES Cell Differentiation

G. Chen, N. Banerjee, M.Q. Zhang [in collaboration with M. Ko, NIA/NIH]

To understand the genetic pathways controlling embryonic stem (ES) cell differentiation, we have been using cDNA microarrays ("NIA mouse 15K microarray") containing 15,000 distinct mouse genes, 50% of which were newly identified and derived from preimplantation mouse embryo cDNA libraries. Undifferentiated mouse R1 ES cells were maintained in the presence of both leukemia inhibitory factor (LIF) and BRL-conditioned media without feeder layers. For the time course experiments, ES cells were cultured and harvested at various time points after withdrawal of LIF and conditioned media: 4 hours, 8 hours, 18 hours, 24 hours, and 36 hours. In addition, aggregates of ES cells were generated using the "hanging-drop" technique. After 7 days in culture, these aggregates contain a heterogeneous mixture of highly differentiated cells. Total RNAs were extracted from these cells and used for cDNA microarray hybridizations. All microarray analyses were done in triplicate, and the F-test was used to identify 3508 genes for their statistically significant expression changes during the ES cell differentiation.

A program based on the *K*-mean analysis was used to group 3508 genes into 36 clusters according to the similarity of their expression patterns. Among them, 12 different clusters containing 1424 genes showed a dramatic decline of gene expression levels at 4 and/or 8 hours after the withdrawal of LIF and conditioned media. Functionally, these genes fall into six categories. The first category contains well-characterized genes, such as *Stat3*, *Oct4*, and *Rex1*, which are known to be down-regulated by the withdrawal of LIF. Genes in the second category, including *Maid* and *Hai2* (hepatocyte growth factor activated inhibitor type 2), are known to function as negative regulators of genes that are important in cell differentiation. Genes in the third category, including *HMG1* and *Brg1*, are involved in chromatin remodeling. Genes in the fourth category, including *Cdc25A* and *p33q1K* kinase, are involved in cell cycle regulation and apoptosis. Genes in the fifth category, including *TGF*-β receptor interacting protein 1 and β-*ig-h3*, are growth factors and growth factor responsive genes. The last category includes 790 newly identified genes.

To elucidate the potential functions of the newly identified genes, we have developed a bipolar clustering program "sdclust." In contrast to other clustering programs, this program can identify both negatively correlated genes and positively correlated genes. For example, we used the *Oct4* gene as the "center of cluster" and identified 65 genes (45 of them novel) whose expression patterns show positive correlation with that of *Oct4*. Further analyses of these novel genes may provide critical insights into the characteristic features of ES cells, such as pluripotency and self-renewal.

Computer-assisted Identification of Cell-cycle-related Genes: New Targets for E2F Transcription Factors

M.Q. Zhang [in collaboration with the laboratories of A. Kel, Russian Academy of Science; P. Farnham, University of Wisconsin; and E. Wingender, Gesellschaft fur Biotechnologische Forschung mbH]

The processes that take place during development and differentiation are performed through coordinated regulation of expression of a large number of genes. One such gene regulatory network provides cell cycle control in eukaryotic organisms and, in this work, we have studied the structural features of the 5′ regulatory regions of cell-cycle-related genes. We developed a new method for identifying composite substructures (modules) in regulatory regions of genes consisting of a binding site for a definite transcription factor and additional contextual motifs—potential targets for other transcription factors that may synergistically regulate gene transcription. Applying this method to cell-cycle-related promoters, we created a program for

context-specific identification of binding sites for E2F transcription factors, key regulators of cell cycle. We found that E2F composite modules are found at a high frequency and in close proximity to the start of transcription in cell-cycle-related promoters in comparison with other promoters. Using this information, we then searched for E2F sites in genomic sequences with the goal of identifying new genes that have important roles in controlling cell proliferation, differentiation, and apoptosis. Using a chromatin immunoprecipitation assay, we then experimentally verified the binding of E2F in vivo to the sites predicted by the computer-assisted methods. Our identification of new E2F target genes provides new insight into gene regulatory networks and provides a framework for continued analysis of the role of promoter context in transcriptional regulation. Tools are available at http://compel.bionet.nsc.ru/FunSite/SiteScan.html.

Identification of CREB Targets in *Drosophila melanogaster*

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

cAMP response element-binding protein (CREB) is a conserved transcription factor present in a number of organisms, including *Homo sapiens* and *Drosophila melanogaster*. It has an important role in long-term memory formation, and it is also implicated in human neurological diseases, such as Alzheimer's. To understand the learning and memory microarray expression results, we set out to identify CREB targets in the fly by searching for the promoter element (CRE). Since there was very little known about fly CRE elements, we decided to use an in silico phylogenetic approach: collecting known CRE elements and CREB targets in higher organisms as the training set and then identifying the corresponding fly orthologs. We obtained the following results: (1) A database of the known CREB targets in various organisms was created and soon will be published; (2) the approach used to produce the ortholog set of genes was validated, and it can be used in the future for other transcription factors research; (3) the matrix for the CRE site was produced, and it can be used in the future for identification of CRE sites; and (4) putative CREB targets were suggested from the set of candidate genes and from the set of potential orthologs of non-*Drosophila* CREB targets.

PUBLICATIONS

Davuluri R.V., Suzuki Y., Sugano S., and Zhang M.Q. 2000. CART classification of human 5'UTR sequences. *Genome Res.* **10:** 1807–1816.

Ioshikhes I.P. and Zhang M.Q. 2000. Large-scale human promoter mapping using CpG islands. *Nat. Genet.* **26:** 61–63.

Liu H.X., Chew S.L., Cartegni L., Zhang M.Q., and Krainer A.R. 2000. Exonic splicing enhancer motif recognized by human SC35 under splicing conditions. *Mol. Cell. Biol.* **20:** 1063–1071.

Machida M., Yamazaki S., Kunihiro S., Tanaka T., Kushida N., Jinnno K., Haikawa Y., Yamazaki J., Yamamoto S., Sekine M., Oguchi A., Nagai Y., Sakai M., Aoki K.I., Ogura K., Kudoh Y., Kikuchi H., Zhang M.Q., and Yanagida M. 2000. A 38-kb segment containing the *cdc2* gene from the left arm of fission yeast chromosome II: Sequence analysis and characterization of the genomic DNA and cDNAs encoded on the segment. *Yeast* **16:** 71–80.

Stamm S., Zhu J., Nakai K., Stoilov P., Stoss O., and Zhang M.Q. 2000. An alternative exon database (AEDB) and its statistical analysis. *DNA Cell Biol.* **19:** 739–756.

Zhang M.Q. 2000. Discriminant analysis and its application in DNA sequence motif recognition. In *EBI Symposium on Genome Based Gene Structure Determination*. Cambridge, U.K. *Briefings in Bioinformatics*, vol. 1, p. 1.

Zhu J. and Zhang M.Q. 2000. Cluster, function and promoter: Analysis of yeast expression array. In *Proceedings of Pacific Symposium on Biocomputing 2000* (ed. R.B. Altman et al.). Honolulu. **5:** 479–490.

In Press

Liu H.X., Chew S.L., Cartegni L., Zhang M.Q., and Krainer A.R. 2001. A mechanism for exon skipping caused by nonsense or missense mutations in *BRCA1* and other genes. *Nat. Genet.* **27:** 55–58.

Wu Q., Zhang T., Cheng J.-F., Kim Y., Grimwood J., Schmulz J., Dickson M., Noonan J.P., Zhang M.Q., Myers R.M., and Maniatis T. 2001. Comparative DNA sequence analysis of mouse and human protocadherin gene clusters. *Genome Res.* (in press).

NEUROSCIENCE

The last generation of neuroscientists has contributed tremendous insight to the cellular basis of neuronal function. A basic electrophysiology and cell biology of the neuron has emerged, which includes molecular models of synaptic plasticity and cellular models of associative processes. Intuitively, we all know that these mechanisms likely underlie the various forms of behavioral plasticity—learning and memory—that animals display. Unbeknownst to those who pioneered this effort in various animal models, the modern-day convergent view holds that these basic mechanisms may be evolutionarily conserved. The "neuron" appears to have been invented once. Hence, study of plasticity in animal models now holds great promise to inform the basic biology of cognition in humans.

The diversity of behavioral responses—both within a given animal and among the species—appears to reside at two levels of biological organization: the network of gene products within a neuron, which interact to yield cellular function, and the network of connections among neurons, which translates cellular function into behavioral response. These two computational networks are enormously complex and largely unresolved. The next generation of neuroscientists will need to derive the computational algorithms at each level of biological organization in order to achieve an adequate understanding of the genetics of memory. From this vertical integration will emerge effective new strategies to diagnose and treat cognitive dysfunction in humans.

PRINCIPLES OF BRAIN DESIGN

D.B. Chklovskii A. Stepanyants

The goal of our laboratory is discovering key organizing principles of brain design and applying these principles to understand brain function. One such principle, that of wiring economy, proved to be particularly useful for understanding the organization of the brain. This principle, which goes back to Cajal, asserts that the brain has evolved under pressure to keep its volume to a minimum. Thus, for a given circuit, the layout of brain components, such as neuronal bodies and synapses, should minimize the length of wiring, i.e., of axons and dendrites. By solving the optimization problem, we establish a link between the connectivity and spatial layout of brain circuits, thus making experimentally testable predictions.

Ocular Dominance Patterns in the Visual Cortex

D.B. Chklovskii [In collaboration with A.A. Koulakov, Salk Institute]

The primary visual area (V1) of the mammalian brain is a thin sheet of neurons. Activity of most neurons is dominated by the inputs from either the right eye or the left eye. The spatial arrangement of neurons dominated by different eyes is known as the ocular dominance (OD) pattern. We propose a theory for OD patterns based on the premise that they are evolutionary adaptations to minimize the length of intracortical connections. Thus, the existing OD patterns are obtained by solving a wire length minimization problem. We divide all the neurons into two classes: right-eye-dominated and left-eye-dominated. We find that if the number of connections of each neuron with the neurons of the same class differs from that with the other class, the segregation of neurons into monocular regions indeed reduces the wire length. The shape of the regions depends on the relative number of neurons in the two classes. If both classes are equally represented, we find that the optimal OD pattern consists of alternating stripes. If one class is less numerous than the other, the optimal OD pattern consists of patches of the underrepresented (ipsilateral) eye-dominated neurons surrounded by the neurons of the other class. We predict the transition from stripes to patches when the fraction of neurons dominated by the ipsilateral eye is about 40%. This prediction agrees with the data in macaque and Cebus monkeys (Fig. 1). Our theory can be applied to other binary cortical systems.

Orientation Preference Patterns in the Visual Cortex

D.B. Chklovskii [in collaboration with A.A. Koulakov, Salk Institute]

Different edge orientations in the visual scene are recognized by different neurons. In the visual cortex,

FIGURE 1 Ocular dominance pattern in macaque visual cortex: (*Gray*) Neurons dominated by the left eye; (*white*) neurons dominated by the right eye. Transition from stripy to patchy pattern takes place when the fraction of left-eye neurons is about 40% (*black line*) as predicted by our theory.

these neurons are arranged in spatial patterns known as orientation preference patterns. The appearance of these patterns varies between different species. We propose that the observed interspecies variability of the orientation preference patterns reflects the variability of intracortical neuronal circuits. By using the wire-length minimization approach, we establish a link between the statistics of interneuronal connectivity and map appearance (Fig. 2). This link can be used to make testable predictions about interneuronal connectivity from map appearance. Because intracortical neuronal circuits underlie visual processing, our results may help to understand how different animals see the world differently. In addition, our work provides the first explanation of "singularities" in the origination preference patterns, i.e., locations where orientation preference changes abruptly (Fig. 2G–J). We suggest that they arise to minimize wiring between neurons of opposite as well as of similar orientation preference.

Information Storage Capacity of the Human Brain

A. Stepanyants, D.B. Chklovskii

How much information can be stored in a human brain? The answer to this question depends on the time scale available for information storage. One major component of information storage underlying learning and memory is the formation and elimination of dendritic spines, which happens on the time scale from a few minutes to a few hours. We estimate the information storage capacity associated with this component. To do this, we derive an expression for the potential number of synapses a neuron could make. Under minimal assumptions, we find that the number of potential synapses is equal to the product of dendritic, axonal, and spine lengths and neuronal density. By using existing anatomical data, we evaluate this expression and find that the ratio of existing to potential synapses is about 0.2. Therefore, the information storage capacity associated with synaptic patterns is about 3 bits per synapse. Because the human brain contains about 10^{15} synapses, the information storage capacity associated with changes in synaptic patterns is on the order of 10^{15} bits or 100 Terabytes.

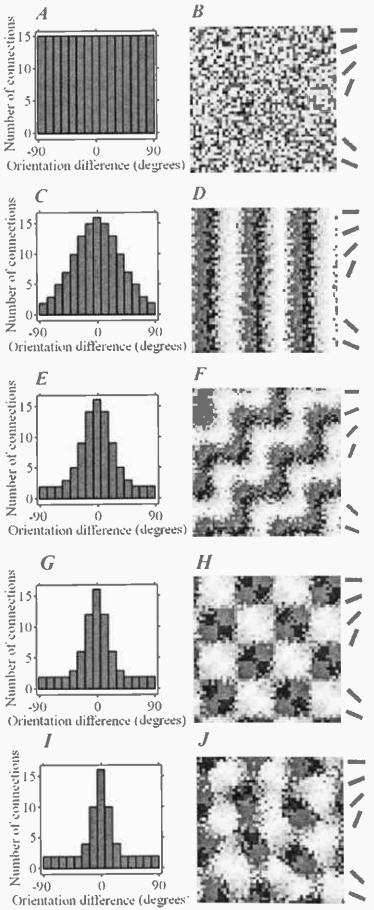


FIGURE 2 Statistics of interneuronal connections in the visual cortex (*left column*) and corresponding orientation preference patterns (*right column*) obtained by minimizing the length of these connections. On the basis of these results, we propose that the interspecies variability in orientation preference patterns can be accounted for by the differences in interneuronal connectivity.

Why Is Each Half of the Brain Responsible for the Opposite Half of the World?

D.B. Chklovskii [in collaboration with A.A. Koulakov, Salk Institute]

A surprising and ubiquitous feature of vertebrate design is that the representation of each half of the external world is localized in the opposite half of the brain. Why should this be? Of course, nerve fibers have to cross the body midline in order to pass information between sensory organs on the one side of the body to the motor organs on the other side. However, this does not answer the original question because the representation of the world can be localized before or after the crossing. Our hypothesis is that the representation of the world is located according to the relative number of sensory inputs and motor outputs. To illustrate this hypothesis, we consider the neuroanatomy of fish. If the number of incoming sensory fibers is greater than the outgoing motor fibers, then the integration unit should be located ipsilaterally to the half of the world it represents. An example of such arrangement is the Mauthner neuron. If the number of motor outputs exceeds the number of sensory inputs, then the integration unit should be located closer to the motor outputs, or contralaterally to the half of the world it represents. An example of such arrangement is optical tectum. This could explain contralateral representation in the mammalian cerebral cortex because it is strongly coupled to the brainstem.

What Determines the Shapes of Dendritic and Axonal Arbors?

A. Stepanyants, D.B. Chklovskii

Dendritic and axonal arbors come in a dazzling variety of different shapes that have been known for over a century but remain unexplained. We have shown in the past that the wiring economy principle explains the sizes (diameters) of arbors as well as the typical mesh size. However, the topology of arbors as characterized by the branching points remains unconstrained. We pursue two hypotheses to explain the branching of dendritic and axonal arbors. The first one (we call it plasticity potential) is due to the fact that the shape of the arbor cannot be completely specified by the genes. Arbor shape must accommodate the inevitable variability in the possible location of synapses. Branching of the processes enhances plasticity potential because branches can be formed or eliminated locally, without global remodeling of the arbor shape. The second hypothesis invokes the cost of neuronal processes being distant from the cell body. This cost could be due to the intracellular transport of material or delay and attenuation of the nerve pulses.

PUBLICATIONS

Chklovskii D.B. 2000. Binocular disparity can explain the orientation of ocular dominance stripes in primate primary visual area (V1). *Vision Res.* **40:** 1765–1773.

Chklovskii D.B. 2000. Optimal sizes of dendritic and axonal arbors in a topographic projection. *J. Neurophysiol.* **83:** 2113–2119.

Chklovskii D.B. 2000. Optimal sizes of axonal and dendritic arbors. In *Advances in neural information processing systems 12* (ed. S.A. Solla et al.), pp. 108–114. MIT Press.

Chklovskii D.B. and Koulakov A.A. 2000. A wire length minimization approach to ocular dominance patterns in mammalian visual cortex. *Physica A* **284:** 318–334.

Chklovskii D.B. and Stevens C.F. 2000. Wiring optimization in the brain. In *Advances in neural information processing systems 12* (ed. S.A. Solla et al.), pp. 103–107. MIT Press.

In Press

Koulakov A.A. and Chklovskii D.B. 2001. Orientation preference maps in mammalian visual cortex: A wire length minimization approach. *Neuron* (in press).

ACTIVITY-DEPENDENT NEURONAL DEVELOPMENT

H. Cline C. Aizenman, K. Bronson, I. Cantallops, I. Cohen, M. Davenne, L. Foa, K. Haas, A. Javaherian, K. Jensen, Z. Li, G. Munoz, E. Rial Verde, E. Ruthazer, W.-C. Sin

Normal brain development requires brain activity, suggesting some kind of positive feedback. We are interested in understanding the function of neuronal activity in brain development. We address this issue by examining the development of the visual system in amphibian tadpoles. These animals are transparent, which allows us to observe brain development in the living animal. Many of the experiments we perform involve in vivo imaging of neuronal structure during periods up to several weeks. We combine these studies with gene transfer methods that allow us to test the function of genes of interest in brain development. In addition, we assess neuronal function using electrophysiological assays of synaptic connectivity and synaptic plasticity. This range of technical approaches allows us to identify key regulatory mechanisms governing the development of brain structure and function.

TECHNICAL ADVANCES

Time-lapse Imaging Using Two-photon Laser Scanning Microscopy

E. Ruthazer [in collaboration with P. O'Brien, B. Burbach, and K. Svoboda, Cold Spring Harbor Laboratory]

E. Ruthazer modified an Olympus Fluoview confocal scan box mounted on an Olympus microscope body to enable two-photon imaging. This work was done under the tutelage of K. Svoboda with technical assistance from P. O'Brien and B. Burbach.

Single-cell Electroporation for Gene Transfer In Vivo

K. Haas, W.-C. Sin, A. Javaherian, Z. Li, H.T. Cline

K. Haas, W.-C. Sin, A. Javaherian, and Z. Li have developed an electroporation technique for targeting gene transfer to individual cells in intact tissue. Electrical stimulation through a micropipette filled with DNA or other macromolecules delivers the DNA into a single cell at the tip of the micropipette. Electroporation of a plasmid encoding enhanced green fluorescent protein (GFP) into the brain of intact *Xenopus* tadpoles or rat hippocampal slices resulted in GFP expression in single neurons (Fig. 1). In vivo imaging showed morphologies, dendritic arbor dynamics, and growth rates characteristic of healthy cells. Coelectroporation of two plasmids resulted in expression of both proteins, whereas electroporation of fluorescent dextrans allowed direct visualization of transfer of molecules into cells. Due to its versatility, this single method can be used to deliver genes, antisense oligonucleotides, fluorescent dyes, drugs, and other macromolecules to a variety of cell types including neurons, muscle cells, and glia in the intact animal. Application of this technique allows unprecedented spatial and temporal control of gene delivery and permits experiments that cannot be performed with current gene transfer technology.

Application of Electroporation to Target Gene Expression to Selected Populations of Neurons in *Xenopus* Brain In Vivo

L. Foa, K. Haas, Z. Li, H.T. Cline

We have made extensive use of viral gene transfer to deliver genes of interest into neurons in the *Xenopus* brain. In addition to the use of vaccinia virus, we now use electroporation to introduce plasmid DNA into neurons within the optic tectum. We obtain widespread gene expression targeted to a single optic tectum. Targeted electroporation allows exquisite spatial and temporal control over gene expression in the brain of living animals.

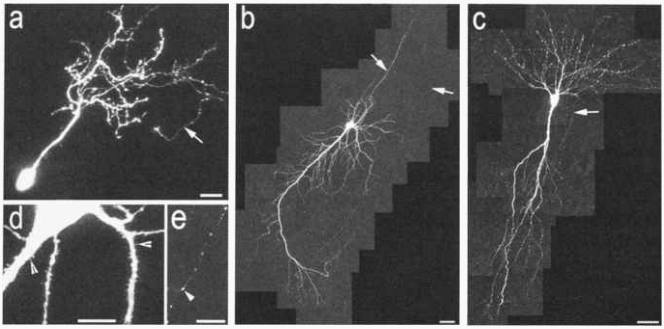


FIGURE 1 Single-cell electroporation with pEGFP targets gene delivery to individual neurons. Shown are confocal images of a single GFP-labeled neuron in the tadpole optic tectum (*a*), CA1 (*b*), and CA3 (*c*) regions of rat hippocampal slice cultures. Arrows point to axons in each cell. Higher magnification of the CA1 pyramidal cell from *b* demonstrates that GFP expressed following electroporation completely fills dendritic spines (*d*, *open arrowheads*) and axonal varicosities (e, *closed arrowhead*). Bars: (*a*,*d*) 10 μm; (*b*,*c*) 50 μm; (*d*,e) 20 μm.

VISUAL SYSTEM DEVELOPMENT

Visual Stimulation Enhances the Development of Tectal Cell Dendrites In Vivo

W.-C. Sin, H.T. Cline

Extensive evidence indicates that sensory system stimulation is required for normal development of central connections pertaining to sensory information. Nevertheless, there has been no direct demonstration of an effect of visual system stimulation of the structural development of neurons within the visual projection. W.-C. Sin addressed this question by collecting time-lapse images of single optic tectal neurons in vivo under conditions in which the animals received a simulated motion stimulus. Sin compared the dendritic arbor growth rates of neurons during a 4-hour period without visual stimulus and a subsequent 4-hour period with visual stimulation. Light stimulation significantly increased dendritic arbor growth rates. The light-induced increase in dendritic arbor growth was abolished when the stimulus was provided in the presence of glutamate receptor blockers. The results indicate that visual stimulation has a direct effect on dendritic arbor growth rate and that this effect requires glutamatergic synaptic transmission.

Competition-based Dynamics of Retinotectal Axon Morphology in *Xenopus* Optic Tectum

E. Ruthazer, H.T. Cline

Axon terminal arbors in the developing brain are highly dynamic structures, capable of rapid morphological rearrangements. E. Ruthazer examined whether dynamics are related to competition-based mechanisms between arbors. He surgically ablated one tectal lobe, which causes the retinal axons that normally innervate that tectum to project to the remaining tectal lobe. The inputs to the dually innervated tectal lobe segregate into eye-specific zones of left-eye and right-eye afferents. Repeated time-lapse imaging of single retinal axons was performed on a two-photon microscope, and the dynamics of individual branches was related to their location within territory from the same or competing eye. Branches tended to be more stable if they were in territory dominated by the competing eye and more dynamic if they were in territory dominated by the same eye. The differences in dynamics were blocked by *N*-methyl-D-aspartate (NMDA) receptor antagonists. The data are consistent with the idea that dynamic branches occur in locations where arbors are actively growing.

Regulation of Dendritic Morphology by RhoA GTPases

Z. Li, H.T. Cline [in collaboration with L. Van Aelst, Cold Spring Harbor Laboratory]

Members of the Rho family of small GTPases, Rac, Cdc42, and RhoA, mediate morphological changes in neurons by regulating the cytoskeleton. Z. Li investigated the function of the GTPases Rac, Cdc42, and RhoA in regulating dendritic arbor development in vivo. She used time-lapse in vivo imaging combined with viral gene transfer to express either constitutively active or dominant negative forms of the GTPases in optic tectal neurons. Li found that decreasing RhoA activity promoted dendritic branch extension. Furthermore, NMDA receptor-mediated dendritic arbor elaboration appears to be mediated through RhoA. Rac and Cdc42 activities enhance the additions of small branches to the dendritic arbor. These data indicate that Rac activity promotes local branch additions to the dendrites. The new branches are maintained (or not) also as a result of Rac activity. Local decreases in RhoA activity, possibly triggered by synaptic NMDA receptor activity, promote the extension of the newly added branches. This sequence of events indicates that the different GTPases have distinct roles in controlling dendritic arbor elaboration.

CANDIDATE PLASTICITY GENES

Candidate Plasticity Genes

I. Cantallops, M. Davenne, L. Foa, K. Haas, K. Jensen, H.T. Cline, K. Bronson [in collaboration with P. Worley, Johns Hopkins University, and E. Nedivi, Massachusetts Institute of Technology]

Our experiments have demonstrated the important role of synaptic activity in promoting the development of the brain. One potential effect of synaptic activity is to induce gene transcription. The activity-induced genes may then promote the further development of the brain. We have continued our investigations of several activity-induced genes to test their potential function in brain development.

The activity-regulated candidate plasticity gene 15 (*cpg15*) encodes a glycosylphosphatidylinositol (GPI)-linked protein. E. Nedivi, G.-Y. Wu, and H.T. Cline previously demonstrated that CPG15 expression in vivo increases the dendritic arbor growth rate of *Xenopus* optic tectal cells. Expression of truncated CPG15, lacking its GPI anchor, does not promote dendritic arbor elaboration.

CPG15 Gene and Protein Expression

A. Javaherian, K. Bronson, H.T. Cline

A. Javaherian cloned *cpg15* from *Xenopus*. This gene is highly homologous to the *cpg15* gene from rats, cats, and humans. The *cpg15* message is highly expressed in retinal ganglion cells, but not in other cells in the retina. In the brain, the *cpg15* message is widely expressed in differentiated neurons. CPG15 protein is targeted to the axons of retinal ganglion cells and to axons of central nervous system neurons.

Postsynaptic Expression of CPG15 Promotes Synaptic Maturation In Vivo

K. Haas, H.T. Cline

K. Haas tested whether CPG15 expression affects synaptic maturation. He made whole-cell voltage-clamp recordings from optic tectal neurons in animals infected with vaccinia virus expressing either β-galactosidase, CPG15, or tCPG15. Haas demonstrated that CPG15 expression promotes synaptic maturation by increasing the α-amino-3-hydroxy-5-methyl-4-isoxazole (AMPA)/NMDA ratio and by decreasing the fraction of silent NMDA-R-only retinotectal synapses. Furthermore, CPG15 increases the frequency of AMPA-R-mediated miniature excitatory postsynaptic currents (mEPSCs). These data indicate that CPG15 enhances the maturation of retinotectal synapses by promoting the insertion of functional AMPA-R into synapses previously mediated solely by NMDA-R. Expression of tCPG15 appears to block the normal maturation of retinotectal synapses by preventing the delivery of functional AMPA-R to synapses. This interpretation is consistent with the decreased AMPA/NMDA ratios and increased fraction of pure NMDA-R synapses in mature neurons, as well as the decreased mEPSC frequency, seen with tCPG15 expression. These data indicate that one function of CPG15 is to promote

synapse maturation. CPG15 may also be required to maintain synapses, since the increased fraction of NMDA-R-only synapses seen with tCPG15 may be due to loss of AMPA-R from synapses.

Postsynaptic Expression of CPG15 Promotes Presynaptic Retinal Axon Elaboration In Vivo

I. Cantallops, H.T. Cline

To test the potential role of CPG15 in regulating presynaptic axonal arbor structure, I. Cantallops imaged single DiI-labeled retinal ganglion cell (RGC) axons within the optic tectum of *Xenopus laevis* tadpoles during a period of 2 days. Cantallops infected their postsynaptic partners, the optic tectal cells, with vaccinia virus expressing the reporter β-galactosidase, with virus expressing CPG15, or with virus expressing a truncated form of CPG15, lacking the GPI consensus sequence.

Cantallops identified two phases of RGC axonal growth in control axon: a fast-growing phase seen in axons with simple morphologies and a slower growing phase seen in more complex axons. Analysis of branch dynamics in simple and complex arbors demonstrates that once axons become more complex, branch dynamics change such that relative rates of branch retractions increase. Postsynaptic expression of CPG15 significantly increased the growth rate of presynaptic retinal axons, in a GPI-linkage-dependent manner. The growth-promoting effect of CPG15 was selective for complex axons, which normally have relatively slow growth rates. Postsynaptic expression of CPG15 enhances growth rates of complex presynaptic axons by maintaining them in a fast-growing and dynamic growth state, characteristic of simple axons.

SYNTHESIS OF OUR DATA ON CPG15

Brain development is characterized by a period of axonal and dendritic arbor elaboration and synaptogenesis. During this period, the elaboration of neuronal morphology and the formation of synaptic connections are intimately entwined. These observations suggest the existence of mechanisms that might coordinate several aspects of circuit formation. One potential scenario for the coordinated development of pre- and postsynaptic neuronal structures and synaptic connections within a circuit is as follows: Newly extended branches on axons and dendrites form synapses with only NMDA-R. As these synapses mature, AMPA-R is recruited to the synaptic sites. Mature AMPA-R-containing synapses stabilize the branches on which the synapses are located. Stabilized branches can then support the addition of new branches. These branches in turn establish new NMDA-R-only synapses, which are either stabilized through the addition of AMPA-R or retracted. As both tectal cell dendrites and RGC axons become more complex, the lifetime and stability of the arbor branches are determined by the strength of the synapses they make: Branches that have established mature synaptic contacts remain, whereas those that fail to add AMPA-R retract. In this way, during the growth phase of the circuit, the overall number of synaptic contacts is increased, as is the complexity of both axons and dendrites.

We propose that CPG15 expression in tectal neurons increases the elaboration of tectal cell dendrites and retinal axons through the same sequence of events including branch initiation, synapse formation, synapse maturation, and branch stabilization. The increased retinal axon arbor growth rate is accomplished by a significant and selective decrease in axon branch retractions. Therefore, a major mechanism of regulating arbor elaboration is through the stabilization of newly added branches. Correlated with the stabilization of axonal branches is an increase in the strength of retinotectal synaptic transmission following CPG15 expression. This suggests that the strengthening of synaptic contacts between axonal and dendritic branches stabilizes the axon arbor branches. Therefore, the increased dendritic and axonal growth rates occur hand in hand with the increased number of mature synapses.

Analysis of Axon Pathfinding In Vivo

L. Foa, K. Jensen, K. Bronson, H.T. Cline [in collaboration with P. Worley, Johns Hopkins]

The establishment of connections within the brain requires that the axonal growth cones navigate to the target region, recognize the target, and elaborate an

axon arbor. Axon guidance is dependent on the detection of environmental cues, the interpretation of the cues as attractive or repulsive, and the transduction of this signal to machinery governing growth cone motility. The Homer family of proteins has been postulated to act as cytosolic scaffold proteins, possibly linking cell surface transmembrane receptors and intracellular calcium stores. New experiments by L. Foa together with earlier experiments by I. Rajan provide evidence that Homer proteins perform a previously unrecognized function in axon pathfinding and target recognition in optic tectal neurons. We suggest that Homer forms part of a protein scaffold complex in axonal growth cones which links guidance receptors to machinery governing growth cone behaviors. K. Jensen is testing whether Homer proteins function in retinal axon guidance.

PUBLICATIONS

Cantallops I. and Cline H.T. 2000. Synapse formation: If it looks like a duck and quacks like a duck. *Curr. Biol.* **10:** R620–R623.

Cantallops I., Haas K., and Cline H.T. 2000. Postsynaptic CPG15 promotes synaptic maturation and presynaptic axon arbor elaboration in vivo. *Nat. Neurosci.* **3:** 556–562.

Li Z., Van Aelst L., and Cline H.T. 2000. Rho GTPases regulate distinct aspects of dendritic arbor growth in *Xenopus* central neurons in vivo. *Nat. Neurosci.* **3:** 217–225.

In Press

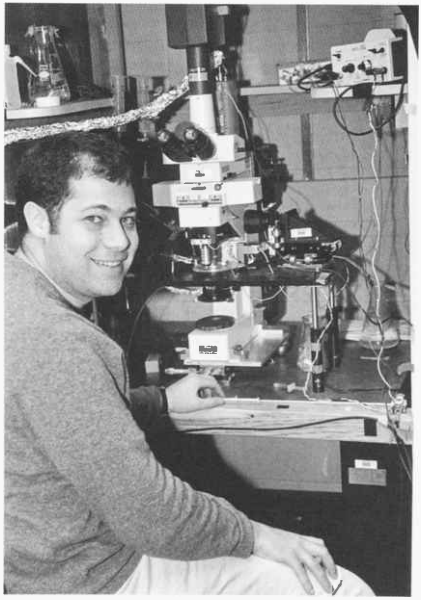
Cline H.T. 2001. Dendritic arbor development and synaptogenesis. *Curr. Opin. Neurobiol.* **11:** 118–126.

Dickson B.J., Cline H.T., Polleux F., and Ghosh A. 2001. Meeting report: New directions in axon guidance. *EMBO J.* (in press).

Foa L., Rajan I., Haas K., Wu G.-Y., Brakeman P., Worley P., and Cline H. 2001. The scaffold protein, Homer1b/c, regulates axon pathfinding in the CNS *in vivo*. *Nat. Neurosci.* **4:** 1–8.

Haas K., Sin W.-C., Javanerian A., Li Z., and Cline H.T. 2001. Single cell electroporation for gene transfer in vivo. *Neuron* **29:** 1–9.

Nedivi E., Javaherian A., Cantallops I., and Cline H.T. 2001. Developmental regulation of CPG15 expression in *Xenopus*. *J. Comp. Neurol.* (in press).



C. Aizenman

SIGNAL TRANSDUCTION AND DIFFERENTIATION

G. Enikolopov J. Hemish, S. John, P. Krasnov, B. Kuzin, T. Michurina, J. Mignone, B. Mish, M. Packer, N. Peunova, I. Pugach, V. Scheinker, Y. Stasiv, A. Vaahtokari, K. Vogeli, N. Nakaya

The long-standing interests of our lab are the signals that guide the cells in the developing and adult organism along pathways of differentiation and how these signals are linked to cell activity. Our efforts have been focused mostly on nitric oxide (NO), a versatile signaling molecule. During the last several years, we have found strong evidence that NO is important for tissue differentiation in a variety of models of animal development, acting as an essential negative regulator of cell proliferation in the developing tissue. We are now focusing on the genetic and molecular interactions of NO with the major signaling pathways in the differentiating cell, especially those that control cell proliferation. Our studies of NO have led us closer to the questions about the biology of stem and progenitor cells, which has now become an important new area of interest for our laboratory. We have generated several mouse models in which selected classes of neural stem cells are marked, and we are using these mice to investigate the origins and plasticity of neural stem cells.

NO and Gene Expression

J. Hemish, B. Mish, N. Nakaya

Many studies of NO signaling have focused on the immediate targets of NO in the cell. However, less is known about the molecular mechanisms involved in long-term effects of NO, such as cessation of cell proliferation, apoptosis, or acquisition of differentiated phenotype, which are presumably due to the ability of NO to affect gene expression. We have used immobilized cDNA arrays to identify NO-induced changes in gene expression and to discern the signaling cascades that mediate the long-term effects of NO. Several gene targets were discovered and NO-induced changes in their expression were validated by Northern or reverse transcriptase–polymerase chain reaction (RT-PCR) analysis. We have found that a subset of these genes is dependent on p53 for activation by NO, as revealed by using cells from knockout mice deficient in p53, whereas other subsets depend on guanylate cyclase, protein kinase C, and PI3-kinase activity, as revealed by using selective inhibitors of these enzymes. More recently, we have extended our studies by using printed microarrays composed of 7000 known genes and expressed sequence tags (ESTs). Results from these experiments have confirmed previously known gene targets of NO, supporting the feasibility of this approach. Furthermore, we have also identified additional new genes whose expression levels are affected by NO. We are currently validating and further characterizing these new candidates. By identifying an extended set of genes whose expression is affected by NO, we seek to determine the direct and indirect targets of NO-related signaling cascades.

NO and *Drosophila* Development

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

NO is directly involved in organ development, synaptogenesis, and response to hypoxia in *Drosophila*. Flies have only one NOS gene, *dNOS*, which is an ortholog of the mammalian neuronal NOS. We found that to a large degree, *dNOS* expression in *Drosophila* is regulated on the posttranscriptional level through the use of alternative splicing. We identified four new alternatively spliced transcripts of the *dNOS* gene. Structurally, all splicing events in the *dNOS* locus can be described as single and multiple exon insertions or deletions and as alternative usage of the splicing sites. Alternative splicing affects both the 5′-end untranslated regions and the

coding region of the gene. All splicing events in the coding region of the gene lead to a premature termination of the open reading frame and production of truncated proteins that lack various carboxy-terminal regions of *dNOS1* (the full-length product). As a result, none of alternative transcripts code for the enzymatically active protein. However, these truncated proteins very effectively inhibit enzymatic activity of the full-length *dNOS1*, when coexpressed in cultured cells, thus acting as dominant negative regulators of NO production. One of the alternative transcripts, *dNOS4*, is widely expressed during fly development. We have shown that overproduction of *dNOS4* in developing *Drosophila* eye under the control of the GMR promoter suppresses the antiproliferative action of *dNOS1*, causing a severe hyperproliferative eye phenotype in adult flies. To further investigate the biological role of *dNOS4*, we generated heat shock promoter-driven *dNOS4* and upstream activation sequence (UAS) (Gal4 target)-driven *dNOS4* transgenic flies and are now studying the consequences of tissue- and organ-specific expression of the *dNOS4* dominant negative regulator on *Drosophila* development.

Xenopus laevis NOS Gene

V. Scheinker, Y. Stasiv, N. Peunova

We have cloned NOS cDNA from *Xenopus laevis* (*XNOS*) and showed that it is an ortholog of the mammalian neuronal isoform of NOS. We found five different transcripts that originate from the *XNOS1* gene. They are generated due to five unique variants of XNOS exon 1 (exons 1A to 1E) which are expressed in a tissue- and developmental-stage-specific manner. To confirm the presence of these alternative exons and of multiple regions of transcription initiation, we cloned and mapped the *XNOS* gene from the *Xenopus* genome. Our data demonstrate a high complexity of the *XNOS* gene structure. The five variants of exon 1 span a region of the genome of at least 100 kb. We used in situ hybridization with each of the variant exon 1 probes and found that their expression is highly tissue- and developmental-stage-specific. The appearance of some of the transcripts, for instance, marks the zygotic induction of the genome, and the appearance of others marks neurulation, organogenesis, etc. Our findings suggest that the biological activity of NO is tightly and specifically regulated during *Xenopus* development by a complex pattern of alternative transcription initiation and splicing.

NO and Early *Xenopus* Development

N. Peunova, V. Scheinker, K. Vogeli

In situ hybridization with *XNOS* probes reveals a complex pattern of alternative promoter activation during early *Xenopus* development. Remarkably, we found that only one of the alternative sites of transcription initiation is used in unfertilized oocytes, producing maternal *XNOS* RNA. Further examination of this maternal transcript demonstrated that it is localized exclusively at the animal pole of the oocyte in a highly asymmetric pattern. This is the first indication that NOS transcripts are present as maternal RNA during organism development. We have generated dominant negative inhibitory *XNOS* variants, and we are currently using these recombinant inhibitors in parallel with chemical inhibitors of NOS to investigate whether maternal *XNOS* may be important for setting the dorsoventral axis of the embryo.

Various alternative transcripts of *XNOS* appear at key steps of early *Xenopus* embryo development and during organogenesis. We have used the tools mentioned above to alter NOS activity in order to investigate the potential role of NO in setting of the germ layers of the embryo and control of cell proliferation during gastrulation. Together, our data suggest that NO may be used at a number of critical steps in *Xenopus* development, extending back from our previous demonstration that NO has an essential antiproliferative role during *Xenopus* brain development.

NO and Mouse Hematopoiesis

T. Michurina, P. Krasnov

We are studying the role of NO in the regulation of hematopoietic maturation in vivo. We have found that NO has a critical function during early stages of hematopoiesis such that we can change the proportion of stem and early progenitor cells in the bone marrow by regulating production of NO. To understand the

mechanisms of NO function in the bone marrow, we searched for the expression of specific isoforms of NO synthase in the bone marrow. Using RT-PCR, we were able to demonstrate the presence of mRNA transcripts for all three NOS isoforms—neuronal, endothelial, and inducible—in mouse bone marrow cells and human umbilical cord blood cells. We characterized the expression of particular NOS isoforms in restricted cell populations in the bone marrow. We have isolated subpopulations of bone marrow cells that are greatly enriched in hematopoietic stem and early progenitor cells. When lysates from such cell populations were examined, Western blots revealed a dramatic enrichment in specific NOS isoforms. Thus, NOS protein is synthesized in hematopoietic stem and progenitor cells, supporting our hypothesis that regulation of NO production has an important role in the control of hematopoietic cell proliferation.

Targeted Disruption of the Active Center of the Mouse Neuronal NO Synthase

Y. Stasiv [in collaboration with A. Grinberg and H. Westphal, NICHD, National Institutes of Health]

To test the role of neuronal NO synthase (nNOS) in development, we decided to inactivate the mouse *nNOS* gene by homologous recombination. Previous attempts to mutate the *nNOS* gene in the mouse genome targeted exon 2 of the gene. The resulting mutants, while providing an invaluable research model, still produce variant *nNOS* isoforms with low levels of enzyme activity. In our experiments, we have chosen to target exon 6 which codes for the essential heme-binding domain. In preliminary experiments with cultured cells, we have proven that with the removal of exon 6, the enzymatic activity of *nNOS* is undetectable. To mutate the *nNOS* gene, we cloned mouse genomic fragments surrounding the sixth exon of the gene into the pPNT vector, introduced them into 129/SVJ embryonic stem (ES) cells, selected transformed clones using G418 and ganciclovir, and identified properly targeted clones by PCR and Southern blots. Two lines of germ-line-transmitting mice from two independently derived ES cell clones were generated and used to obtain homozygous *nNOS*(–/–) mutants. We are currently analyzing the phenotypes of these *nNOS* knockouts, focusing on the patterns of cell division during development.

NO in Neuronal Differentiation and Cell Death in the Brain

M. Packer [in collaboration with S. Goldman, Cornell Medical School]

NO has been shown by our group to be an important modulator of cell growth in various models. We are looking at how this small free radical molecule controls the proliferation, differentiation, and survival of neural cells in adult animals. We have developed transgenic mice that overexpress the neuronal form of NO synthase under the calcium/calmodulin protein kinase type IIα (CaMKIIα) promoter. This promoter is strongly activated beginning 2 weeks postnatally in the brain regions close to and overlapping with the zones of adult neurogenesis and remains active throughout adult life. We are using these mice to look at the effects of NO on the life of the new cells in these regions. We are using surgical placement of miniosmotic pumps to infuse the brains of adult animals with drugs that modulate the activity of NO synthase. With these two systems combined with the use of bromodeoxyuridine (BrdU), a reagent used to label dividing cells, serial sectioning, immunohistochemistry, and various imaging techniques, we have shown that NO exerts a cytostatic influence on the pool of progenitor cells. Furthermore, under some treatments, we have been able to dramatically increase the rates of proliferation of cells in the brain areas that normally have very little adult neurogenesis. Our results suggest that it may be possible to use NO-related compounds as therapies for treating neurodegenerative disorders or trauma where benefits might be gained by repopulation of neural cells.

Neural Stem and Progenitor Cells in the Developing and Adult Brain

J. Mignone

We have generated transgenic mice that express green fluorescent protein (GFP) under the temporal and spatial control of the nestin gene regulatory elements. These mice allow the selective marking of neural stem and progenitor cells by GFP expression. We are now in a position to study the biological roles that these cells perform in the brain throughout the life of the animal, by being able to visualize and follow individual stem

cells while they are still alive. This work is further supplemented by the use of transgenic lines that have been generated in our lab and that use differential labeling of various lineages of the brain cells. By using mice with differentially labeled subgroups of cells, we will be able to pursue the fate of a neural stem cell. Currently, with Mike Packer in the lab, we are performing transplantation of the nestin-GFP cells to the brains of recipient animals to further understand the plasticity of these cells as well as their ability to functionally integrate into the recipient neural network. Furthermore, in collaboration with Sang Yong Kim here at the Laboratory, we have been transplanting the nestin-GFP cells to mouse blastocysts to determine the true potential of these cells. We want not only to understand their ability to initiate various neuronal lineages, but also to discern to what degree these cells are multipotent and are able to contribute to other, non-neuronal, lineages in the developing organism.

A Transgenic Mouse Model for Studying Cell Cycle Progression of Neural Stem and Progenitor Cells

A. Vaahtokari

To gain insight into the regulation of the cell cycle of neural stem and progenitor cells, we generated transgenic mice that express a modified version of GFP in neural stem cells in a cell-cycle-dependent manner. When the expression pattern of modified GFP was analyzed in embryonic and adult transgenic mice, several striking features were discovered, including clustering of dividing cells in the developing nervous system. We are currently testing the hypothesis that the distribution of GFP in these transgenic animals reflects the synchronous cell cycle of clonally related neural stem and progenitor cells. We have also developed an in vitro method for growing embryonic cortical explants that is suitable for time-lapse imaging. Thus, we can analyze dynamic changes in the expression of GFP in neuronal tissue. In addition, we are using our tissue culture system to study the effects of various factors on cell cycle progression of neuronal stem and progenitor cells, e.g., epidermal growth factor and fibroblast growth factor.

PUBLICATIONS

Krasnov P.A. and Enikolopov G. 2000. Targeting of synaptotagmin terminals in differentiated PC12 cells. *J. Cell Sci.* **113:** 1389–1404.

Kuzin B., Regulski M., Stasiv Y., Scheinker V., Tully T., and Enikolopov G. 2000. Nitric oxide interacts with the retinoblastoma pathway to control eye development in *Drosophila*. *Curr. Biol.* **10:** 459–462.

Nakaya N., Lowe S.W., Taya Y., Chenchik A., and Enikolopov G. 2000. Specific pattern of p53 phosphorylation during nitric oxide-induced cell cycle arrest. *Oncogene* **19:** 6369–6375.

CONSTRUCTION AND EXPERIENCE-DEPENDENT REFINEMENT OF NEOCORTICAL CIRCUITS

Z.J. Huang H. Higashiyama
J. Walls
B. Chattopadhyaya

We are interested in understanding the basic design principles of the neocortical circuits and the role of experience in shaping the function of these circuits. Although the cellular architecture of the neocortex is exceedingly complex, there are well-defined cell types and stereotyped synaptic connectivity patterns that are strikingly conserved between different cortical areas and among different mammalian species. One of the best examples of such conservation comes from the GABAergic inhibitory circuits, which control cortical excitability and information processing at precise spatial and temporal domains. A fascinating yet puzzling feature of the cortical GABAergic system is the rich array of interneuron cell types. These cell types often display remarkably distinct morphology, intrinsic physiological properties, synaptic input and output patterns, and gene expression profiles. In addition, the functional maturation of the GABAergic system is strongly influenced by experience deprivation during early postnatal ages and, as a result, may contribute to the experience-dependent refinement of neocortical circuits. Our general hypotheses are that (1) distinct GABAergic cell types have evolved to execute defined types of inhibitory mechanisms which regulate specific aspects of the development and function of cortical circuits and (2) there are molecular mechanisms that determine the highly stereotyped synaptic innervation patterns among different GABAergic cell types.

To test these hypotheses, it is necessary to manipulate the physiological property and visualize the morphology of defined types of GABAergic interneurons in living tissue and in vivo. For decades, GABAergic cell-type-specific manipulations have not been possible. We are now using the mouse genetic approach, including bacterial artificial chromosome (BAC) transgenics and knockins, to achieve such cell-type-specific manipulations. Our strategy is based on the observation that an increasing number of genes are shown to be expressed in distinct classes of GABAergic interneurons. BACs containing the transcriptional promoters of these genes can therefore be engineered to express either a functional gene or a green fluorescent protein (GFP)-based fluorescent marker in these specific cell types. Combined with physiological and imaging techniques, we are studying the following set of questions in the mouse visual cortex: How do different types of interneurons mature morphologically and physiologically during the critical period of visual cortical development? What are the effects of visual deprivation on the maturation of GABAergic circuits? How do neurotrophic factors regulate the experience-dependent maturation of GABAergic interneurons? How do specific types of GABAergic circuits contribute to critical period plasticity?

Maturation of GABAergic Circuits during the Critical Period

H. Higashiyama, J. Walls, Z.J. Huang [in collaboration with K. Svoboda, Cold Spring Harbor Laboratory]

In the visual cortex, the fast-spiking (FS) basket-type interneurons send off horizontal axons, selectively innervate the soma of target neurons, are capable of high-frequency nonadaptive firing, and express parvalbumin. On the other hand, the low-threshold spiking (LTS) bitufted cells send off vertical axons, selectively innervate the distal dendrites of targets, fire broader spikes that show pronounced adaptation of firing frequency, and express somatostatin. How do GABAergic interneurons develop such distinct morphology, synaptic connectivity, and physiologic properties? To address these questions, we are using a series of GABAergic transcriptional promoters to systematically label specific GABAergic cell types with GFP variants using BAC transgenic mice. These promoters include GABA synthetic enzymes (GAD67), calcium-binding proteins, and neuropeptides. For example, we have constructed BACs expressing yellow fluorescent protein (YFP) under the control of the

somatostatin promoter, and GAD67 promoter. We have also constructed BACs expressing GFP under the control of the parvalbumin promoter and have produced transgenic founders (Pv-GFP). These transgenic mice will allow us (1) to characterize the intra- or interlaminar patterns of axon growth in basket and bitufted interneurons, (2) to characterize the development of somatic versus distal dendritic patterns of synaptic innervation in basket and bitufted interneurons, (3) to examine the maturation of distinct firing properties of these interneuron cell types, and (4) to examine the effects of visual deprivation (e.g., dark rearing) on the maturation of these interneuron cell types.

Role of GABAergic Inhibition in Critical Period Plasticity in the 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 the 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). However, the specific types of GABAergic circuits and inhibitory mechanisms involved in the regulation of OD plasticity remain unknown. Because GAD65$^{-/-}$ mice do not show critical period plasticity 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 constructed BACs expressing GAD65 under the control of the parvalbumin promoter and have produced transgenic founders. The same strategy is being applied to the somatostatin promoter. We will introduce BAC transgenes expressing GAD65 in either the basket or bitufted interneuons 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.

Role of BDNF in the Experience-dependent Maturation of GABAergic Circuits

B. Chattopadhyaya, Z.J. Huang

The brain-derived neurotrophic factor (BDNF) has been an attractive candidate for mediating activity-dependent plasticity. The production of BDNF in the visual cortex is stimulated by neuronal activity and by visual experience. BDNF in turn has potent effects on neurite growth and synaptic strength. We have shown previously that BDNF regulates the critical period of OD plasticity in part by promoting the maturation of GABAergic inhibitory circuits. However, the specific GABAergic cell types that respond to BDNF and the cellular mechanisms of BDNF regulation remain unclear. BAC transgenic mice expressing GFP in specific GABA cell types will allow us to address these questions. For example, we hypothesize that BDNF regulates the synaptic innervation of basket interneurons to pyramidal neurons during the critical period. To test this, we will prepare visual cortical slices at different ages from Pv-GFP transgenic mice. We will then cotransfect cortical pyramidal neurons with constructs expressing BDNF and RFP (red fluorescent protein) or RFP alone. The low frequency of biolistic transfection allows single RFP-transfected pyramidal neurons to be visualized in the background of GFP-labeled GABAergic neurons. This will allow us to answer the following questions: Is the effect of BDNF on promoting GABAergic innervation "cell autonomous," or does it also spread to nearby non-BDNF overexpressing neurons? Is the effect of BDNF on promoting GABAergic innervation restricted to the soma region of transfected neurons or does it also occur in apical and basal dendrites? What is the time course of BDNF-stimulated GABAergic innervation? This preparation will also allow pharmacological treatment to study the role of neuronal activity and signaling pathways in BDNF-stimulated GABAergic innervation.

Cre/loxP Recombination-activated Neuronal Markers in Mouse Brain

Z.J. Huang

We are developing a Cre/loxP recombination-activated GFP marker system in the mouse brain. This binary

system consists of a reporter strain and a deleter strain. In reporter mice, the expression of a reporter gene is driven by a strong and ubiquitous promoter but is prevented from expression by the presence of a transcription STOP cassette. In deleter mice, the Cre recombinase is driven by a cell-type-specific promoter. In a double transgenic strain, the expression of the GFP-based reporter gene is activated by the removal of transcription STOP cassette upon Cre/loxP recombination. The advantages of such a system include its combinatorial power and compatibility with a Cre/loxP-mediated gene knockout. We have previously demonstrated the feasibility of this system using the dendrite-targeted GFP reporter strain. We have now produced a reporter strain in which a fusion gene expressing a membrane-targeted GFP (which is expected to label the entire neuron) is "knocked-in" to the strong and ubiquitous Rosa26 locus. We are now breeding this strain with several lines of Cre transgenic mice to access the quality of cell labeling in this reporter strain.

J. Walls

NEURAL ORGANIZATION OF OLFACTORY PERCEPTION AND BEHAVIOR

Z. Mainen S. Macknik V. Egger
R. Gasperini N. Caporale
N. Uchida

Our laboratory is working toward understanding the general principles of neural organization that allow organisms to perceive, remember, anticipate, and act on complex sensory information. The goal is an integrative understanding that relates information processing at the microscopic level (individual synapses and neurons) to its macroscopic function. Our research is currently centered on the olfactory system. The principles of chemical sensation are still poorly understood, but the olfactory system offers numerous experimental advantages including known receptor genes linked directly to the initial encoding of stimuli, accessibility by optical imaging techniques, a simpler neuronal architecture compared to the neocortex, and a very direct connection between sensory periphery and central processing.

Neural Correlates of Olfactory Perception

S. Macknik, N. Uchida, Z. Mainen

Why do flowers smell more similar to one another than to trees? What is the neural basis for the perceived qualities, similarities, and differences between odors? To address these questions, we are combining optical imaging techniques to probe neural representations with human and rat psychophysical techniques to quantify perceptual characteristics of odors. Because each glomerulus in the olfactory bulb is targeted by neurons expressing a single olfactory receptor type, it is thought that the olfactory glomeruli form a map of chemical structure. Any odor, either a single molecule or a mixture of many different molecules, activates a specific subset of odor receptors and hence a particular pattern in the olfactory bulb. Because the location of particular receptors within the glomerular map is preserved from individual to individual, the receptor map could provide the basis for the invariant perceptual qualities of odors. Our main hypothesis is that perceptual qualities of odors are direct correlates of the spatial patterns of activity produced across the glomerular input layer to the olfactory bulb (Fig. 1). Alternative hypotheses include the possibility that such perceptual maps are only formed later in the olfactory pathway, such as in the olfactory cortex, and the possibility that perceptual qualities are not directly related to any kind of spatial map, but instead rely on nonspatial factors such as synchronization of neur-

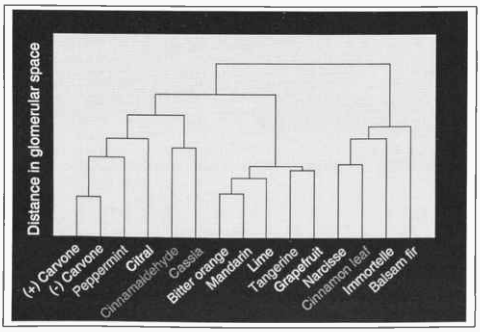


FIGURE 1 Do glomerular representations in the olfactory bulb code for the qualities of odor sensations? This figure plots the similarity of glomerular responses to several natural odorants (measured in the anesthetized rat with intrinsic signal imaging). It can be seen that odorants with similar sensations (e.g., citrus fruits) produced more similar glomerular patterns.

al activity. We are testing the main hypothesis by comparing the perceptual discriminability of pairs of odors, measured by errors made by rats in a behavioral task, with the similarity of their glomerular activity patterns, measured by intrinsic imaging of odor-evoked responses in anesthetized rats.

Neural Representation of Individual Identity

N. Caporale, Z. Mainen

Do "grandmother" neurons exist? Primate social behavior relies heavily on visual (and auditory) communication. Recent work in monkeys and humans has revealed neural systems that appear to be devoted specifically to processing socially significant stimuli such as eyes and faces. The origins of social behavior lie in chemical communication. In the rodent, both the main olfactory and the vomeronasal system are known from behavioral studies to be major channels of social information, but very little is known about the neural mechanisms underlying social olfactory function. Using a combination of behavior, intrinsic imaging, and neurophysiology, we are studying the neural basis of one important building block of social organization, the recognition of individual identity.

Psychophysics of Human and Rat Olfactory Quality

S. Macknik, Z. Mainen

How different are two odors from each other? Standard psychophysical measurements involve finding the perceptual difference between two intensities of the same stimulus. The way one does this is by adding (or taking away) more and more stimulus until the subject notices that the stimulus has changed in perceptual intensity. This unit, in which the subject notices the smallest possible difference between intensities, has been named the just-noticeable-difference (JND). We are extending the concept of the JND to mixtures of equally intense but qualitatively dissimilar odors. We systematically vary the mixture of two odorants from a single pure odorant through a series of stepwise mixtures of the two odorants, until we arrive at the other pure odorant. The task of the subject is to notice when the mixture has changed. By counting the number of JNDs there are between two pure odors, we can obtain a *quantitative* measure of the *qualitative* difference between those odors. These experiments are being carried out first in humans and will subsequently be transferred to a similarly designed rodent behavioral paradigm. Finally, we will optically image the olfactory bulb of rats while presenting the same stimuli in order to measure the physiological change associated with a minimal qualitative perceptual change.

Coupling of Blood Flow to Neural Activity

N. Uchida, Z. Mainen

Several important modern imaging techniques, such as fMRI and intrinsic optical imaging, make use of correlations between neural activity and blood flow. It is apparent that blood flow parameters such as the relative concentration of oxyhemoglobin and deoxyhemoglobin are modulated by the level of local neural activity, but the details of the relationship are still mostly unknown. Our aim is to describe a quantitative relationship between neural activity parameters such as presynaptic release rate or postsynaptic firing and these blood flow signals. To do so, we are using a combination of intrinsic optical imaging and pharmacology in the olfactory bulb (Fig. 2). Because of the unique anatomy of this preparation, it is possible to pharmacologically manipulate the olfactory glomerular signals without fear of interfering with upstream circuitry (as is a major concern in the neocortex). The results of these studies will have implications not only for the interpretation of optical signals in the olfactory bulb, but more generally for our understanding of the relation of blood flow imaging to the underlying neural activity.

Two-photon Imaging of Calcium Dynamics of a Rat Olfactory Bulb Synapse

V. Egger, Z. Mainen

The goal of this project is a better understanding of early olfactory processing, in which the large dendro-

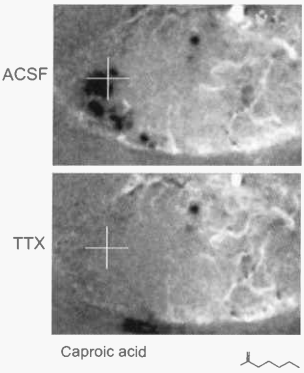


FIGURE 2 Pharmacology of intrinsic signal images from olfactory bulb. Control solution (ACSF) and subsequently solution containing tetrodotoxin (TTX, 20 μM) were pressure-injected at a site in the anterior OB (cross). TTX injection produced local blockade of blood flow responses.

dendritic reciprocal synapse between mitral cells and granule cells is a main player. The approach is based on simultaneous application of two-photon imaging of calcium dynamics and conventional electrophysiology of synaptic transmission in vitro. Given a long-term goal of understanding the nature of neuronal processing of olfactory information in the bulb, two lines of research are ongoing: First, we aim to measure the influence of the emotion- and arousal-related neuromodulator norepinephrine on calcium transients and on long-term plasticity in the mitral cell–granule cell synapse. There are strong neuromodulatory projections into the olfactory bulb, and neuromodulatory action on this synapse has been implicated in olfactory learning and memory.

Second, we aim to investigate transmission at the mitral cell–granule cell reciprocal synapse in the context of a more intact circuit, including the influence of distributed glomerular activation and centrifugal inputs from the olfactory cortex. To do so, we are developing an "integrative" slice preparation containing extrabulbar elements including olfactory cortex or the olfactory epithelium. Together, these two lines of research will help to elucidate the function of an important synapse in olfactory function.

PUBLICATIONS

Mainen Z.F. 2000. Thinking about feeling. *Nat. Neurosci.* **3:** 857.

Malinow R., Mainen Z.F., and Hayashi Y. 2000. LTP mechanisms: From silence to four-lane traffic. *Curr. Opin. Neurobiol.* **10:** 352–357.

Maravall M., Mainen Z.F., Sabatini B.L., and Svoboda K. 2000. Estimating intracellular calcium concentrations and buffering without wavelength ratioing. *Biophys. J.* **78:** 2655–2667.

TRANSMISSION AND PLASTICITY IN MAMMALIAN CENTRAL SYNAPSES

R. Malinow A. Barria, N. Dawkins, I. Ehrlich, J. Esteban, Y. Hayashi, F. Kamenetz, A. Piccini, K. Seidenman, S. Shi, T. Takahashi, J. Zhu, J. D'amore, P. Waseling

This laboratory addresses issues 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. This allows us to examine a cellular and molecular basis for changes in electrophysiological function. It is our philosophy that synapses have key properties whose understanding will provide insight into phenomena at higher levels of complexity.

The main result of this year was to begin to decipher the mechanisms by which glutamate receptors are delivered to synapses during plasticity. We find that during long-term potentiation (LTP), the GluR1 subunit of α-amino-3-hydroxy-5-methyl-4-isoxazole receptors (AMPA-Rs) has a critical role: It must interact with PDZ domain proteins for LTP to occur. A similar process occurs during synaptic potentiation early in postnatal development. This involves the synaptic delivery of GluR4-containing AMPA-Rs; this is driven by "spontaneous" neural activity. Once these GluR4-containing AMPA-Rs are delivered, they can be replaced by a different class of AMPA-Rs, those containing GluR2. This subunit-specific delivery and replacement can account for long-term changes in synaptic transmission.

DELIVERY OF GLUTAMATE RECEPTORS TO SYNAPSES DURING LTP

To elucidate mechanisms controlling and executing activity-dependent synaptic plasticity, AMPA-Rs with an electrophysiological tag were expressed in hippocampal neurons. LTP or increased activity of the calcium/calmodulin-dependent protein kinase II (CaMKII) induced delivery of tagged AMPA-Rs into synapses. This effect was not diminished by mutating the CaMKII phosphorylation site on the GluR1 AMPA-R subunit, but was blocked by mutating a predicted PDZ domain interaction site. These results show that LTP and CaMKII activity drive AMPA-Rs to synapses by a mechanism that requires the association between GluR1 and a PDZ domain protein. We are currently trying to identify which PDZ domain proteins interact with GluR1 to bring about LTP.

SPONTANEOUS ACTIVITY DELIVERS GLUR4-CONTAINING AMPA-Rs INTO SILENT SYNAPSES DURING EARLY DEVELOPMENT

Spontaneous activity early in postnatal development is crucial for the formation of functional neuronal circuits. During this early period, many glutamatergic synapses contain only *N*-methyl-D aspartate receptors (NMDA-Rs) and are largely "silent," becoming functional by acquiring AMPA-Rs. In this series of studies, we show that spontaneous oscillatory activity in the hippocampus makes silent synapses functional by selectively delivering GluR4-containing AMPA-Rs. This differs from LTP seen in older animals, which delivers GluR1-containing AMPA-Rs and requires CaMKII activity. Consistent with this special role, GluR4 expression in the hippocampus is largely restricted to the first postnatal week. Once delivered by activity, synaptic GluR4–AMPA-Rs are exchanged with GluR2-containing AMPA-Rs in a manner that requires little neuronal activity and maintains the enhanced strength of the synapse despite protein turnover. This "delivery and exchange" represents a new form of activity-dependent long-term synaptic plasticity that may be responsible for the initial establishment of functional neuronal circuitry.

SYNAPTIC DELIVERY OF NMDA RECEPTORS

NMDA-Rs have a critical role in plasticity, distinct from AMPA-Rs. NMDA-Rs likely have a different set of

rules governing their trafficking. Thus, we have developed optical and electrophysiological tools to monitor and perturb the delivery of NMDA-Rs to synapses. We use gene-gun technology to deliver engineered NMDA subunit genes into neurons. Optically, we can monitor the appearance of green fluorescent protein (GFP)-tagged NMDA-R subunits at spines. As an electrophysiological monitor of receptor delivery, we have developed an "electrophysiologically tagged" NMDA-R. We engineer the NR1 pore so that it is insensitive to Mg^{++}, and this renders the receptor voltage insensitive; thus, when this receptor reaches synapses, an NMDA-R-mediated response can be seen at –60 mV. We are using these tools to determine the mechanisms underlying the delivery of NMDA-Rs to synapses.

QUANTITATIVE OPTICAL MEASUREMENTS OF RECEPTOR DELIVERY TO SPINES

We have developed quantitative techniques to measure the relative amounts of recombinant GFP-tagged proteins within small neural structures. To achieve this, we first deliver recombinant genes of interest encoding GFP-tagged proteins into neurons using gene-gun or viral transfection. After expression is allowed to take place, the neurons expressing the GFP-tagged protein are identified, and a vital dye, Texas Red, is delivered intracellularly using a whole-cell patch pipette. After the dye is allowed to equilibrate within the cell (~30 minutes), we obtain two-photon scanning laser microscopic three-dimensional images, capturing emitted photons in the green (GFP) as well as the red (Texas Red) channels. We can use the red channel to demarcate the volume anatomy of small structures, whereas with the green channel, we can determine how much of the GFP-tagged protein is in this structure. With this technique, we have shown that AMPA-Rs traffic differently, depending on their composition. GluR1-containing AMPA-Rs are delivered throughout dendritic arbors, but they are restricted from entering dendritic spines, sites of synaptic contacts. In contrast, GluR2-containing AMPA-Rs traffic to dendrites and dendritic spines. We are using this technique to characterize where proteins go and do not go, and how much variability there is within a single neuron.

β-AMYLOID AS A HOMEOSTATIC NEGATIVE REGULATOR OF SYNAPTIC TRANSMISSION

We are testing the hypothesis that β-amyloid acts as a homeostatic negative regulator of synaptic transmission. That is, we propose that (increased neural activity)→(increased β-amyloid formation)→(decreased synaptic transmission)→(decreased neural activity). In support of this, we find that organotypic slices secrete less β-amyloid if transmission is depressed and they secrete more β-amyloid if transmission is enhanced. Furthermore, we find that acute overexpression of amyloid precursor protein (APP) in neurons leads to increased secretion of β-amyloid and depression of synaptic transmission onto those neurons. The physiological effect (depression of synaptic transmission) requires formation of β-amyloid from APP because (1) mutations in APP that prevent β-amyloid formation block this effect and (2) drugs that block β-amyloid from APP (γ-secretase inhibitors) also block this effect. Furthermore, if neural activity is pharmacologically depressed onto these APP-expressing neurons, then normal transmission is preserved. If this hypothesis is correct, it will have a significant impact on the proposed therapeutic use of agents that reduce β-amyloid secretion for Alzheimer's disease.

In conclusion, we are continuing to elucidate the basic mechanisms involved in central synaptic transmission and plasticity.

PUBLICATIONS

Carlezon W.A., Jr., Haile C.N., Coopersmith R., Hayashi Y., Malinow R., Neve R.L., and Nestler E.J. 2000. Distinct sites of opiate reward and aversion within the midbrain identified using a herpes simplex virus vector expressing GluR1. *J. Neurosci.* **20:** RC62.

Hayashi Y., Shi S.H., Esteban J.A., Piccini A., Poncer J.C., and Malinow R. 2000. Driving AMPA receptors into synapses by CaMKII or LTP and CaMKII: Requirement for GluR1 and PDZ domain interaction. *Science* **287:** 2262–2267.

Malinow R., Mainen Z.F., and Hayashi Y. 2000. LTP mechanisms: From silence to four-lane traffic. *Curr. Opin. Neurobiol.* **10:** 352–357.

Zaman S.H., Parent A., Laskey A., Lee M.K., Borchelt D.R., Sisodia S.S., and Malinow R. 2000. Enhanced synaptic potentiation in transgenic mice expressing presenilin 1 familial Alzheimer's disease mutation is normalized with a benzodiazepine. *Neurobiol. Dis.* **7:** 54–63.

Zhu J.J., Esteban J.A., Hayashi Y., and Malinow R. 2000. Postnatal synaptic potentiation: Delivery of GluR4-containing AMPA receptors by spontaneous activity. *Nat. Neurosci.* **3:** 1098–1106.

In Press

Shi S.-H., Hayashi Y., Esteban J., and Malinow R. 2001. Subunit-specific rules governing AMPA receptor trafficking to synapses in hippocampal pyramidal neurons. *Cell* (in press).

PLASTICITY OF CORTICAL NEURONS AND THEIR CIRCUITS

K. Svoboda B. Burbach, B. Chen, K. Greenwood, M. Maravall, E. Nimchinski, P. O'Brien, T. Oertner, T. Pologruto, B. Sabatini, E. Stern, R. Yasuda, C. Zhang, K. Zito

The neocortex underlies most cognitive functions in mammals. Even though these functions are extremely diverse, the underlying anatomy is relatively uniform across functional areas and is arranged in a modular fashion into columns. However, cortical tissue is dauntingly complex: 1 mm^3 of tissue contains nearly 1 million neurons, each of which connects to thousands of other neurons. How does electrical and biochemical dynamics in this awesome network produce our perception of the world? How does this network change in response to experience? Answers to these questions will profoundly change our understanding of the function and diseases of the brain.

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. For this purpose, we build and use sensitive optical tools: Two-photon laser-scanning microscopy (TPLSM) allows us to detect the excitation of single synapses in brain slices and in the intact brain by measuring intracellular calcium accumulation associated with activation of synaptic receptors. Intrinsic signal imaging allows us to keep track of the dynamics of networks of neurons in vivo. 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. For our in vivo measurements, 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. For our in vitro measurements, we use brain slices from barrel cortex or hippocampus.

Ca^{++} in Single Dendritic Spines

B. Sabatini, R. Yasuda

Long-term changes in synaptic efficacy occur when a postsynaptic neuron consistently fires an action potential within approximately 10 msec after the arrival of a particular synaptic input. These changes are triggered by increases in [Ca^{++}] in the postsynaptic neuron. We have used whole-cell patch-clamp recordings and TPLSM 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 know everything we ever wanted to know about [Ca^{++}] handling in individual spines: After Ca^{++} ions enter the cell, 95% are bound to intracellular buffers, whereas 5% remain free. The lifetime of Ca^{++} in the cytoplasm is remarkably brief, about 10 msec; 50% of the ions are sequestered into the SER, and the other 50% are pumped out of the cell. Diffusion from spine into dendrite has a negligible role in Ca^{++} clearance from spines. Therefore, spine heads operate as isolated compartments and subserve extremely rapid Ca^{++} signaling. Steep gradients in [Ca^{++}] can be maintained between spine head and dendrite on a micrometer length scale.

We also developed a new type of optical fluctuations analysis to estimate the number, location, and properties of voltage-sensitive calcium channels and

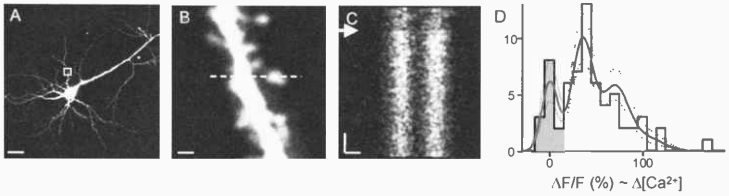


FIGURE 1 Two-photon imaging of calcium dynamics in small neuronal compartments. (*A*) Neuron labeled with a calcium-sensitive fluorophore (Bar, 20 μm). (*B*) Blow-up of boxed region in *A*, showing dendritic spines (Bar, 1 μm). (*C*) Line-scan image along dashed line in *B*, showing temporal dynamics of calcium in response to an action potential (*arrow*) (Bars: horizontal, 1 μm; vertical, 20 msec). (*D*) Histogram of responses to single action potentials (*solid line*). The histogram shows failures of responses (*gray shaded region*), a large peak of responses mediated by single channels, and a smaller peak due to two channels. Overlaid is the expected Poisson distribution (*black curved line*).

synaptic receptors. We find that $[Ca^{++}]$ responses to back-propagating action potentials often open only a single channel (Fig. 1). Similarly, only a few *N*-methyl-D-aspartate receptors (NMDA-Rs) are opened with the release of a synaptic vesicle. Therefore, synapses transmit quanta of information using only a few signaling molecules.

Optical Studies of Single Synapses

T. Oertner, E. Nimchinsky

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: 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^{++} that 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. One question we have addressed is: Can a synapse release only single vesicles of neurotransmitter or can it also simultaneously release multiple vesicles? To address this question, we stimulate single synapses under a variety of conditions that are known to modulate the probability of vesicle release. If multivesicle release does occur, conditions of high release probability should favor the simultaneous release of two or more vesicles. Such multivesicular release would be reflected in larger-amplitude Ca^{++} responses. Contrary to the textbook view of synaptic transmission, we find clear evidence for multivesicular release.

A second question concerns the cross-talk between neighboring synapses. 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 address this question, 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. To understand this remarkable observation, we are now using pharmacological and molecular tools to understand the mechanisms of clearance of glutamate from the extracellular space.

Spine Morphogenesis and the Cellular Basis of the Fragile X Syndrome

E. Nimchinsky, K. Greenwood

The Fragile X mental retardation syndrome is the most common form of inherited mental retardation. The molecular basis of the syndrome is a large expansion

of a triple repeat (CGG) in the 5′-untranslated region (UTR) of the *FMR1* gene that renders it transcriptionally silent. The protein encoded by this gene, called FMRP (Fragile X mental retardation protein) is an RNA-binding protein of unknown function. One suggested function is the transport of mRNA out of the nucleus and into neuronal dendrites. Interestingly, the only cellular abnormality in the brains of affected individuals appears to be restricted to dendritic spines, the targets of excitatory synapses. These spines have been described as unusually long, thin, and tortuous and more numerous than in normal brains. Since the gene is functionally knocked out in humans suffering from Fragile X syndrome, a reasonable animal model of this disease is a mouse lacking *FMR1*. In these mice, as in patients, dendritic spines have been described as being longer and more numerous than in control mice. Long, thin spines are suggestive of immature spines, found early in development, that are highly motile and presumably are in search of the correct presynaptic partners.

To understand the nature of this abnormality, we characterized the developmental aspects of structural defects. We used in vivo transfection of mouse barrel cortex neurons with green fluorescent protein (GFP)-expressing virus. Fluorescent neurons were analyzed postmortem. We find that there is a transient large abnormality in spine structure and density in the developing brain (PND 14) that decreases as the brain matures. To investigate the dynamic aspects of these spines, we are studying the motility of spines in slice cultures prepared from mutant mice at a time when dendritic spines are known normally to be highly motile. Finally, we are investigating the effects of reintroduction of FMRP into knockout mice, on both morphology and motility.

Morphological Basis of Experience-dependent Plasticity In Vivo

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 TPLSM of layer-2/3 pyramidal neurons in developing rat barrel cortex to image the structural dynamics of dendritic spines and filopodia. We have previously shown that protrusions are highly motile throughout development: Spines and filopodia appeared, disappeared, or changed shape over tens of minutes, suggesting that synaptic lifetimes during development might be relatively short in vivo (~10–100 hours). Motility of spines is driven by sensory experience. We are now using transgenic mice that express GFP and other fluorescent proteins in specific subpopulations of their neurons. These animals allow us to look at the structure of neurons over chronic time scales of approximately days, allowing us to measure the lifetime of synapses in the adult brain. Furthermore, we are trying to determine if the structure of neurons and their circuits is stable in the adult brain and if this stability can be perturbed by sensory experience.

Neocortical Experience-dependent Plasticity In Vivo

E. Stern, I. Bureau

We are exploring the experience-dependent physiological properties of neocortical neurons in vivo. How does sensory experience early in life shape the development of topographic maps in the barrel cortex? In contrast to previous studies that have employed extracellular recordings, we are using intracellular recordings in vivo. In addition to neuronal spiking activity, intracellular recordings measure synaptic potentials that reflect the structure of neural circuits. We find that brief whisker trimming (a couple of days) has a profound effect on the structure of sensory maps in layer 2/3. However, layer 4, the input layer to cortex, is not perturbed by sensory experience. The large scale of the defects in deprived animals suggests large-scale rearrangements of axonal structure. Our results show that sensory experience is required for the construction of well-ordered sensory maps.

Experience-dependent Changes in the Properties of Neocortical Pyramidal Neurons

M. Maravall

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 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. So far, we have not found significant differences in membrane excitability between deprived and spared animals. These results strongly point toward primarily a synaptic locus for experience-dependent plasticity.

Experience-dependent Gene Expression in the Neocortex

K. Zito, B. Burbach

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. We are presently using Affymetrix oligonucleotide arrays. Fluorescence images of the arrays are analyzed for patterns of expression.

Algorithms for Automated Morphometric Analysis

E. Nimchinsky, K. Zito [in collaboration with I. Koh and B. Lindquist, SUNY Stony Brook]

Changes in the structure of neurons are intimately related to neural development and plasticity and its dysfunctions. It is of interest to define the signaling systems that control aspects of neuronal shape. We use the gene gun to transfect neurons with candidate genes together with GFP and subsequently analyze the structure of transfected cells down to the level of single synapses. An underappreciated problem with this simple approach is that structural data are extremely difficult to analyze. Mind-numbing, laborious manual analysis invariably limits the throughput in such studies. For these reasons, we have invested considerable effort in developing software that allows essentially automated analysis of neuronal structure. Our approach consists of three steps: digital filtering by deconvolution; image segmentation; and image analysis. We now have a reliable and user-friendly algorithm running.

Instrumentation

K. Svoboda, P. O'Brien, B. Sabatini, T. Pologruto [in collaboration with R. Eifert and E. Ruthazer, Cold Spring Harbor Laboratory]

Our instrumentation efforts during the last year have focused on getting further microscopy/physiology stations working and others updated. We are also currently developing new software tools for image acquisition and microscope control. We think that this software will have many advantages over our current system; for example, it will accomplish many of the tasks that previously required custom electronics.

PUBLICATIONS

Chen B.E., Lendvai B., Nimchinsky E.A., Burbach B., Fox K., and Svoboda K. 2000. Imaging high-resolution structure of GFP-expressing neurons in neocortex in vivo. *Learn. Mem.* **7:** 433–441.

Cox C.L., Denk W., Tank D.W., and Svoboda K. 2000. Action potentials reliably invade axonal arbors of rat neocortical neurons. *Proc. Natl. Acad. Sci.* **97:** 9724–9728.

Lendvai B., Stern E.A, Chen B., and Svoboda K. 2000. Experience-dependent plasticity of dendritic spines in the developing rat barrel cortex in vivo. *Nature* **404:** 876–881.

Maravall M., Mainen Z.F., Sabatini B.L., and Svoboda K. 2000. Estimating intracellular calcium concentrations and buffering without wavelength ratioing. *Biophys. J.* **78:** 2655–2667.

Sabatini B.L. and Svoboda K. 2000. Analysis of calcium channels in single spines using optical fluctuation analysis. *Nature* **408:** 589–593.

In Press

Sabatini B.S., Maravall M., and Svoboda K. 2001. Ca^{2+} signaling in spines. *Curr. Opin. Neurobiol.* (in press).

MOLECULAR NEUROBIOLOGY OF LEARNING AND MEMORY IN *DROSOPHILA*

T. Tully J. Christensen J. DeZazzo J. DiLeo J. Dubnau R. Gasperini S. Gossweiler L. Grady S. Pinto M. Regulski P. Smith S. Xia L. Zhang

We have felt the impact of genome sequencing first-hand. In the latter part of the 1980s, we identified four mutants with defective olfactory memory. Most of the 1990s then was consumed by the basic molecular-genetic identification of the relevant transcription unit disrupted in these mutant flies. To date, we have published on three of these four "memory" genes. In the latter part of the 1990s, we identified another 57 candidate mutants with defective 1-day memory after spaced training. Because the full genome sequence now is available in *Drosophila*, molecular-genetic identification has been accomplished for 40 of these mutants. This effort triples the number of "memory" mutants identified in *Drosophila* and greatly expands our potential to understand the molecular mechanisms underlying this form of behavioral plasticity.

Identification of New Genes Involved with Associative Learning

T. Tully, S. Pinto, J. Christensen, J. DeZazzo, P. Smith, L. Grady

The Hartford Screen: The behavioral screen (forward genetics) for new Pavlovian learning/memory mutants is complete. In all, more than 6700 transposant lines were generated and assayed for 1-day memory after spaced training using a 48 array of Robotrainers. From this screen, we have identified 107 candidate mutants. Of these candidate mutants, 47 have abnormally low memory scores but normal (>90%) learning and sensorimotor scores. We designate these as "memory" mutants. Ten candidate mutants have abnormally low scores for both learning and memory. We designate these as "learning" mutants. This yields a total of 57 new learning/memory mutants, which triples the number of mutants known to affect olfactory memory in *Drosophila*. We have named these mutants after Pavlov's dogs.

To date, 40 of these 57 mutations have been identified molecularly. The candidate transcription units comprise a variety of cell biologies, including gene regulation, RNA processing, translational control, trafficking, signal transduction, and cell adhesion.

Genomics with *Drosophila* DNA Chips

T. Tully, J. Dubnau, S. Gossweiler, J. DiLeo
[in collaboration with M. Zhang, Cold Spring Harbor Laboratory, and G. Latter, Cold Spring Harbor Laboratory and Helicon Therapeutics, Inc.]

Functional Genomics: Using a prototype fly DNA chip from Affymetrix containing 1542 genes (12% of the genome), we are trying to identify candidate genes involved in long-term memory formation in normal flies. By comparing chips hybridized with probes derived from the whole heads of wild-type flies subjected to spaced versus massed training, we can focus our search for those transcripts specifically involved in transcription-dependent long-term memory formation. By looking at several time points after training, we are identifying clusters of coordinately regulated genes, using our 129 statistically significant candidate memory genes (CMGs) as focal points.

Pharmacogenomics: A drawback of the approach above derives from the notion that olfactory memory formation may occur only in a few cells in the brain. For DNA chip hybridizations, however, we can extract RNA only from whole heads. Thus, a significant signal-averaging may result. To address this issue, we have taken advantage of the well-established fact that olfactory associative learning in *Drosophila* requires normal cAMP signaling. By feeding flies drugs that enhance cAMP signaling systemically, the signal-to-noise ratio from cAMP-responsive transcription can be boosted. This approach has yielded 75 statistically

significant candidate genes, 8 of which are also included among the CMGs.

Dysfunctional Genomics: Another approach to increase the signal-to-noise ratio from our DNA chip analyses is to compare levels of gene expression of wild-type (normal) flies to those of a single-gene mutant. Here again, these differences may exist in many more cells. To date, we have compared whole-head RNA from wild-type flies to that from *amnesiac* and from *nalyot* mutants. The former appears to encode a neuropeptide that may activate adenylyl cyclase during middle-term memory formation; the latter encodes the ADF1 transcription factor, which appears to be involved in long-term memory formation. Wild-type versus *amnesiac* comparisons have yielded 162 candidate genes, 9 of which are also included among the CMGs. Wild-type versus *nalyot* comparisons have yielded 86 candidate genes, 7 of which are included among the CMGs. Interestingly, 24 candidates were in common between the pharmacological treatment and the *amnesiac* mutant comparisons, and 18 candidates were in common between the pharmacological treatment and the *nalyot* mutant comparisons.

Bioinformatics: Issues exist here on two levels. The first concerns the basic signal-to-noise analysis of data from DNA chips. We have addressed this problem with traditional statistical approaches. Proper consideration of underlying assumptions has greatly improved our ability to identify true positives and to eliminate false positives. The second concerns true bioinformatics: cluster analysis of coregulated genes and promoter analysis among genes in a cluster. Our ongoing analysis suggests that the quality of bioinformatics improves as our signal-to-noise analysis improves. The two issues go hand in hand.

Behavioral Analyses

T. Tully, S. Xia, J. Dubnau [in collaboration with T. Kitamoto, Beckman Research Institute of the City of Hope]

Olfactory Discrimination: For the proper behavioral identification of associative learning/memory mutants, sensorimotor responses must be shown to be normal. To this end, we have developed "task relevant" assays of olfactory acuity and shock reactivity for our Pavlovian conditioning paradigm. This conditioning procedure is inherently discriminative, however, requiring trained flies to choose between two differentially reinforced odors during a test trial. To do so, the flies must be able to discriminate between the two odors when they are presented simultaneously in the T-maze. This "quality choice" may be distinctly different from the "quantity choice" presented to the flies in our olfactory acuity assay. Hence, we have been interested in developing a valid assay of odor discrimination. Traditional assays of discrimination, however, have trained animals with associative protocols to yield a discriminative response between two stimuli. Obviously, such an approach cannot be used when trying to identify associative learning mutants.

With this caveat in mind, we have developed three different measures of discrimination, each of which does not depend on associative learning per se. Most of the extant learning/memory mutants show normal responses in each of these discriminability assays, supporting the notion that they have been validly identified. We now seek single-gene mutations that specifically disrupt discriminability.

Acute, Reversible Disruption of Synaptic Transmission: Across the animal kingdom, memory has been shown to exist in two basic forms, first as a transient, labile short-term memory (STM) and then as a persistent, stable long-term memory (LTM). Some theories have suggested that the neural basis of STM is a "reverberating circuit" that keeps newly acquired information online until the LTM (structural change) can be formed. We have begun to test this theory in *Drosophila* using a novel genetic tool.

Mutant *shibire*^{ts1} flies originally were identified in a screen for temperature-sensitive paralytics. Subsequent molecular cloning of this gene revealed it to encode the fly homolog of dynamin, a synaptic protein involved in vesicle recycling. In *shi*^{ts1} mutants, synaptic transmission fails within seconds after shifting flies to restrictive temperature. We generated UAS-*shi*^{ts1} transgenic flies carrying the mutant form of dynamin. When overexpressed widely using a central nervous system (CNS)-wide GAL4 driver line, these transgenic flies become paralyzed within seconds after shifting to the restrictive temperature (29°C) and recover within seconds after shifting back to the permissive temperature (20°C). When expression of UAS-*shi*^{ts1} is restricted to the mushroom body, in contrast, the perceptions of odors and footshock are normal at both permissive and

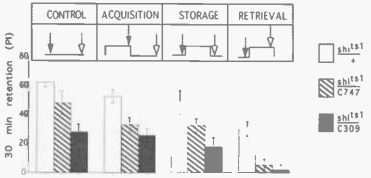


FIGURE 1 Disruption of synaptic transmission in the mushroom body abolishes memory retrieval but not acquisition or storage. CONTROL: When trained (*closed arrows*) and then tested (*open arrows*) 30 min thereafter at permissive temperature (20ºC; see stimulus schedules above each panel), all three genotypes display robust performance (although some nonspecific effects on 30-min memory are apparent in UAS-shi^{ts1}/C747 and UAS-shi^{ts1}/C309 transgenic lines). ACQUISITION: When first shifted to restrictive temperature for 30 min, trained, and then shifted back to permissive temperature for 30 min before testing, memory retention levels are unaffected in all three genotypes. STORAGE: When trained at permissive temperature, shifted to restrictive temperature for a 30-min retention interval, and then shifted back to permissive temperature for 5 min before testing, memory retention levels again are unaffected in all three genotypes. RETRIEVAL: When trained at permissive temperature, shifted to restrictive temperature immediately thereafter, and then tested for 30-min retention, memory is normal in control flies but abolished in transgenic shi^{ts1} lines.

restrictive temperatures, revealing that synaptic transmission from mushroom body neurons is not required for the normal sensorimotor responses used during olfactory associative learning.

We then looked at Pavlovian learning. With the rapid, reversible nature of the transgenic tool, however, we were able to restrict the disruption of synaptic transmission spatially (only in the mushroom body) and temporally (only during acquisition, storage, or retrieval of olfactory memory) in our Pavlovian experiments. Surprisingly, we found that blocking synaptic transmission during acquisition or the first 30 minutes of memory storage had no effect on 30-minute retention. In contrast, blocking synaptic transmission during memory retrieval completely blocked 30-minute retention (see Fig. 1).

Taken together, these data indicate that a persistent neural activity (reverberating circuit) in the mushroom body is not required for the acquisition and storage of olfactory memory. Rather, it is needed for its retrieval. Moreover, these data suggest that the Hebbian neuronal process underlying this associative process must reside in the dendrites of mushroom body neurons. This view now permits the development of computational models in the fly model system to "vertically integrate" neuronal function with conditioned behavioral responses.

PUBLICATIONS

DeZazzo J., Sandstrom D., de Belle S., Velinzon K., Smith P., Grady L., DelVecchio M., Ramaswami M., and Tully T. 2000. *nalyot*, a mutation of the *Drosophila* myb-related *Adf1* transcription factor, disrupts synapse formation and olfactory memory. *Neuron* **27:** 145–158.

Kuzin B., Regulski M., Stasiv Y., Scheinker V., Tully T., and Enikolopov G. 2000. Nitric oxide interacts with the retinoblastoma pathway to control eye development in *Drosophila*. *Curr. Biol.* **10:** 459–462.

Saitoe M. and Tully T. 2000. Making connections between synaptic and behavioral plasticity in *Drosophila*. In *Toward a theory of neuroplasticity* (ed. J. McEachern and C. Shaw), pp. 193–220. Psychology Press, New York.

LONG-TERM MEMORY FORMATION IN *DROSOPHILA*

J. Yin M. Cowan, E. Drier, R. Filipkowski, E. Friedman, J. Horiuchi, Z. Lin, C. Margulies, K. Seidenman, M. Stebbins, M. Tello, P. Wu, H. Zhou

Atypical PKC and Memory Formation

E. Drier, M. Cowan, M. Tello, P. Wu [in collaboration with T. Sacktor, SUNY Health Science Center, Brooklyn, New York]

Transgenic flies carrying mouse, atypical, full-length (*MaPKCζ*), or truncated (*MaPKCζ*) genes have been analyzed for their effects on memory formation. Induction-dependent enhancement of memory formation occurs when the *MaPKMζ* transgene is induced after a single training trial or after massed trials, but not spaced trials. This enhancement requires persistent kinase activity, since neither the full-length kinase (PKCζ) nor a kinase-dead point mutation on the truncated gene (KD-*PKMζ*) can enhance. We can detect induction-dependent expression of all of the different kinases and can measure an induction-dependent increase in atypical (calcium-independent and DAG-independent) PKC activity in crude fly extracts. There is a striking temporal window to the enhancement; enhancement only occurs if the transgene is induced after behavioral training, but within a 2-hour time window.

Chelerythrine, a specific inhibitor of atypical PKM kinase activity (when used at the proper concentration), inhibits memory formation after massed training, but does not affect learning (immediate memory after a single training trial). The kinase-dead *PKMζ* transgene functions as a dominant negative molecule and inhibits memory formation after massed training, but does not affect learning (single-trial training). Therefore, induction of the *PKMζ* transgene is sufficient to enhance memory formation, and atypical PKC activity is necessary for memory formation. Since manipulating atypical PKC activity can enhance or disrupt memory formation, but does not affect learning, we believe that atypical PKC activity is involved in the maintenance of memories, not their establishment. These results, and this interpretation, are consistent with experimental data in the hippocampal slice culture, where "gain-of-function" and "loss-of-function" phenotypes can be achieved with exactly the same proteins (T. Sacktor et al., pers. comm.). In that context, these authors interpret their results to show that PKMζ is involved in the maintenance, but not induction, of long-term potentiation (LTP).

The 2-hour temporal window is a particularly striking result. Experiments in hippocampal slices and *Aplysia* cultured neurons have led to a hypothetical concept, the "synaptic tag," which has a 2–3-hour duration. Although it may be coincidental, we believe that the PKMζ temporal window is intimately related to the duration of the tagging system and that PKMζ may be directly acted upon by the tagging system or may participate in it. Cell biological experiments on atypical PKC proteins in development suggest that atypical PKC is part of a general marking process which evolved very early and may be utilized in neurons for more specialized purposes.

Characterizing the *DaPKC* Gene

M. Tello, E. Drier, P. Wu [in collaboration with T. Sacktor, SUNY Health Science Center, Brooklyn, New York]

Analysis of the completed fly genome indicates that flies contain only a single atypical PKC gene, although there are a total of seven PKC genes. A number of characteristics distinguish an atypical gene from its closely related conventional and novel genes, including domain structures (which are ultimately responsible for cofactor binding) and distinctive amino acid substitutions. We have been characterizing

the gene structure of the *DaPKC* gene, as well as its mRNA and protein products.

The fly gene is distributed over approximately 20 kb and consists of nine coding exons, with a large 12-kb intron between exons 2 and 3. Overall, there is more than 60% identity between the fly gene and the mouse or human *PKCζ* genes and slightly less when compared to the other mammalian atypical gene, *PKCι/λ*. Multiple antibodies suggest the existence of two bands in fly extracts. Consistent with the available information on the mammalian *PKCζ* gene, it is likely that the larger protein band corresponds to the full-length protein, and the shorter band corresponds to the PKMζ protein.

When adult head mRNA is analyzed using Northern blots, a probe from the 5′ end of the gene results in a single mRNA transcript of about 5 kb. When a probe is made from the 3′ end of the gene, two additional transcripts are recognized. The 3′ probe corresponds to regions of the gene 3′ to the large 12-kb intron. Consistent with the rat *PKCζ* gene, this pattern of transcripts suggests that there is alternative transcription initiation, or splicing, of the *DaPKC* gene.

Recently, a report described the participation of the *DaPKC* gene, and its products, in asymmetric cell division in the neuroblast. The authors describe a P-element insertion, which results in a homozygous recessive, lethal phenotype during early development. Mapping of the insertion site shows that the P-element is located within the 12-kb intron, approximately 3 kb from the 3′ end of the intron. We are currently analyzing imprecise revertants of this insertion. It is our expectation that some existing revertants may remove an internal promoter that is primarily responsible for initiating the two 3′-specific transcripts.

In addition to alternative promoter usage, evidence in the literature suggests that the PKMζ protein may be produced through calpain-mediated, proteolytic cleavage of the PKCζ protein. Calpain is a calcium-activated protease, and it is suspected that it is one of the early events following synaptic activation and calcium influx at the nerve terminal. It is known that the PKMζ protein, which is found in all regions of the brain, is distributed in a punctate pattern in dendrites, near synaptic terminals. Our behavioral analysis of transgenic flies shows that enhancement of memory formation requires induction of a (truncated) *PKMζ*, but not (full-length) *PKCζ*, transgene. We are looking for behavioral conditions that activate calpain and thus allow the full-length protein to enhance memory formation.

Role of Circadian Genes and Sleep in Memory Formation

M. Cowan, P. Wu [in collaboration with J. Hendricks, University of Pennsylvania]

Ever since our demonstration of periodic cycling CRE-binding transcription in transgenic flies, we have been interested in the possible connection between this nighttime activity and memory formation. After some initial experiments using manipulations of light:dark to try to disrupt circadian rhythms, we have resorted to manipulating genes that are known players in the circadian system. Induction of a dominant negative clock protein affects long-term memory, without affecting memory after massed training. This inhibition can occur even if the transgene is induced 24 hours after the end of training. Preliminary experiments show that mutations in the *per* gene, a central component of the circadian clock, affects memory formation. It is unclear if these effects are due to (1) effects on the circadian system (which indirectly affects memory formation), (2) effects on the circadian system affecting sleep (which affects memory formation), or (3) if the proteins themselves participate directly in the process of memory formation.

Tetracycline-inducible Expression Systems

M. Stebbins [in collaboration with G. Byrne, Nextran, and W. Hillen, Erlangen]

We have completed our optimization and analysis of the Tet-Off and Tet-On systems in transgenic flies. For each system, we have characterized both a ubiquitously expressed version and a spatially restricted version. In addition, we have compared the effects of altering *trans*-activators. Altering the *trans*-activator includes removal of a cryptic splicing site in the TetR portion of each *trans*-activator, reformatting codon usage for mammalian expression, and using SCS and SCS′ insulator elements to remove the effects of position effects.

For the ubiquitous Tet-Off system, we have characterized the dosage and kinetics for gene repression (addition of antibiotic to fly food) and gene induction (drug withdrawal), the leakiness, and the amount of induction. Total repression can be easily achieved

within 12 hours of placing flies on drug-containing food, and total induction occurs within 24 hours of placing flies on drug-free food. There is virtually no leakiness (defined as the difference between target gene expression in single transgenic flies without a *trans*-activator and expression when double transgenic flies are fed antibiotic). Drug withdrawal results in about 125-fold induction, and the amount of induction is dosage-sensitive to the copy number of the *trans*-activator gene. The system works equally well in adult flies, embryos, and larvae.

By combining the Gal4 and Tet-Off transgenes in a tripartite system, it is possible to achieve robust induction of target genes that is both temporally and spatially regulated. From the widely available collection of Gal4 drivers, a desired driver line is crossed together with a UAS-tTA transgene, resulting in Tet-Off *trans*-activator expression in the tissues where Gal4 is expressed. When the TetO-target transgene is also crossed into the same fly, doxycycline delivery or withdrawal regulates target gene expression. In the adult eye, 115-fold induction occurs upon doxycycline withdrawal, with little leakiness. Similar spatially restricted expression occurs in embryos when appropriate drivers are used and the mothers are fed dox-free or dox-containing food. Collectively, these data show that the spatially restricted expression system is comparable in its properties to the ubiquitously expressed system in terms of induction ratio, leakiness, and kinetics.

The ubiquitous Tet-On *trans*-activator is now a viable system for gene disruption. Two modifications were key to the improvement of this system: altering the *trans*-activator, which resulted in greater expression, and the development of a second-generation Tet-On *trans*-activator. This *trans*-activator resulted from a second mutagenesis screen for mutations that reversed the binding properties of the TetR protein. This new *trans*-activator outperforms the first-generation Tet-On protein and, when altered, results in induction ratios that are about 50% of the Tet-Off protein. Dosage and kinetic experiments show that maximal induction occurs within 24–48 hours when flies are fed very practical concentrations of doxycycline in regular fly food. Maximal induction can occur within 30 minutes if flies are directly injected with the antibiotic. The leakiness is slightly greater than with the Tet-Off systems, but it is insignificant when compared to the induction ratio. Gene-dosage experiments indicate that the amount of *trans*-activator still limits the induction ratio. The system works well in adult flies, embryos, or larvae.

The spatially restricted Tet-On system has properties similar to those of its ubiquitous version. We are currently characterizing the use of this system both for tissue-specific gene induction and for tissue-specific ablation of cells. The ablation system works well during development and is currently being tested for its efficacy in postmitotic cells.

The Tetracycline System in Primary Neurons

Z. Lin

The study of gene function in neurons would benefit greatly from an inducible system. We have tested the altered, first-, and second-generation Tet-On *trans*-activators in transiently transfected, rat cortical, primary neurons. The altered, first-generation Tet-On *trans*-activator performs poorly in primary neurons, whereas the second-generation Tet-On *trans*-activator results in 20-fold induction of target gene expression, shows little leakiness in the absence of antibiotic, and only requires 24 hours of drug treatment to achieve maximal induction.

Three different target genes can be induced in a doxycycline-dependent manner, indicating that robust induction is not just a function of particular target genes. When enhanced green fluorescent protein (EGFP) is used as the target gene, all of the transfected neurons, as judged from EGFP-mediated fluorescence, also show green fluorescence. Thus, it is likely that the amount of induction in each transfected neuron is relatively equal, or at least great enough to result in saturated green fluorescence.

Regulation of dCREB2 Activity

J. Horiuchi, P. Wu

We have shown previously that a set of phosphorylation sites inhibit dCREB2 DNA binding in vitro. These sites can be phosphorylated by the endogenous *Drosophila* casein kinase II protein in crude, cell-free extracts. Mutation of these residues to alanine not only allows DNA binding in vitro, but also changes the subcellular fractionation properties of the protein.

Most of the endogenous or transgenically overproduced dCREB2-b protein fractionates in the cytoplasmic fraction, and mutation of these casein kinase sites results in much greater nuclear localization of the protein. All dCREB2 protein isoforms contain a consensus nuclear localization signal.

We suspect that the dCREB2-b protein normally exists in a cytoplasmic, multiprotein complex and that phosphorylation of the casein kinase sites may be necessary for complex formation. We are initiating biochemical analyses of crude extracts to test this hypothesis. The existence of this complex may help explain the cytoplamic localization of the dCREB2-b protein and its inability to bind DNA and lead to an understanding of how this protein inhibits memory formation.

Functional Anatomy of Memory Formation

C. Margulies, P. Wu

We are using the Gal4 system to determine which parts of the adult fly brain are needed for long-term memory formation. We are expressing the dCREB2-b transgene in different regions of the brain and are asking which expression patterns disrupt long-term memory formation. Ultimately, we anticipate using the Tet-On spatially restricted system to refine this analysis. Temporal and spatial regulation will remove any doubt about the acute, as opposed to developmental, role of particular brain regions in memory formation.

Mouse Behavior

E. Friedman, R. Filipkowski

We have established training procedures for the Morris water maze and contextual discrimination that produce long-lasting memory, or shorter-lasting memory, depending on training parameters. A constant number of training trials are given, but the intervals between individual training trials, or blocks of trials, are varied. Using these procedures, we are examining the effects of pharmacological agents for their effects on memory formation, especially their ability to enhance these processes.

M. Tello-Ruiz, P. Wu

NEURAL SUBSTRATE OF SELECTIVE AUDITORY ATTENTION IN THE CORTEX

A. Zador M. DeWeese L.-H. Tai
M. Morgan G. Tervo
S. Edgar

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 most peripheral (i.e., near the retina) areas of the visual cortex. This enhancement is surprising because the brain 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 lab 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.

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 (see Fig. 1). These data provide clues about the representations with which the cortex solves hard problems in auditory processing.

Activity-dependent GFP as a Real-time Measure of Synaptic Activity In Vivo

M. DeWeese [in collaboration with J. Sullivan, Salk Institute]

With conventional electrophysiological recording techniques, it is difficult to study how one population of neurons influences the activity of another population. For example, when a neuron in the primary auditory cortex responds to a sound, how much of the activity arises from the upstream regions (e.g., the thalamus) and how much from the cortex? To address such questions, we are using an engineered synaptic vesicle protein that has been tagged with green fluorescent protein (GFP) and which can be delivered to cortical neurons in vivo. This engineered protein fluoresces during the vesicular fusion

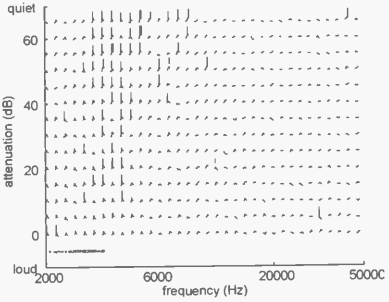


FIGURE 1 Whole-cell patch-clamp recording of sound-evoked responses in an auditory cortical neuron. Each trace represents the membrane voltage response to 25-msec pure tone pips of variable frequency and intensity. Only 15% of the pips caused spikes, but more than half caused robust subthreshold responses. These subthreshold responses provide insights into how neurons in the auditory cortex generate selective responses.

events underlying synaptic activity, and so can be used to monitor the synaptic activity in an anatomically or genetically defined subpopulation of neurons. This new technology—a kind of population-specific local field potential—may emerge as an important complement to conventional electrophysiological methods in vivo.

Statistical Structure of Natural Sounds

G. Tervo

To develop the best strategy for processing a complex signal, such as a natural sound, we need to understand the properties of the sounds likely to be encountered. We are therefore using a variety of mathematical approaches to characterize the statistical structure of natural sounds. An understanding of these statistics provides a foundation for understanding the constraints on any algorithm, biological or artificial, that solves the cocktail party problem.

Behavioral Paradigm for Studying Selective Auditory Attention

G. Tervo, L.-H. Tai, S. Edgar, M. DeWeese

More than a decade has passed since the first reports of a neural correlate of selective attention in monkeys, but the mechanism underlying the enhancement of neural activity remains a mystery. We are therefore developing a simpler preparation within which to study the mechanism. We are training rodents to perform a simple selective auditory attention task. We will then use extracellular recording methods (tetrodes) to look for a neural correlate—a change in the firing rate—of this selective attention in the auditory cortex. This simple rodent preparation will have many advantages over the existing primate preparation, as it will permit us to bring to bear the full cellular and molecular armamentarium available to modern neurobiology.

PUBLICATIONS

deCharms R.C. and Zador A. 2000. Neural representation and the cortical code. *Annu. Rev. Neurosci.* **23:** 613–647.

DeWeese M. and Zador A. 2000. *In vivo* whole cell recordings of synaptic responses to acoustic stimuli in rat auditory cortex. *Soc. Neurosci Abstr.* 637.14

Zador A. 2000. The basic unit of computation. *Nat. Neurosci.* **3:** 1167.

NEURAL BASIS OF LEARNING AND MEMORY IN *DROSOPHILA*

Y. Zhong F. Hannan J. Tong
H.-F. Guo I. Hakker
Y. Wang Y.-H. Zhu
J. Pathenveettil

We are interested in the neural basis of learning and memory and are taking an approach of combining functional analyses with genetic manipulation in the study of *Drosophila*. Currently, we are pursuing two major projects. First, we are establishing *Drosophila* models for studying genes involved in human neurodisorders that impair learning and memory. In particular, we are interested in genes known to contribute to neurofibromatosis 1 (*Nf1*) and Alzheimer's disease. *Nf1* patients are identified by neurofibromas and other symptoms including learning defects. We are investigating the hypothesis that the tumor-suppressor gene *Nf1* not only acts as a Ras-specific GTPase-activating protein (GAP), but also is involved in mediating G-protein-stimulated activation of adenylyl cyclase (AC), and this NF1-dependent AC pathway is required for learning. In the case of Alzheimer's disease, our study has been limited to examining how presenilin and accumulation of Aβ affect age-dependent decline of learning ability. This approach, on the one hand, serves as a new way to identify biochemical cascades underlying learning and memory in *Drosophila*. On the other hand, it may also provide insights into pathogenesis of the diseases. Second, we are investigating how odors are encoded by population neuronal activity in the fly brain and will ultimately study learning and memory at the level of population neural activity. Such studies are carried out by optical recordings from living flies. The specific projects are described below.

Site-directed Mutagenesis in Human NF1

F. Hannan, I. Hakker

The tumor-suppressor gene *Nf1* encodes a large protein containing a fragment homologous to Ras-GAPs, which inhibit Ras activity. *Drosophila* NF1 is highly conserved since 60% of its 2803 amino acids are identical to human NF1. Our previous electrophysiological, biochemical, and behavioral analyses of *Drosophila NF1* mutants have indicated that, in addition to functioning as a Ras-GAP, NF1 regulates activation of adenylyl cyclase (AC). This NF1-dependent cAMP pathway is crucial for learning and memory. Our recent effort in biochemical assay of AC activity has revealed that G-protein-stimulated AC activity consists of two components, i.e., NF1-dependent and NF1-independent. The NF1-dependent component is largely mediated via the *rutabaga* (*rut*)-encoded AC, which is required for learning in *Drosophila*. To understand how NF1 regulates AC activity, we are investigating how point mutations identified in NF1 patients affect cAMP-pathway-related functions. We have shown that, in transgenic flies, the human *NF1* gene is capable of rescuing NF1 mutation-induced phenotypes or defects, including small body size, reduction in G-protein-stimulated AC activity, and olfactory learning. Transgenic flies carrying different mutations, two within the GAP domain (directly relating to Ras activity) and one outside of the GAP domain, have been generated, and we are in the process of testing how these mutations affect the ability of human NF1 to rescue NF1 defects in mutant flies. We are hoping to gain insight into whether Ras activity is required for AC regulation and whether there is a specific domain in the NF1 molecule designated to AC regulation.

NF1-regulated cAMP Pathway in Vertebrates

J. Tong, Y.-H. Zhu

All studies related to NF1-regulated AC activity have been confined to *Drosophila*. Thus, it is important to determine whether NF1 also regulates AC activity in vertebrates in order to determine whether a defect in such regulation may contribute to the wide range of symptoms observed in NF1 patients. The observation that expression of human NF1 rescues phenotypes in flies resulting from impaired NF1-regulated AC activity suggests that the structure and function of NF1 molecules are conserved across invertebrates and ver-

tebrates. During last year, we studied this issue by assaying AC activity in an *Nf1* mouse knockout. Since these *Nf1* mutant mice are embryonically lethal at the E13.5 stage, we used brain tissue from embryos at E12.5 to E13.5. Data from assays of dissected brain tissues or from assays of cultured neurons all consistently indicated that GTPγS-stimulated AC activity was reduced in homozygous NF1 nulls as compared to heterozygous or wild type.

Modeling Alzheimer's Disease in *Drosophila*

Y. Wang, J. Pathenveettil, D. Yom, Y. Zhong

The most common cause of familial Alzheimer's disease is mutation of the gene encoding presenilin 1 and 2, which alters γ-secretase activity to increase the production of the highly amyloidogenic $A\beta_{42}$. *Drosophila* has a highly conserved homolog of the human *presenilin* gene. By overexpressing the wild-type *Drosophila presenilin* homolog gene, or by introducing one with mutations found in Alzheimer's disease, we are trying to build a simple *Drosophila* model to study Alzheimer's disease. Our preliminary results show that the fly's life span is greatly reduced by expression of both wild-type *presenilin* and the mutated genes. In addition, learning ability shows age-dependent deterioration. This may afford us a simple system to understand the pathological mechanisms of Alzheimer's disease.

Notch in Learning and Neural Plasticity

H.-F. Guo

Another known target of presenilin is Notch, which has been shown to be a key signaling molecule in determination of cell lineage across different species. Notch is also expressed in the adult nervous system, but its function in the adult remains to be revealed. As a substrate of presenilin, it has been speculated that Notch may have an important role in the pathogenesis of Alzheimer's disease. However, it has been difficult to investigate Notch's role in the adult nervous system because of lethality induced by mutations in the gene. In *Drosophila*, the existence of temperature-sensitive mutants and heat-shock-inducible active or dominant negative Notch may facilitate our understanding of its role in the adult. Our preliminary data indicate that Notch activity has a role in learning and memory and in activity-dependent nerve terminal plasticity.

Role of *Drosophila* Mushroom Bodies in Odor Perception: Imaging and Behavioral Studies

Y. Wang

Drosophila mushroom bodies (MBs) are the olfactory-related associative learning center. Using a Ca^{++} imaging technique, we have recorded neural activities in the MBs in response to odor stimulation. It is shown that odors evoke spatial neural activities in the MB that are both odorant- and concentration-specific. In an olfactory-binding protein mutant where the response to an attractive odor is disrupted, the spatial activity pattern in the MB loses its dependency on the odor concentration. This has led us to a hypothesis that the MB may be required only for perceiving attractive odors. To test this hypothesis, we introduced a *shibire*^{ts1} transgene to the MB, which blocks synaptic transmission upon elevating the temperature to over 28°C. We found that although temperature-dependent expression of *shibire*^{ts1} disrupted the response to attractive odors, it had no obvious effect on repulsive odors. In future studies, in addition to testing more odors, we will investigate the changes in neural activities in the MB with Ca^{++} imaging.

PUBLICATIONS

Guo H.-F., Tong J., Hannan F., Luo L., and Zhong Y. 2000. A neurofibromatosis-1-regulated pathway is required for learning in *Drosophila*. *Nature* **403:** 895–898.

In Press

Wang Y., Wright N., Guo H.-F., Malinow R., Smith D.P., An J., and Zhong Y. 2001. Genetic manipulation of the distributed odor-evoked representation in the *Drosophila* mushroom body. *Neuron* **29:** 267–276.

Wright N.J.D., Feany M.B., Quinn W.G., and Zhong Y. 2001. Electrophysiological responses induced by putative amnesiac neuropeptides in *Drosophila* CNS neurons. *J. Neurosci.* (in press).

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 W. 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, will end her fellowship in June, 2001, when she will become an assistant professor at the Laboratory.

In 2000, two new CSHL Fellows joined the Laboratory. Terence Strick 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 properties of these enzymes. Gilbert (Lee) Henry joined us from Doug Melton's laboratory at Harvard University where he earned his Ph.D. for studies on *Xenopus* development. Lee is studying the mechanisms that regulate the formation of taste buds and the maintenance of their structure in the adult. Lee is not new to CSHL. He was a participant in the Undergraduate Research Program for three consecutive summers in the labs of David Helfman and Winship Herr from 1990 to 1992.

L. Henry
T. Strick
M. Timmermans

The Development and Maintenance of Taste Bud Structure

L. Henry

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 cells of the bud undergo membrane depolarization 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 newly formed receptor cells extend their axons back toward glomeruli in the olfactory bulb, newly formed taste receptor cells synapse onto sensory afferents that remain at the bud during its entire lifetime.

Taste buds are embedded in epithelial specializations called papillae. In most mammals, there are three distinct classes of papillae. 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. For both findings, basic morphological criteria were used to evaluate the dependency of each structure on innervation.

Turnover in the adult taste bud creates special constraints on this structure. As new receptor cells are formed, they must acquire an appropriate synapse with one or more sensory afferents. It is possible that the molecular biology of this process is very similar to that required for the initial formation of the taste bud. For this reason, we believe that there will be overlapping mechanisms in the formation and maintenance of taste bud structure. Keeping this possibility in mind, we will specifically address the following problems. How do papillae form in the appropriate pattern during late gestation and what role do innervating sensory afferents play in this process? How do taste buds form and are the already present afferents somehow involved? How diverse is the mature receptor cell population? Is there a true stem cell in the adult taste bud whose daughters become mature differentiated receptor cells? Do sensory afferents in the bud regulate either progenitor or receptor cell development? To tackle these problems, we will use a variety of molecular biological, organ culture, and genomics techniques.

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 papilla 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 are being isolated, and using a recently described recombination technique, we will insert GFP 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, tongue-specific macroarrays, 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.

HOW DIVERSE ARE BOTH THE MATURE RECEPTOR CELL POPULATION AND THE PROGENITOR POOL FROM WHICH IT IS DERIVED?

Morphologically, there are three types of cells within the adult taste bud. Highly elongated differentiated receptor cells are found at the apex of the bud. Mitotic progenitor cells are found almost entirely at the bud's base. Packed in between these two cell types are less elongated cells that are believed to be the daughters of the progenitors. Detailed electron microscopic observations have further divided the mature receptor cell population into two to three distinct classes. The recent identification of taste-specific seven-transmembrane receptors and heterotrimeric G-proteins has demonstrated that within the mature receptor cell population, there is a great deal of cellular diversity at the level of expression of these genes.

We would like to analyze the molecular diversity of both mature receptor and mitotic basal cells in detail, without a bias toward a particular gene family. In an approach that is similar to what is planned for the developing papillae (see above), we will isolate both murine and amphibian taste buds and generate transcriptional profiles from single cells. We will screen

microarrays and taste-bud-specific macroarrays and in some cases, directly sequence cDNA to obtain relevant profiles. Once the internal make-up of the taste bud is understood, it will be possible to analyze a number of issues. Does synapse formation have an instructive or permissive role in receptor cell maturation? Are the progenitors identical at the molecular level? If so, then how is receptor cell diversity achieved? If not, then how are the progenitors themselves created?

DEVELOPMENT OF AN IN VITRO SYSTEM FOR THE STUDY OF TASTE BUD FORMATION

There already exist in vitro culture methods for the study of papilla formation and their innervation. There is, however, no equivalent method for the study of taste bud formation at the postnatal period. We will attempt to culture either whole papillae or slices of P7–P10 tongue. Both approaches have the drawback that sensory afferents will be disturbed. The soma for these neurons are located near the brain stem far from the tongue, which makes it very difficult if not impossible to imagine a preparation that could contain both the lingual epithelium and the sensory afferents that provide its innervation. However, taste bud formation might resemble papilla formation in that innervation is not required for the earliest stages of the process.

Single-molecule Analysis of Enzyme Kinetics

T.R. Strick

In the next 3 years, our lab will develop and use single-molecule techniques to quantitatively study DNA supercoiling and its interactions with proteins involved in transcription, replication, and chromosome compaction. Supercoiling describes the wrapping of the two strands of DNA one about the other, as well as the writhing of the double helix through space (a twisted phone cord is a somewhat useful analogy). Controlled in vivo by the topoisomerase enzymes, it is both a regulator and a by-product of DNA transcription, replication, and packaging. Although supercoiling has been extensively studied in the past using bulk experimental techniques such as sedimentation or gel electrophoresis, new methods now make it possible to mechanically control the supercoiling of an individual DNA molecule. The major advantage of performing experiments at the level of single molecules is that it allows one to observe in real time the behavior of the molecule, eventually giving the researcher access to the time course of a reaction between the DNA and a protein. In this report, I briefly describe the methods to be used as well as some of the results obtained previously in the study of DNA-topoisomerase interactions.

The single-molecule experiment we have just implemented is depicted in Figure 1. An approximately 10-kbp linear DNA molecule is shown anchored at one end to a glass surface and at the other end to a 1-micron-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 depends solely on the distance between the magnets and the sample (the closer the magnets, the higher the force), whereas the twist imparted to the DNA is exactly equal to the number of clockwise or counterclockwise turns performed by the magnets. The double helix is thus quantitatively and reversibly supercoiled. By determining the position of the magnetic bead, one measures the end-to-end exten-

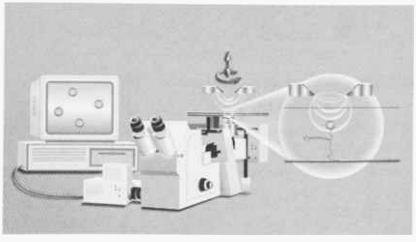


FIGURE 1 Sketch of the experimental setup. A glass capillary tube is mounted on an inverted microscope whose focus is controlled by a computer. The computer also controls the displacements (translation and rotation) of the magnets used to manipulate the bead. A CCD camera connected to the microscope 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 applied stretching force and the DNA's end-to-end extension.

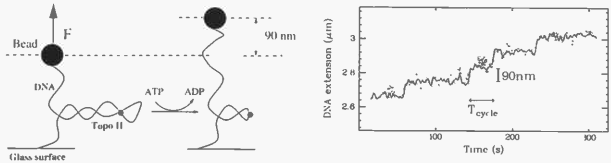


FIGURE 2 (*Left*) Sketch of the relaxation of supercoils by a topoisomerase II molecule. (*Right*) Discrete steps in the DNA's extension were observed in the presence of limiting amounts of ATP and topoisomerase II, corresponding to single enzymatic cycles of the enzyme.

sion of the DNA molecule, and thus its mechanical response to stretching and twisting.

Three types of structural transitions have been observed in supercoiled DNA using this micromanipulation technique. For low levels of mechanical torsion, and in a manner which is identical for positive or negative supercoiling, the molecule will writhe and trace out loops ("plectonemes") similar to those observed on a twisted phone cord (see Fig. 2, left). This is evidenced by a rapid contraction of the bead toward the surface. If the torsion becomes greater, the double helix responds by locally hypertwisting (when it is positively supercoiled) or by locally denaturing (when it is negatively supercoiled). One of the topics of research for the coming year in our lab will be to study the onset of denaturation on a single supercoiled DNA. Indeed, preliminary results indicate that the supercoiled DNA undergoes reversible thermal melting on an approximately 30-bp scale and that this process has a very slow dynamic. A better understanding of this denaturation will then be used to study processes such as promoter melting prior to transcription and the initiation of replication.

We will also pursue the analysis of plectonemes using this apparatus, since these structures are very useful in the analysis of DNA-protein interactions. As an example, the interactions between topoisomerase II and supercoiled DNA were recently studied at the single molecule level. Type II DNA topoisomerases are ubiquitous ATP-dependent enzymes capable of transporting a DNA through a transient double-strand break in a second DNA segment. This enables them to untangle DNA and relax the plectonemes that arise in twisted DNA. In vivo, they are responsible for untangling replicated chromosomes and their absence at mitosis or meiosis ultimately causes cell death. Here, we monitored the supercoiled DNA's extension in the presence of ATP and topoisomerase II and directly observed the removal of two supercoils during a single catalytic turnover (see Fig. 2, right). Furthermore, specific steps of the enzymatic cycle could be studied by withholding ATP or by modulating the applied stretching force. These results demonstrate that single-molecule experiments are a powerful new tool for the study of DNA-protein interactions.

Plant Developmental Genetics

M. Timmermans, M. Juarez, P. Vahab, J. Thomas, W. Songnuan

The long-term objective of the research in our lab is to elucidate the molecular basis underlying the initiation and patterning of lateral organs in plants. Pattern formation during organogenesis in plants and animals is likely a distinct process. For instance, small protein morphogens such as *wingless* and *hedgehog* have not been identified in the *Arabidopsis* genome. Plants are also unique in that organogenesis continues beyond the period of embryogenesis. Lateral organs such as leaves arise progressively from a group of stem cells in the shoot apical meristem. In the first step in organogenesis, founder cells are recruited from the meristem and new developmental axes are specified relative to the main body axis. Subsequently, the fate of the organ becomes determined, resulting in the differentiation of appropriate cell types in response to the basic pattern. 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 that affect

lateral organ initiation and patterning. In particular, we are analyzing the roles of two genes: *Leafbladeless1* (*Lbl1*) and *Rough sheath2* (*Rs2*). *Lbl1* is required to establish dorsal cell identity in leaves and leaf-like lateral organs. *Rs2* has an important role in the repression of stem cell fate during organ initiation and growth. A better understanding of the function of these and other genes involved in pattern formation will provide new insights into fundamental concepts of plant development. Variations in the expression patterns of such genes are likely to contribute to the diversity of leaf shapes among many plant species. More importantly, however, the isolation and characterization of genes involved in the patterning of leaves may facilitate the manipulation of plant architecture, which has become increasingly more important for aspects of crop yield such as shade tolerance.

ROLE OF *ROUGH SHEATH2* IN THE REPRESSION OF STEM CELL FATE

Homeobox genes related to *Knotted1*, the *Knox* genes, are expressed in the shoot apical meristem and are required for stem cell fate. Determinate lateral organs such as leaves are initiated by the recruitment of founder cells, which is facilitated by the down-regulation of *Knox* gene expression in a subset of cells within the meristem. Dominant neomorphic mutations in *Knox* genes in maize result in displacement of proximal leaf features, sheath, ligule, and auricle to more distal positions within the leaf blade. The recessive *rs2* mutant causes a similar proximodistal patterning defect. We have shown that the *rs2* phenotype results from the misexpression of KNOX proteins in leaf primordia, suggesting that *Rs2* acts as a negative regulator of *Knox* gene expression.

We have previously shown that the *Rs2* gene encodes a Myb-domain protein related to the *Antirrhinum* gene *Phantastica* and that it is expressed in young 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* may thus prevent differentiated daughter cells from reverting into indeterminate stem cells. We have generated RS2-specific monoclonal and polyclonal antibodies that we are using to determine more precisely the temporal and spatial expression patterns of RS2 and the KNOX proteins and to analyze changes in RS2 accumulation in other maize mutants affecting leaf initiation and patterning.

We are also studying the mechanism by which *Rs2* regulates *Knox* gene expression by identifying genes that act together with *Rs2*. We have constructed and screened a two-hybrid library made from young maize leaf primordia cDNAs. We have identified just over 200 cDNA clones that interact with RS2, and these include homologs of several genes with known functions in transcriptional repression or chromatin remodeling, but the specificity of their interaction has not yet been confirmed.

DORSOVENTRAL PATTERNING OF LATERAL ORGANS IN MAIZE

Normal maize leaves develop as flattened dorsoventral organs with distinct cell types on the adaxial/dorsal and abaxial/ventral sides. We have previously shown that the recessive *leafbladeless1* (*lbl1*) mutation causes a loss of dorsal cell types. Partial 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. In addition, we have shown that the number of founder cells incorporated into *lbl1* leaf primordia is strongly reduced. These observations suggest (1) that *Lbl1* has a direct or indirect role in the down-regulation of *Knox* genes during organ initiation, (2) that dorsoventral patterning of the maize leaf primordium occurs within the meristem during initiation, and (3) that lateral growth of the organ occurs at the boundary between dorsal and ventral domains. This latter point is particular intriguing because lateral growth of insect wings occurs similarly at the dorsoventral boundary.

We have identified three new alleles of *lbl1* from EMS- and transposon-mutagenized populations, and we have introgressed these alleles into diverse maize backgrounds which affect the severity of their phenotypes. The *ragged seedling1* (*lbl1-rgd1*) allele is the

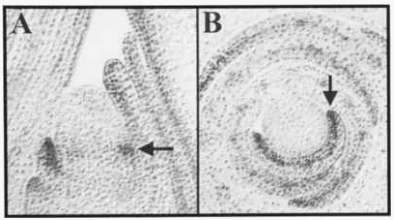


FIGURE 3 Expression pattern of the maize *yabby* gene, *Zmyab9*, in the vegetative SAM. (*A*) Longitudinal section; (*B*) transverse section. Not unlike the *Arabidopsis YABBY* genes, *Zmyab9* is expressed in the incipient primordium (arrow in *A*), and in older leaf primordia, expression becomes restricted to the vasculature and the margins of the leaf. However, in contrast to the ventral expression pattern of the *Arabidopsis YABBY* genes, expression of *Zmyab9* is restricted to the dorsal side of the leaf (arrow in *B*). The thin black lines highlight the circumference of some leaf primordia.

most severe allele, and in particular maize backgrounds, this allele results in embryo lethality. The most penetrant *lbl1* phenotype is male sterility. We are currently analyzing the embryo-lethal and male-sterile phenotypes in more detail. Our data suggest that homozygous *lbl1-rgd1* embryos develop a distorted and more rounded scutellum (comparable to a cotyledon) and lack a shoot apical meristem (SAM). These results suggest that establishment of dorsoventral polarity in the scutellum may be required to initiate or maintain a SAM, and they reveal a mutual relationship between the dorsal leaf domain and SAM function. Signals from the SAM are required to specify the dorsal leaf domain, and signals from the dorsal domain of the scutellum (embryonic leaf) are required to initiate or maintain normal meristem function.

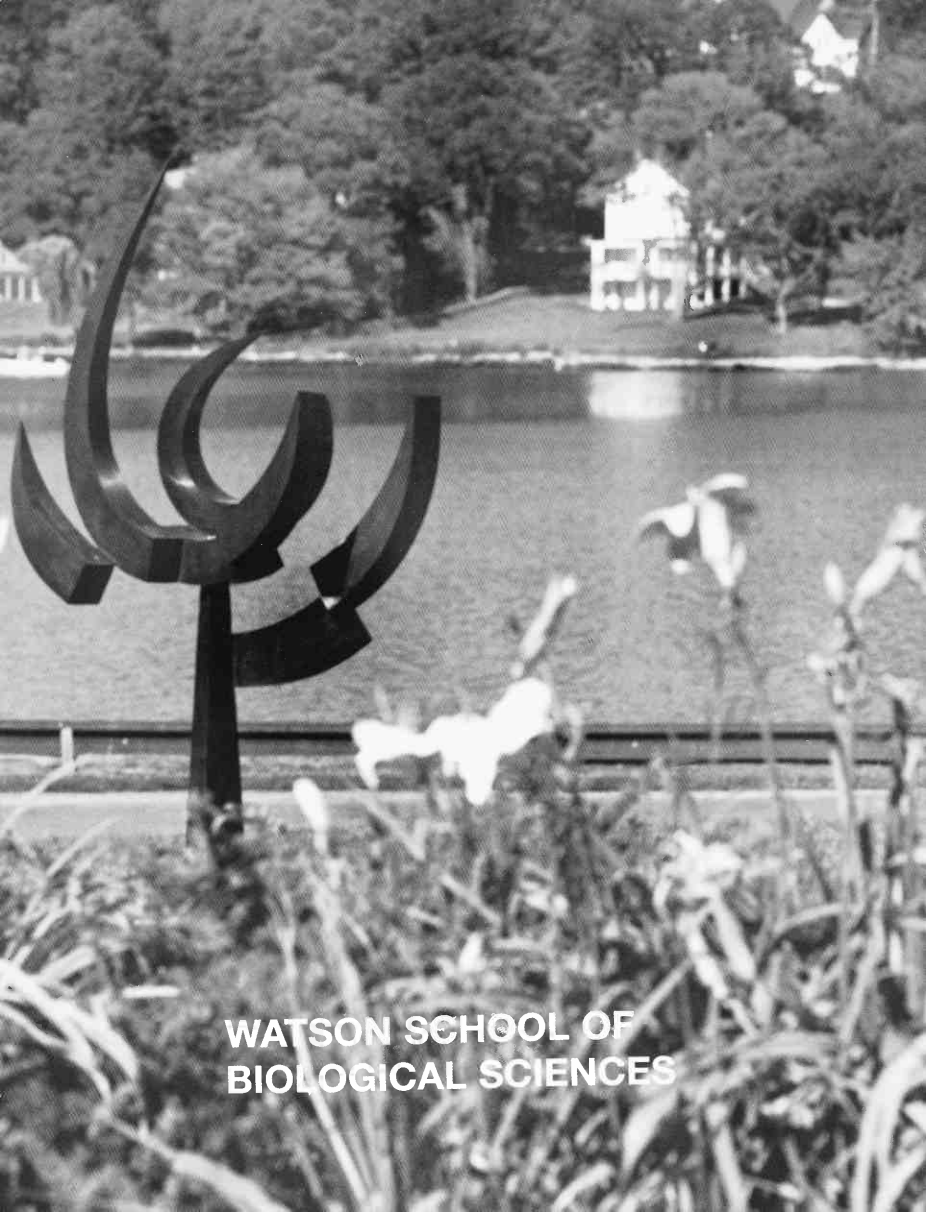
We have isolated several other mutants that affect dorsoventral patterning. The phenotypes of two of these mutations resemble *lbl1*, but both mutations are not allelic to *lbl1*. A third mutant is an allele of *Rolled1* (*Rld1*) in maize. This is a semidominant mutant that causes an inversion in leaf polarity. We have begun double-mutant analyses between the different polarity mutants. The double mutant between *Rld1* and *lbl1* resulted in a mutual suppression of both phenotypes, suggesting that *lbl1* and *rld1* act in an opposing fashion on the same pathway or that *lbl1* and *rld1* negatively regulate each other.

To characterize *lbl1* and the new dorsoventral patterning mutants in more detail, we have cloned the maize homologs of several *Arabidopsis* genes that are expressed specifically in either the dorsal or ventral domains of the leaf (Fig. 3). The *Arabidopsis YABBY* genes act in the ventral domain of lateral organs and are required for the maintenance or interpretation of dorsoventral signals. We have isolated cDNAs for several *Yabby* homologs from maize. We are currently in the process of analyzing their expression patterns in wild type and in the different dorsoventral patterning mutants in maize. Preliminary data suggest that the expression pattern of the maize *Yabby* genes is different. As in *Arabidopsis*, the maize *Yabby* genes are expressed throughout the incipient primordium, but interestingly, expression later in development becomes restricted to the dorsal side and to the margins of the leaf. The basis for and the consequence of the opposite *Yabby* expression patterns in *Arabidopsis* and maize is currently under investigation.

In Press

Byrne M., Timmermans M., Kidner C., and Martienssen R. 2001. Development of leaf shape. *Curr. Opin. Plant Biol.* **4:** 38–43.

AUTHOR INDEX


WATSON SCHOOL OF
BIOLOGICAL SCIENCES

Winship Herr, Ph.D., *Dean*
Lilian Gann, Ph.D., M.B.A., *Assistant Dean*
Janet Duffy, B.A., *Admissions and Academic Records Administrator*

EXECUTIVE COMMITTEE

Chair:	Winship Herr	
Faculty Members:	Hollis Cline	Scott Lowe
	David Helfman	William Tansey
	Michael Hengartner	Jan Witkowski
Student Representatives:	John Mignone, SUNY Stony Brook (Jan.–Nov.) Michelle Cilia, Watson School (Jan.–Nov.) Ajit Janardhan, SUNY Stony Brook (Nov.–Dec.) Amy Caudy, Watson School (Nov.–Dec.)	
Secretary:	Lilian Gann	

ADMISSIONS COMMITTEE

Chair:	Winship Herr	
Faculty Members:	Shiv Grewal	Leemor Joshua-Tor
	Gregory Hannon	Lincoln Stein
	Nouria Hernandez	Jerry Yin
	David Jackson	Anthony Zador
Secretary:	Janet Duffy	

QUALIFYING EXAM COMMITTEE

Chair:	Winship Herr
Faculty Members:	Gregory Hannon Robert Martienssen Linda Van Aelst
Secretary:	Lilian Gann

EXTERNAL ADVISORY COMMITTEE

Keith Yamamoto (Chair)
Professor and Chairman, Department of Cellular and Molecular Pharmacology, University of California, San Francisco

Gail Mandel
Professor, Department of Neurobiology and Behavior, SUNY Stony Brook
Investigator, Howard Hughes Medical Institute

Marguerite Mangin
Research Associate, The Rockefeller University

Barbara Meyer
Professor of Genetics and Development, Department of Molecular and Cell Biology, University of California, Berkeley

Frank Solomon
Professor, Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology

Shirley M. Tilghman
Professor, Department of Molecular Biology, Princeton University

WATSON SCHOOL OF BIOLOGICAL SCIENCES DEAN'S REPORT

We made it! The Watson School survived its first year! Happily, there are no casualties to report, as all of the students in the first entering class of 1999 finished the year in good standing. The faculty and School administration also survived. The first academic year of the Watson School doctoral program was a great learning experience for all—the constructive feedback and advice from students, faculty, and advisors improved and streamlined the curriculum as the year progressed. In addition, on August 28, we opened the doors to a new class of nine students from across the country and around the world. It was truly an exciting year.

The Spring Curriculum

If the first Fall Course term in 1999 was intensive, the following Spring term from January to May was hardly a walk in the park. Students returned from a 2-week holiday recess at the beginning of January to face (1) laboratory rotations, (2) teaching at the DNA Learning Center, (3) a Topics in Biology course, and, all importantly, (4) selection of a research mentor. The laboratory rotations ran the course of the spring curriculum together with teaching at the DNA Learning Center. The Topics in Biology course, a week-long "retreat" to study a specialized topic that lies outside the expertise of the Cold Spring Harbor Laboratory faculty, was held in the fourth week of February. By the close of the spring term, the students had sampled three different laboratory rotations and, after a week of discussion and reflection on possible mentors, had made decisions concerning their selection of a research mentor.

Teaching at the DNA Learning Center

One of our goals for the graduate program has been to educate biologists who can communicate effectively with nonscientists. This goal is one of the reasons that we devote a core course to scientific exposition during the fall term. For the spring, the School has taken advantage of the Laboratory's unique DNA Learning Center, situated in Cold Spring Harbor village, to provide students hands-on experience in communication to the layperson. The DNA Learning Center pioneered the teaching of DNA science with laboratory instruction to high school and middle school students. The Watson School students were given the opportunity to lead classes at the Center as part of a teaching program developed especially for them.

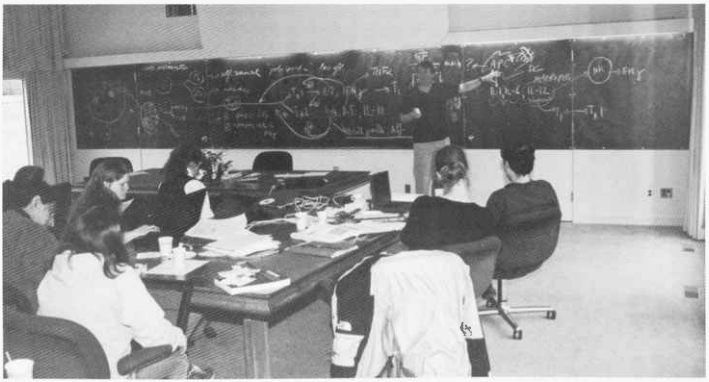
In the first week of January, DNA Learning Center Director David Micklos met with all of the students and described how the Center's instructors, led by Scott Bronson and Trisha Maskiell, would teach the students how to lead the high school and middle school classes. The students would work in pairs and begin by observing the instructors before progressing to co-teaching with the instructors and eventually teaching on their own. Once on their own, the students had to develop individual strategies to explain the lessons being taught to the young students and be prepared to answer the questions of the inquisitive minds of the students.

I sat in on three classes, one taught by each of the three pairs of students—Elizabeth Thomas and Emiliano Rial Verde, Michelle Cilia and Niraj Tolia, and Amy Caudy and Ahmet Denli—and was impressed with both the quality and diversity of the lesson plans developed by the students. In the end, the teaching experience was a great success and, were it not for the impending qualifying exam in June and the need to focus on their thesis research, the students would have enjoyed continuing teaching longer.

Topics in Biology Course

Two other goals of the Watson School are to provide a broad education in the biological sciences—thus demonstrating to the students how discoveries in seemingly unrelated fields influence one another—and to teach that learning is a lifelong process that goes hand in hand with research. Providing a broad education in the biological sciences at a small institution like Cold Spring Harbor Laboratory, with its focus on plant genetics, cancer, neuroscience, and bioinformatics, is a challenge. To help meet this challenge, we take advantage of the Laboratory's Banbury Conference Center, where its director Jan Witkowski, together with Lilian Gann, organized the first Topics in Biology course this spring. After making a collective decision that immunology was an important area in which we could not offer firsthand instruction, Jan identified Hidde Ploegh as just the person to organize and teach a 1-week course on this topic. I was excited with the selection because I have known Hidde since we were both graduate students at Harvard during the 1970s.

The selection was a resounding success. Hidde came to Banbury with his family to spend the last week of February teaching what turned out to be an outstanding course; he is a lecturer par excellence and provided an outstanding overview of immunology. He was aided by two visiting lecturers, Diane Mathis and Uli von Adrian, and three teaching fellows, Margot Furman, Ben Gewurz, and Madelon Maurice. Perhaps the most indicative measure of his success was that three non-Watson School students who chose to audit the course stayed from beginning to end. The success of the course was further enhanced by the beautiful Robertson House on the Banbury estate where Katya Davey hosted dinner for the students and instructors each evening. In toto, students and teachers alike felt that the course was an unqualified success.



Hidde Ploegh teaching the Topics in Biology course.

Laboratory Rotations and Selection of a Research Mentor

Throughout the spring term, the students participated in laboratory rotations and attended building-wide group meetings. The goals of the 6-week rotations were to provide students with hands-on laboratory experience, to give students and faculty opportunities to get to know each other, and to explore possibilities for doctoral thesis research. The rotations were short but, nevertheless, by the end of each

ENTERING CLASS OF 1999
DOCTORAL THESIS RESEARCH

Student	Academic Mentor	Research Mentor	Thesis Research
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* *Beckman Graduate Student*	Nouria Hernandez	David Jackson	Mechanisms of intercellular trafficking via plasmodesmata
Ahmet M. Denli *David Koch Fellow*	Adrian R. Krainer	Gregory Hannon	Biochemical analysis of post-transcriptional gene silencing in plants
Emiliano M. Rial Verde *David and Fanny Luke Fellow* *Howard Hughes Medical Institute Predoctoral Fellow*	Jan Witkowski	Hollis Cline	In vivo analysis of Arc function in *Xenopus* visual system development
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 framework and molecular mechanism of caspase-9 activation

rotation, the students were able to make short presentations of their studies to the other students, their rotation advisors and academic mentors, and the Scientific Exposition and Ethics core course instructors, who commented on the students' performances. These talks allowed the students to share their laboratory experiences, while providing them with another opportunity to hone their presentation skills.

At the end of the three rotations in mid May, the students spent a week discussing possibilities for thesis research with potential research mentors, their academic mentors, and others. All of the students felt comfortable making their decisions by the end of the week. I was pleased that, in the end, the students displayed a diverse set of interests and joined labs representing nearly all segments of the Laboratory's research program.

Emiliano Rial Verde with Lisa Foa during his laboratory rotation in Hollis Cline's lab.

Qualifying Exam

At the end of June, the students took an oral qualifying exam. The students were expected to possess a broad basic knowledge of biology and to display the ability to acquire and articulate in-depth scientific information by defending their knowledge of two assigned topics. The exam also tested the limits

of each student's knowledge in the assigned topics. The aim of the exam was to assess whether students had learned how, on their own, to acquire and synthesize critically scientific information on a new topic. With this goal in mind, each student orally defended their knowledge in two topics—individually assigned to each student at the end of May—over the course of 2 hours in front of three examiners. One of the assigned topics was in the area of the student's planned thesis research, whereas the second topic lay outside the thesis research area. Students were encouraged to meet with their examiners to discuss the limits and expectations for the designated topics.

A critical element of this process was to ensure that the oral exams, which by their very nature will differ in content and style from one student to the next, were fair and equitable to the students. The School therefore established a "parent" committee—the Qualifying Exam Committee (QEC)—to oversee the process. The inaugural QEC was composed of Gregory Hannon, Robert Martienssen, and Linda Van Aelst, with me as its chair. The QEC established the list of topics offered to the students for examination and made the final topic selections. The three examiners at each individual qualifying exam were all members of the Watson School faculty. Greg, Rob, and Linda served as both examiners and the chairs of two examining committees, while I sat in on each exam as a nonparticipating observer to ensure parity in the examining process. The QEC assessed the reports of each examining committee and prepared reports for the Watson School Executive Committee, which made the final decisions concerning the outcome of the qualifying exam. The QEC did an excellent job in making this critical element of the School's curriculum a success. Strengths and weaknesses were revealed through the course of the qualifying exam process, but in the end, all of the students successfully completed this element of their curriculum and were able to begin focusing on their doctoral research in earnest.

Elizabeth Thomas Spends the Fall Term at MIT

From the very beginning, we were cognizant that, in establishing a doctoral degree program at Cold Spring Harbor Laboratory, there would be areas in which we could not provide expertise on site. One such area is instruction in mathematics. Even before she decided to join the founding class of the Watson School, Farish-Gerry fellow Elizabeth Thomas had expressed her desire to improve her mathematical skills so that she could complete a doctoral thesis in the area of bioinformatics. Therefore, this fall, Elizabeth attended courses in mathematics at the Massachusetts Institute of Technology. We were pleased to learn at the end of the year that she had completed the courses successfully, and we welcomed her back to Cold Spring Harbor.

Michelle Cilia performing thesis research in David Jackson's lab.

Thesis Proposal

Toward the end of the year, as the first class entered its second year of studies, the students began the process of preparing and orally defending a written thesis proposal. The preparation and defense of the thesis proposal is a defining step in our curriculum. With the proposal, the students provide a clear outline of the goals and specific aims of their thesis research and place the proposed research in the larger context of the ongoing debates in the field. It is the time, early in the doctoral thesis work, for the students to think deeply about their research. It is also the time when students can receive extensive guidance and mentoring from diverse sources such as their research and academic mentors, thesis committee members, and the Scientific Exposition and Ethics core course instructors. Therefore, the Watson School has devoted much attention to developing a process for the thesis proposal that is most beneficial to the students and mentors alike.

ENTERING CLASS OF 2000

Santanu Chakraborty, Indian Institute of Technology, Bombay
George A. and Marjorie H. Anderson Fellow

Elena S. Ejkova, Moscow State University
Engelhorn Scholar

Rebecca C. Ewald, King's College, London
Engelhorn Scholar

Ira Hall, University of California, Berkeley
Arnold and Mabel Beckman Graduate Student

Guillaume Lettre, Université de Sherbrooke, Québec
George A. and Marjorie H. Anderson Fellow

Zachary Bela Lippman, Cornell University
Arnold and Mabel Beckman Graduate Student

Marco Mangone, La Sapienza University, Rome
Charles A. Dana Foundation Fellow

Masafumi Muratani, University of Tsukuba, Japan
George A. and Marjorie H. Anderson Fellow

Patrick J. Paddison, Evergreen State College, Olympia, Washington
Arnold and Mabel Beckman Graduate Student

Back row (left to right): Patrick Paddison; Guillaume Lettre; Masafumi Muratani; Santanu Chakraborty
Front row (left to right): Elena Ejkova; Marco Mangone; Ira Hall; Zachary Lippman; Rebecca Ewald

Preparation of the proposals began in October with the selection of thesis proposal committees. The thesis proposal committee is composed of four members—the academic mentor and three faculty members, one of whom may be external to Cold Spring Harbor Laboratory. At the end of the thesis proposal defense, the research mentor joins the committee, at which time it becomes the student's thesis advisory committee.

The written thesis proposal is ten pages long and follows the format of a National Institutes of Health postdoctoral fellowship proposal, with the specific aims of the proposed research, an in-depth background and significance, and research design and methods provided. The experimental plan was expected to be the result of extensive interactions between the student and research mentor and to be focused on a specific biological question that can be appropriately addressed in the course of approximately two and a half years of laboratory research. To aid the students—and true to form—the Scientific Exposition and Ethics core course instructors, Adrian Krainer, William Tansey, and Jan Witkowski, ran an excellent workshop in early November on the mechanics of the thesis proposal preparation. Furthermore, they provided individual tutoring and guidance to each student once a draft of the proposal had been prepared. Except for two justified exceptions for Elizabeth Thomas and Niraj Tolia, the students submitted their written proposals in mid December and were expected to defend them orally in front of their respective thesis proposal committee in January.

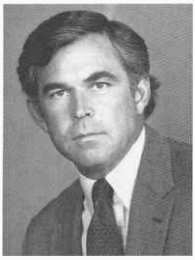
Recruiting a New Entering Class

Since the idea of a graduate school at Cold Spring Harbor Laboratory was first mentioned by James Watson at a Trustees meeting in November 1995, the process of establishing the Watson School—from developing a curriculum to obtaining accreditation to recruiting and teaching students—has been a series of new challenges. This year, for the first time, those challenges began to repeat themselves with the recruitment of a second entering class. Thus, for the first time, the School had to think of more than one class at a time. I am pleased to report that the School administration and faculty both were up to the challenge.

This year, Janet Duffy took over the reins of organizing the admissions process and working with the Admissions Committee. The deadline for applications was advanced from February 1 to January 1 to allow more time for the admissions and recruitment process. The number of applications increased to more than 150, and the School was fortunate that all the members of the first year's Admissions Committee agreed to stay on for a second year, bringing their collective experience to bear on the task ahead. We selected 25 applicants for interviews, of whom 24 agreed to come for a 1- to 3-day visit. Each visit was organized by Lilian Gann, who orchestrated approximately 300 different individual interviews with faculty! Among the most dedicated were James Watson, who met with all of the candidates, and Bruce Stillman, who met with all but one of the candidates. In the end, we made 12 offers of which 9 were accepted, an unexpectedly high acceptance rate of 75%. The 9 students who accepted come from across the United States—California, New York, and Washington—and abroad—India, Italy, Japan, England, Russia, and Canada—and are interested in a wide range of fields (see box on page 193).

The Engelhorn Scholars Program Is Launched

This year saw the start of the new Engelhorn Scholars program. This program, endowed by a gift from Curt Engelhorn through the European Foundation for the Advancement of Medicine, provides the necessary 4-year support for one or two European students a year to enter the Watson School and perform their doctoral studies. In its inaugural year, we had two Engelhorn Scholars, Elena Ejkova from Russia and Rebecca Ewald from Germany. We look forward to a rich tradition of Engelhorn Scholars.



Nicholas C. Forstmann
1947–2001

The Watson School shares the widespread sense of personal loss occasioned by the tragically early death of the very public-spirited investment banker, Nicholas C. Forstmann. Two years ago, Les Quick invited Nick to have lunch on his yacht, after first giving him a tour of our Lab, and learn about our new Ph.D. granting program. In my office, I captured Nick's highly curious mind by saying that the really big objective ahead in brain research, if not for all of biology, was to find how information, say a telephone number, is represented by precise synaptic connections between groups of nerve cells. Then I conveyed my hope that Watson School students would soon join the still limited number of bright minds trying now to decipher the language by which information is stored in our brains.

By the time Nick's subsequent boat deck lunch with Les and Bruce Stillman was over, Nick knew how he wanted to help us—through a generous gift toward the endowment of our core course on Scientific Exposition and Ethics. Through it we aim to give our students an overview of how science should proceed at the human level. That Nick chose this way to help us was a natural extension of his athlete's sense of fair play and the pleasure that comes from being part of endeavors in which competitors respect and learn from each other.

Nick was born in New York in 1947 and educated at the Lawrenceville School and Georgetown University from which he received a Bachelor's Degree in Business Administration. Starting his career at J.P. Morgan, he soon moved to join Kohlberg, Kravis and Roberts as the era of leveraged buyouts began. In 1978, he and his older brother Teddy and Brian Little founded their own buyout firm, Forstmann, Little and Company. With time, it became spectacularly successful. So Nick had the means to respond to his deep-felt philanthropic impulses, particularly through his leadership role in Catholic educational charities, among them the Inner-city Scholarship Fund that helps economically destitute grammar and high schools.

Just a year ago, uncharacteristically feeling run down, Nick learned he had advanced small cell lung cancer. Though Nick all too well knew his chances of beating it were small, he was determined to beat the odds. He loved so much his life, in particular, his talented wife Lana and their three very young children. But, alas, the best of anti-cancer drugs remained not equal to the worst of all cigarette-caused cancers. On February 2, at the age of only 54, he died in his home in Manhattan. The funeral mass celebrated in a very filled St. Patrick's Cathedral was an occasion of much grief for a universally admired man of exemplary honesty, generosity, and fortitude.

James D. Watson

A Dedicated Endowment Grows

One of the very special features of the Watson School is that, in large part through an endowment, the School covers the costs of the 4-year training of the graduate students, including tuition, stipend, and research expenses. In this manner, the School can guarantee that the education of each student remains focused on his or her academic development. The Watson School's capital campaign has continued to be immensely successful largely owing to the dedicated efforts of its chairman David Luke and Richard Cosnotti, Chief Development Officer, to whom we are most thankful for all their efforts. Among many generous contributions this year, I am also very thankful to Cynthia and Leon Polsky for establishing the Dean's Chair in Lita Annenberg Hazen's name. This chair is what cements the School together by providing the essential administrative support that has been so important to its success. Also critical this year was a gift to support four students and core course instruction over a 5-year period by the Arnold and Mabel Beckman Foundation. This gift could not have been more timely with the one or two extra incoming students who joined us this year.

Undergraduate Research Program Alumni Join the Watson School

Since 1959, the Cold Spring Harbor Laboratory Undergraduate Research Program has been recruiting outstanding undergraduate students for a 10-week summer sojourn at the Laboratory to perform hands-on research. One of our hopes has been that we would be able to recruit such high-quality students to our new graduate program. This hope is rapidly becoming a reality. In the first class, Elizabeth Thomas is an alumna of the URP class of 1997 and, in the second class, three URP alumni joined the class: Masafumi Muratani from the class of 1998 and Rebecca Ewald and Marco Mangone from the class of 1999. The School is most grateful for the outstanding work that URP co-directors Michael Hengartner and Leemor Joshua-Tor and administrator Jane Reader do to make the program so successful each year.

A Second Fall Course Curriculum

The new students arrived during August and matriculated on August 28. This time, we were much more prepared—we had been there once before!

With the advent of a new Fall Course term, many of the courses underwent changes and improvements. The faculty teaching the School's flagship core course, the Leslie C. Quick, Jr. Core Course on Scientific Reasoning and Logic, rotated. David Jackson decided to devote more time to his research efforts, and W. Richard McCombie became an instructor of the new Specialized Disciplines course on the genome. In their place, Gregory Hannon and Leemor Joshua-Tor joined the Scientific Reasoning and Logic course. This year, the course became more integrated and the faculty made an extraordinarily successful effort to give students timely feedback on their efforts by grading the weekly problem sets within an agreed upon and very tight schedule. On the Monday following each weekly module, the faculty responsible for that module returned the graded problem sets and met with the students to review both the problem sets and themes of the previous week. Additionally, at David Helfman's prompting, a final exam was instituted. This exam provided the students an opportunity to review the 12 different weekly modules and to integrate what they had learned over the fall term. Some of the students—while regretting having to say so!—commented that the exam had served its purpose well.

The Norris and Henriette Darrell Core Course on Scientific Exposition and Ethics also had a change in teaching faculty. Deborah Barnes, a founding course instructor, left the Laboratory early this year, and a replacement had to be found. William Tansey, the course's lead instructor, was able to interest Jan Witkowski, who has a long-standing interest in writing and ethics, to join as an instructor. I also took the opportunity to join the course as a guest instructor. This year, the instructors provided the course with more cohesion by adopting a novel format in which the syllabus was organized around the process by which scientific research is accomplished—from the original idea for an experiment to the results, presentations and publication, funding, and the impact on scientists and society. The new format was most engaging. I found that participating in the course was invigorating and valued my involvement in the core course for the opportunity it afforded me to get to know the new crop of students.

The Specialized Disciplines courses also underwent an evolution. This year, the Specialized Disciplines course on bioinformatics was replaced by a course on the genome led by Robert Martienssen, with W. Richard McCombie and Lincoln Stein as co-instructors. The course retained a heavy emphasis on bioinformatics and had the original bioinformatics course instructors Andrew Neuwald and Michael Zhang as guest lecturers. It was also strengthened by more emphasis on the biological import of genome structure and function. The transcription course taught by William Tansey and me also evolved. This year, we placed much more emphasis on recent developments in our understanding of transcriptional control in both prokaryotes and eukaryotes, while maintaining a cherished historical perspective. We also engaged the students much more effectively in the course by having them read and discuss in class two selected articles during each week of the 3-week lecture segment of the course. Hollis Cline, Roberto Malinow, and Karel Svoboda also came back this year for a repeat performance of their very successful neurobiology course.

In all, the Fall Course term was a success, showing improvements over the first year as the School and its faculty gained experience.

Two-tier Mentoring

One of the special attributes of the Watson School curriculum is the high degree of mentoring and tutoring offered to the students, and its most innovative feature in this regard is the two-tier mentoring program in which each student eventually acquires both an academic and a research mentor. The idea of two-tier mentoring originated with William Tansey, and he leads its academic mentoring program, which continues to go from strength to strength. Shortly after matriculation, each student is assigned a faculty member as an academic mentor. To make the mentor assignments, self-selected faculty put their names forward as eligible mentors, and students interview those of their choice. After the interviews, the preferences of students and faculty are identified before mentors and students are matched.

This year was most encouraging as the number of faculty who put themselves forward as potential mentors more than doubled—from 12 to 26. Listed below are the pairings of the new students with mentors.

Student	Mentor
Santanu Chakraborty	David Helfman
Elena S. Ejkova	Jan Witkowski
Rebecca C. Ewald	Bruce Stillman
Ira Hall	Shiv Grewal
Guillaume Lettre	Zachary Mainen
Zachary Bela Lippman	William Tansey
Marco Mangone	Linda Van Aelst
Masafumi Muratani	Nouria Hernandez
Patrick J. Paddison	Adrian R. Krainer

Through regular meetings over the course of their graduate education, the academic mentors will follow each student's progress and guide them through their academic development.

Shared Graduate Programs

Not only nine Watson School students joined the Laboratory in 2000. Although the Laboratory only became a degree-granting institution in 1998, it has been involved in graduate education leading to the Ph.D. degree in the biological sciences for more than 25 years. Graduate students from institutions

NEW STUDENTS FROM SHARED GRADUATE PROGRAMS

Student	CSHL Research Mentor	Affiliation
Nila Banerjee	Michael Zhang	George Mason University, School of Computational Sciences
Michelle Carmell	Gregory Hannon	SUNY Stony Brook, Genetics
Bidisha Chattopadhyaya	Z. Josh Huang	SUNY Stony Brook, Neurobiology and Behavior
Brian Chen	Karel Svoboda	SUNY Stony Brook, Neurobiology and Behavior
Michelle Juárez	Robert Martienssen Marja Timmermans	SUNY Stony Brook, Genetics
Jason Kinchen	Michael Hengartner	SUNY Stony Brook, Molecular Genetics and Microbiology
Zaher Nahle	Scott Lowe	SUNY Stony Brook, Physiology and Biophysics
Thomas Pologruto	Karel Svoboda	Harvard University, Biophysics Program
Irina Pugach	Grigori Enikolopov	SUNY Stony Brook, Neurobiology and Behavior
Ashish Saxena	Nouria Hernandez	SUNY Stony Brook, Genetics
Stephanie Shaw	Adrian Krainer	SUNY Stony Brook, Molecular and Cellular Biology
Kathryn Tworkowski	William Tansey	SUNY Stony Brook, Molecular and Cellular Biology

Michelle Carmell, a student in the Genetics Program with SUNY Stony Brook is carrying out her thesis research in Greg Hannon's lab.

with Ph.D. degree-granting authority, most notably the State University of New York (SUNY) at Stony Brook, have performed their doctoral research at Cold Spring Harbor Laboratory. The Laboratory remains heavily involved in many shared graduate programs with SUNY Stony Brook. These programs help make Long Island a vibrant environment for graduate studies. This year, we welcomed 12 new students to Cold Spring Harbor Laboratory (see box).

Special Events

Alternative Careers in the Bioscience Industry

This year, we were most fortunate in being able to take advantage of the excellent series of seminars on "Alternative Careers in the Bioscience Industry" that were hosted by the Center for Biotechnology at SUNY Stony Brook. This seminar series explored the diverse career opportunities present in the bioscience industry and was aimed at seniors and graduate students in engineering, life sciences, and bioscience. It provided an opportunity for them to explore alternative career paths in industry outside academia and featured key industry speakers from the various fields of corporate R&D, finance, business development, regulatory affairs, and entrepreneurial ventures. Seminar titles included:

- The Strategy Beyond the Science—Careers in Business Development
- A Dollar and a Dream—Life as an Entrepreneur
- $$ High Risk, High Returns $$"—Explore Exciting Career Opportunities in the Financial and Commercial Development of Pharmaceutical and Biotechnology Ventures

Gavin Borden Lecture

The sixth Gavin Borden Visiting Fellow Lecture (so named after the energetic and charismatic publisher of *The Molecular Biology of the Cell*, who died in 1991 of cancer) was given by Douglas A. Melton, Ph.D., of the Department of Molecular and Cellular Biology, Harvard University, and the Howard Hughes Medical Institute. The lecture, entitled "Construction of the Pancreas," was followed by a reception and dinner for the speaker with the graduate students. The next day Dr. Melton met with graduate students for an informal discussion.

Holiday Party

This year saw the start of what we expect to become an annual tradition—the Graduate Student Holiday Party. An organizing committee made up of students Emily Bernstein, Ira Hall, Michelle Juárez, Andy Samuelson, Yvette Seger, and Joe West worked relentlessly with Charles Prizzi, Director of Special Events, and Lilian Gann to make this event happen (with only three weeks notice!). The party included a D.J., Karaoke, and the first annual "Wheel of Science" faculty-student challenge. In true graduate-student party tradition, the faculty were "roasted" in an evening filled with challenges, games, quizzes, and much more! We all eagerly await the same fate next year.

Michelle Juárez, Yvette Seger, and Emily Bernstein in fluorescent wigs at the Graduate Student Holiday Party.

Curriculum Development and Oversight

To develop a 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 a dedicated and wise executive committee. The Watson School Executive Committee meets monthly to address the wide range of issues involved in overseeing the program and the progress of the students. Issues the committee addresses are numerous and include:

- Determining the roles of faculty in the program.
- Overseeing the committees for admissions and qualifying examinations.
- Monitoring graduate student progress.
- Developing and monitoring the curriculum.

The faculty and students on the committee have been instrumental in the School's development. The Executive Committee meetings are characterized by thoughtful and lively discussions of issues important to the School. Its members have devoted considerable time and intellectual resources to the program's development, for which I am most grateful.

We have also been particularly fortunate to have two student representatives, John Mignone representing SUNY Stony Brook students and Michelle Cilia representing Watson School students. I am most thankful to John for helping us at a time when the Watson School did not yet exist and the impact

the new school would have on the existing graduate student population was still unknown. He displayed loyalty and vision in his advice on how to make the transition successful and the Watson School special. At the end of the year, two new student representatives replaced John and Michelle; Amy Caudy for the Watson School and Ajit Janardhan for SUNY Stony Brook. We look forward to working with them over the course of the next year.

External Advisory Committee

In such an endeavor as establishing a new doctoral program, internal advice and guidance alone are not sufficient and the School is most fortunate to have an outstanding external advisory committee led by Keith Yamamoto. The Watson School External Advisory Committee showed its dedication to the School by visiting the lab twice, once in January and the second time in July, during its inaugural year. In January, the committee reviewed the first Fall Course term. A major observation of the Advisory Committee was that we need to ensure that the students are learning concepts and principles and integrating the information being presented. The committee was concerned that because of the intensity of the fall term and the modular structure of the Scientific Reasoning and Logic core course, the students might perform a "brain dump" at the end of each week and forget what they had learned before moving on to the next week's assignments. These and other astute observations have been a major impetus for the aforementioned changes to the Fall Course curriculum.

During the Committee's second visit, in addition to reviewing the spring curriculum and our changes for this fall, they encouraged me to reduce my level of involvement in some of the clearly successful components of the School to focus my energy on new challenges and opportunities that will emerge in the future. With this advice in mind, in December, I stepped down from the School's Admissions Committee and, at the Watson School Executive Committee's suggestion, handed over the reins to Nouria Hernandez. At year's end, she, Janet Duffy, and the Committee had their work cut out for them as there was more than a 50% increase in the number of applications received over the previous year!

Michael Hengartner Announces His Imminent Departure

Not everything could be as we desired this year. We learned from one of the founders of the Watson School curriculum, Michael Hengartner, that he would be leaving us early in 2001 for an endowed professorship at the University of Zurich. Such news was most unwelcome, but it offered a chance to reflect on how valuable the faculty have been in establishing a novel curriculum. Michael joined the Laboratory staff in 1994, fresh from his doctoral studies with Robert Horvitz at MIT. From the very beginning, it was evident that Michael was one of our most enthusiastic, energetic, and smart colleagues. Immediately, graduate students gravitated to his lab and in him I saw a future leader of the Undergraduate Research Program, of which he became director in 1995. From the start, Michael took an active interest in graduate education and became heavily involved in the Genetics Program at SUNY Stony Brook, where he was a part of its admissions and executive committees.

Michael Hengartner

For me, Michael's most important contribution was in developing the Watson School curriculum. He was a frequent attendee of the weekly roundtable discussions in the fall of 1997 at which the essence of the curriculum was developed. He was particularly interested in teaching students scientific reasoning and logic. Together with Scott Lowe, he developed the first exemplary problem set of the Scientific Reasoning and Logic Core Course—of course, on his favorite topic: apoptosis!—which was used to gain our accreditation from the

New York State Education Department as a degree-granting institution. When we actually began to teach the course, he naturally became the founding lead instructor. His teaching capabilities were an inspiration to us all as he was consistently rated one of the best lecturers by the students. And yet, not only was he involved in a course, however important it may have been, he was also a founding member of the Watson School Executive Committee and the Admissions Committee. He will be sorely missed, and we can only hope that we will be able to rely on him in new ways in the future.

A Heartfelt Thank You

The preparation of this annual report makes one reflect on how much the Watson School depends on so many people for its success. The first and second classes of students have shown extreme loyalty to the new program, offering much constructive advice as we try things for the very first time. The lean—but not mean!—School administration has performed flawlessly. Without the devotion, intelligence, tact, care, and hard work of Lilian Gann and Janet Duffy, the School would be nowhere near as successful as it has been. Where would the School be without the financial support provided by David Luke's leadership and our many generous benefactors, or without the sage advice of our external advisory committee led by Keith Yamamoto? The Laboratory's administration led by Bruce Stillman and James Watson has supported the School at every step, while offering it the opportunity to spread its wings. And it is a never-ending wonder to me that, at an institution known to attract scientists for the freedom to pursue their research, there has been so much commitment and devotion by the faculty to teaching, student mentoring, and developing new ideas for the curriculum. The School would pale in comparison were it not for all that caring people have done for it, and for this I am most thankful.

April, 2001 **Winship Herr**

Topics in Biology: Immunology

ARRANGED BY	**Lilian Gann** **Jan Witkowski**
FUNDED IN PART BY	**The Daniel E. Koshland, Jr. Visiting Lectureship** **The Fairchild Martindale Visiting Lectureship** **The Lucy and Mark Ptashne Visiting Lectureship** **The Michel David-Weill Visiting Lectureship**
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 **Ben Gewurz,** Harvard Medical School **Madelon Maurice,** Harvard Medical School

Each year, one or a team of invited instructors offer a 7-day course at the Banbury Conference Center to explore specialized topics outside the expertise of the Cold Spring Harbor Laboratory faculty. The course includes morning or evening lectures as well as afternoon sessions during which students read assigned papers. For Spring 2000, the topic was immunology. 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 Sunday, February 20–27, and was organized and largely taught by Hidde Ploegh. Two guests—Diane Mathis and Uli von Andrian—also lectured in the course, and three teaching fellows participated in all aspects of the course. The course was highly rated by all of the student participants, including course auditors.

Back row (left to right): Hidde Ploegh, Tomasz Swigut, Eric Gillitzer, Niraj Tolia
Middle row (left to right): Ahmet Denli, Margot Furman, Ben Gewurz, Madelon Maurice
Front row (left to right): Amy Caudy, Michelle Cilia, Takashi Odawara, Elizabeth Thomas, Emiliano Rial Verde

Teaching Experience at the DNA Learning Center

INSTRUCTOR **David A. Micklos**

CO-INSTRUCTORS **Scott Bronson**
Trisha Maskiell

As science plays an increasing role in society, there is an increasing need for biologists to educate non-scientists of all ages about biology. The Watson School of Biological Sciences doctoral program offers its students unique teaching experiences through the Laboratory's DNA Learning Center, where students teach laboratory courses. 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 a week for 12 weeks. During this time, the students had the opportunity to teach both high school and middle school students. In the initial weeks, the course instructors taught the students the didactic process—it was not until the fifth week that the students taught on their own. At the end of the 12 weeks, the students were very excited about their teaching experience.

Laboratory Rotations

ROTATION MENTORS

Hollis Cline	**David Jackson**	**Karel Svoboda**
Grigori Enikolopov	**Leemor Joshua-Tor**	**Michael Wigler**
Shiv Grewal	**Lincoln Stein**	**Anthony Zador**
Gregory Hannon	**Bruce Stillman**	**Michael Zhang**
Michael Hengartner		

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 a short presentation 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, the instructors of the Scientific Exposition and Ethics core course attend the talks and give individual feedback to students on their presentations. This year, 13 CSHL faculty members (listed above) served as rotation mentors.

FALL COURSE CURRICULUM

CORE COURSES

The Leslie C. Quick, Jr. Core Course on Scientific Reasoning and Logic

INSTRUCTORS	**Michael Hengartner (lead)**	**David Helfman**	
	Grigori Enikolopov	**Leemor Joshua-Tor**	
	Gregory Hannon	**Scott Lowe**	
GUEST LECTURERS	**Alexander A.F. Gann**	**Robert Martienssen**	**Nick Tonks**
	Bruce A. Futcher	**Jacek Skowronski**	**Linda Van Aelst**
	David Jackson	**David L. Spector**	**Michael Wigler**
	Winship Herr	**Arne Stenlund**	**Jan Witkowski**
	Adrian R. Krainer	**Bruce Stillman**	**Rui-Ming Xu**
	Yuri Lazebnik		

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. This course consisted of 12 weekly segments, each of which had a different theme. Each week, students read an assigned set of research articles (generally five 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. This year, the course culminated with a final exam in the last week. 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	DNA Replication
Week 3	RNA Processing
Week 4	Protein Kinesis
Week 5	Signal Transduction
Week 6	Cell Cycle Regulation
Week 7	Cancer Genes
Week 8	Apoptosis
Week 10	Cell–Cell Communication
Week 11	Mobile Genetic Elements
Week 12	Development
Week 13	Final Exam

The Norris and Henriette Darrell Core Course on Scientific Exposition and Ethics

FUNDED IN PART BY **The Edward H. Gerry Visiting Lectureship**
The John P. and Rita M. Cleary Visiting Lectureship
The Martha F. Gerry Visiting Lectureship
The Susan T. and Charles E. Harris Visiting Lectureship

INSTRUCTORS **William Tansey (lead)**
Adrian R. Krainer
Jan Witkowski

GUEST INSTRUCTOR **Winship Herr**

GUEST LECTURER **Terri Grodzicker**

VISITING LECTURERS **Robert P. Charrow, Esq.,** Crowell & Moring LLP
Maria Freire, Director, Office of Technology Transfer, NIH
Joe Palca, Senior Science Correspondent, National Public Radio
Nancy Wexler, Department of Psychiatry, Columbia University

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 took on a novel format 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. An added benefit of this course was that three of the visiting lecturers, Robert P. Charrow, Maria Freire, and Joe Palca, also lectured to the entire Cold Spring Harbor Laboratory community.

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. The students learned how to approach important problems in biology. These seminars, together with the annual fall in-house symposium, provide students with a basis for selecting laboratories in which to do rotations. The weekly speakers were:

Week 1 Ryuji Kobayashi, Andrew Neuwald, Rui-Ming Xu
Week 2 Leemor Joshua-Tor, Arne Stenlund, Bruce Stillman

Week 3	David L. Spector, Michael Zhang
Week 4	Hollis Cline, David Jackson, Jacek Skowronski
Week 5	Linda Van Aelst, Jerry Yin, Yi Zhong
Week 6	Robert Lucito, William Tansey
Week 7	Shiv Grewal, Gregory Hannon, Tatsuya Hirano
Week 8	Masaaki Hamaguchi, Scott Lowe, Michael Wigler
Week 9	Michael Hengartner, Nouria Hernandez, Adrian R. Krainer
Week 10	Winship Herr, Yuri Lazebnik, Lincoln Stein
Week 11	David Helfman, Karel Svoboda, Roberto Malinow
Week 12	Tim Tully, Z. Josh Huang
Week 13	W. Richard McCombie, Nicholas Tonks
Week 14	Dmitri Chklovskii, Zachary Mainen, Anthony Zador

SPECIALIZED DISCIPLINES COURSES

The Genome

FUNDED BY	**The George W., Jr. and Lucy Cutting Lectureship** **The Quick & Reilly Lectureship** **The George B. Rathmann Lectureship**
INSTRUCTORS	**Robert Martienssen** **W. Richard McCombie** **Lincoln Stein**
GUEST LECTURERS	**Robert Lucito** **Vivek Mittal** **Andrew Neuwald** **Michael Wigler** **Jan Witkowski** **Michael Zhang**
VISITING LECTURER	**Benjamin Burr,** Brookhaven National Laboratory

The first draft of the human genome sequence was completed this year, and the new science of genomics promises to revolutionize biological concepts and approaches, making the computer as essential a tool for research 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.

Mechanisms of Transcriptional Regulation: From *E. coli* to Elephants

FUNDED IN PART BY **The Mary D. Lindsay Lectureship**

INSTRUCTORS **Winship Herr**
William Tansey

GUEST LECTURERS **Alexander A.F. Gann**
Nouria Hernandez
Leemor Joshua-Tor
David L. Spector

This year, a draft of the complete sequence of the human genome has been determined, thus presenting a complete set of instructions for human life. These instructions are read through the process of gene transcription, and regulation of this process is central to the control of gene expression. This course presented an integrated description of transcriptional regulation in prokaryotes and eukaryotes. An underlying theme of the course was that, as postulated by Jacques Monod, what is true for *E. coli* is true for elephants. The course was presented from both a historical perspective—to illustrate how the ideas in the field developed—and a current perspective—to provide today's view of transcriptional regulation in normal and diseased states.

Mechanisms of Synaptic Plasticity and Learning

FUNDED IN PART BY **The Edward H. and Martha F. Gerry Lectureship**
The Klingenstein Lectureship

INSTRUCTORS **Hollis Cline**
Roberto Malinow
Karel Svoboda

This course explored the cellular plasticity that underlies changes in brain function associated with development and with learning and memory. The study of the development of the visual system shows how organized topographic sensory projections develop in the central nervous system. Discussions of research on the mammalian hippocampus using behavioral, cellular, and molecular approaches were analyzed in order to describe the cellular basis of learning and memory in the adult brain. Ultimately, students gained an understanding of neural plasticity in development and in learning and memory.

UNDERGRADUATE RESEARCH

Program Co-Directors: Michael Hengartner, Ph.D.
Leemor Joshua-Tor, Ph.D.

Program Administrator: Jane Reader

An important aspect of the summer program at Cold Spring Harbor Laboratory is the participation of college undergraduate students in active research projects under the supervision of full-time laboratory staff members. The program was initiated in 1959. Since that year, 552 students have participated in the course, and many have gone on to productive careers in biological science.

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, and molecular and cellular biology; and (4) a personal acquaintance with research, research workers, and centers for study. The following students, selected from 546 applicants, took part in the program:

Michelle A. Aaron, Clarion University
Sponsor: National Science Foundation
Advisor: **Dr. Adrian Krainer**
Exon definition and alternative splice site selection in AT-AC intron splicing.

Sarah Addou, University College, London
Sponsor: Emanual Ax Fund
Advisor: **Dr. Lincoln Stein**
Genetic map display for ACeDB.

Bottom row, left to right: Sarah Hart, Despina Siolas, Ramya Rajagopalan, Michell Aaron, Daniela Cohen, Frederick Tan, Maria Vichnevskaia
Middle row, left to right: Charles Kopec, Dougal Tervo, Kevin Vogeli, Sarah Addou, Doublas Weinstein, Trevor Yeung, Eileen Wood
Top row, left to right: Mario Ezaguirre-Sierra, Tariq Ahmad, Michael Ryczko, Abullah Ozer, David Schlesinger, Ahmed Elewa, Guillermo Munoz-Elias
Not pictured: Heather Cosel-Pieper, Natalia Caporale, Joan Hu, Sarah Archer-Evans, John D'Amore, Wisuwat Songnuan

Tariq Ahmad, New York University
Sponsor: National Science Foundation
Advisor: **Dr. Ryuji Kobayashi**
Phosphorylation analysis of p62dok.

Sarah Archer-Evans, University of Texas
Sponsor: National Science Foundation
Advisor: **Dr. David Jackson**
Subcellular localization of *fasciated ear2* in maize.

Natalia Caporale, University of Buenos Aires
Sponsor: Olney Fund
Advisor: **Dr. Zachary Mainen**
Individual recognition and its neuronal representation in the olfactory bulb.

Daniela Cohen, Yale University
Sponsor: National Science Foundation
Advisor: **Dr. Yi Zhong**
Role of Notch in activity-dependent synaptic plasticity.

Heather Cosel-Pieper, New York University
Sponsor: The JM Foundation
Advisor: **Dr. Scott Lowe**
Toward an understanding of the cellular response to chemotherapy.

John D'Amore, Harvard University
Sponsor: The JM Foundation
Advisor: **Dr. Robert Malinow**
The surface expression of NMDA receptors.

Ahmed Elewa, Cairo University
Sponsor: Garfield Fund
Advisor: **Dr. Michael Hengartner**
Measuring proliferation kinetics in the germ line of *Caenorhabditis elegans*.

Sarah Hart, Cambridge University
Sponsor: Burroughs Wellcome
Advisor: **Dr. William P. Tansey**
Characterization of the F-box protein BAA7.

Joan Hu, Washington University
Sponsor: National Science Foundation
Advisor: **Dr. Rui-Ming Xu**
Toward the structural study of pre-mRNA splicing factors.

Mario Izaguirre-Sierra, National Autonomous University of Mexico
Sponsors: Glass Fund & Libby Fund
Advisor: **Dr. David Spector**
Does actin play a role in nuclear structure?

Charles Kopec, Rutgers University
Sponsor: The JM Foundation
Advisor: **Dr. Robert Martienssen**
Expanding on a model of RA1s function in *Zea mays*.

Guillermo Munoz-Elias, Rutgers University
Sponsor: Frederica Von Stade Fund
Advisor: **Hollis T. Cline**
Lending ears to silent synapses: Regulation and expression of calcium permeable AMPA receptors in the developing retinotectal system of *Xenopus*.

Abdullah Ozer, Bilkent University
Sponsor: Burroughs Wellcome Fund
Advisor: **Dr. Yuri Lazebnik**
Construction of single-chain antibodies against caspase-9, caspase-7, and APAF-1.

Ramya Rajagopalan, Cornell University
Sponsor: Burroughs Wellcome
Advisor: **Dr. W. Richard McCombie**
Sequencing of a tomato BAC; analysis of promoter regions of nodulin-like genes in *Arabidopsis thaliana*.

Michael Ryczko, Laurentian University
Sponsor: Bliss Memorial Fund
Advisor: **Dr. Tim Tully**
Adf1 transcription factor and synapse formation in *Drosophila melonagaster*.

David Schlesinger, Brigham Young University
Sponsor: Jephson Educational Trust
Advisor: **Dr. Linda Van Aelst**
Molecular characterization of oligophrenin-1.

Despina Siolas, St. John's University
Sponsor: National Science Foundation
Advisor: **Dr. Gregory Hannon**
Developing a procedure for creating a phenotype array using RNA interference in *Drosophila* cells.

Wisuwat Songnuan, Duke University
Sponsor: Shakespeare Fund
Advisor: **Dr. Marja Timmermans**
Repression of homeobox genes by Rough Sheath2 in maize lateral organ primordia.

Frederick Tan, Worcester Polytechnic Institute
Sponsor: Jephson Educational Trust, Read Fund
Advisor: **Dr. Andrew Neuwald**
Rapid sequence alignment against hidden Markov models.

Dougal (Gowan) Tervo, Oxford University
Sponsor: Burroughs Wellcome
Advisor: **Dr. Anthony Zador**
An electrophysiological and behavioral investigation of pitch in the rat.

Maria Vichnevskaia, University of Bridgeport
Sponsor: Burroughs Wellcome Fund
Advisor: **Dr. Michael Zhang**
Identification of CREB targets in *Drosophila melanogaster.*

Kevin Vogeli, University of California, Berkeley
Sponsor: National Science Foundation
Advisor: **Dr. Grigori Enikolopov**
Role of nitric oxide signaling in early *Xenopus* development.

Douglas H. Weinstein, Duke University
Sponsor: The JM Foundation
Advisor: **Dr. Leemor Joshua-Tor**
Replication in the papillomavirus: A structural study.

Eileen Woo, Harvard University
Sponsor: National Science Foundation
Advisor: **Dr. Bruce Stillman**
Isolation and characterization of human Rad1, Rad9, and Hus1: A putative PCNA-like complex.

Trevor M.-Y. Yeung, Clare College, Cambridge University
Sponsor: Burroughs Wellcome Fund
Advisor: **Dr. David Helfman**
An investigation into the importance of a 13-amino-acid trigger sequence in mediating the dimerization of LMW tropomyosin.

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 and Nature Photography.

During the summer of 2000, a total of 350 students participated in 27 courses within the program. The classes were held outdoors, weather permitting, at the West Side School. The Laboratory has equipped and maintains classroom and laboratory facilities as well as a darkroom at West Side School. This facility is used as a base for the student's exploration of the local environment. Field classes are held on Laboratory grounds, St. John's Preserve, Fire Island National Seashore, the Nature Conservancy, Caumsett State Park, the Cold Spring Harbor Fish Hatchery and Aquarium, and other local preserves and sanctuaries.

In addition to the three 2-week sessions, the Adventure Education course met on one Friday for a 12-mile canoe trip on the Nissequogue River to navigate and explore the waters of Long Island.

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., English, Adephi University
Margot Gallowitsch, B.S., Biology, Fairfield University
Amy Friedank, B.S., Marine Science, Long Island University/Southampton College
Steve Gravano, School of Visual Arts, New York City
Patricia Grimaldi, B.S., Biology, SUNY at Stony Brook

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 thoroughly 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 harbor area.

Frogs, Flippers, and Fins (Grades 3–4): Designed for younger students as an introduction to aquatic ecosystems. Fresh water and marine habitats are explored.

Pebble Pups (Grades 3–4): Elementary geology for students interested in making 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 in this course.

Freshwater Life (Grades 5–7): Students study the vertebrate, invertebrate, and plant life found in area bogs, ponds, lakes, and streams.

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.

Adventure Education (Grades 6–10): This course is a 12-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. This course provides field trip, dissection, microscope, and laboratory experiment experiences.

Nature Photography (Grades 8–10): Students take pictures and use the darkroom to learn techniques of printing and developing. Photographic techniques relating to nature photography are emphasized. Each student supplies their own camera and film; darkroom supplies are provided.

COLD SPRING HARBOR LABORATORY MEETINGS AND COURSES

The Academic Program in 2000 brought scientists from all over the world to the Laboratory to teach, learn, and communicate about the latest results and methodologies in a wide range of areas. The program this year included 25 laboratory and lecture courses and 18 meetings, including the Cold Spring Harbor Laboratory Symposium, which focused on Biological Responses to DNA Damage.

The course program ran from March through November and covered areas ranging from molecular biology and biochemistry, genetics and genomics, and bioinformatics to structural biology and neurobiology. In the summer, 3-week laboratory courses were held, as well as lecture and workshop courses at the Laboratory's Banbury Center. A series of 1- and 2-week courses continue to be held in the spring and summer. Regardless of length and topic, the courses emphasize teaching the latest concepts and techniques that can be immediately applied to the student's own research. The timeliness and unique usefulness of the courses are reflected in the number and quality of the students who apply to take them. For example, there were almost 300 applicants for the two bioinformatics courses that were given in the fall. All of the courses were oversubscribed. The students who attended ranged from graduate students and postdoctoral fellows to senior faculty. This unique mix of students made the course labs a very lively place to be. Of course, the popularity of the courses depends, in large measure, on the wonderful instructors who come from around the world to teach them. Since the courses extend from the morning until late into the evening, the instructors' energy and stamina as well as expertise and knowledge are called into play. The course instructors, assistants, and lecturers are listed in the Postgraduate Courses Section, and we are very grateful to them for their fabulous work.

The courses are supported by grants from the National Institutes of Health, the National Science Foundation, the Howard Hughes Medical Institute, and the Grass Foundation. A variety of companies, which are listed following the Postgraduate Courses Section, also lend valuable equipment and supplies.

A series of meetings and biotechnology workshops were held this year in the Grace Auditorium. The highlight of the meetings season is always the Symposium, which was very exciting this year. This heavily oversubscribed meeting, organized by Bruce Stillman, brought scientists from all over the world to discuss their latest results in a rapidly moving area. Other oversubscribed meetings included those on Translational Control, Genome Sequencing and Biology, and Zebrafish Development and Genetics. In fact, we are saying farewell this year to the Zebrafish meeting, which was started here several years ago and has now grown so much that it must seek a larger venue. The organizers, session chairs, and session topics for the 2000 meetings are listed in the following pages.

One of the reasons that so many outstanding scientists are willing to teach courses and organize meetings is the huge amount of skilled help that is provided by staff at the Laboratory. They include Andrea Stephenson, the Course Registrar; Ed Campodonico, the Manager of Audiovisual Services; and Barbara Zane, the Course Coordinator. Staff of the Meetings and Courses office handle an ever-increasing and complex workload with efficiency, skill, and good humor. They include those listed above, as well as Michael Glaessgen, Drew Mendelsohn, Mary Smith, Marge Stellabotte, and Jenna Williams. Housing and front-desk services are provided by Nancy Weeks, the Head Concierge, and by Donna Dykemen and Andrea Newell. Audiovisual services are ably provided by Ed Campodonico, William Dickerson, John Parsons, and the part-time audiovisual staff. Staff from several other departments also do crucial work for the courses and meetings, including Wendy Crowley, the Education Grants Manager; Cliff Sutkevich 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. The Information Services Department, headed by Jerry Latter, provides ever-increasing computer support for all of the courses and meetings.

Terri Grodzicker
Assistant Director for Academic Affairs

David Stewart
Director of Meetings and Courses

65th COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

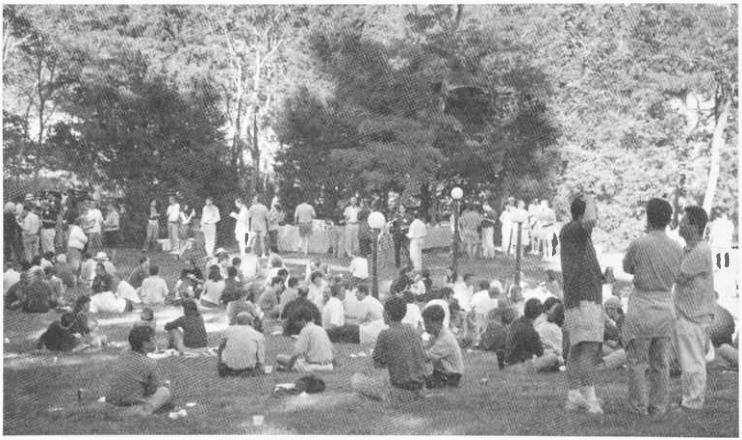
Biological Responses to DNA Damage

May 31–June 5 451 participants

ARRANGED BY **Bruce Stillman,** Cold Spring Harbor Laboratory

There are few times in science when all the planets align, allowing what were apparently different areas of investigation to come together to present a new view of a field. Such appeared to be the case for the varied fields that focused on the diverse aspects of the biological responses to DNA damage. DNA repair, genotoxic stress, telomere maintenance, and cell division cycle checkpoint control were, not so long ago, fields of research that did not obviously overlap. Now they are so intertwined that ignoring developments in any one area would limit one's view of how cells respond to biological damage. Indeed, Barbara McClintock's long-held notion that the genome can sense what is going on in its environment does not now appear to be far from the truth. Moreover, understanding DNA damage control is providing great insight into the mechanisms of cancer progression because cancer is a disease caused by genome instability. Perhaps most surprisingly, genome damage controls are now intimately tied into pathways that, when altered, cause premature aging of cells and when overexpressed, promote life extension. Combining these areas of research into a single symposium was too much of an opportunity to pass by.

There has not been a Cold Spring Harbor Symposium devoted entirely to this topic, which is perhaps surprising since Evelyn Witkin initiated studies with Milislav Demerec on ultraviolet-induced mutations in bacteria at Cold Spring Harbor in 1944. This ultimately led to her major contributions to uncovering the SOS response. Discussion of the nature of mutations and genetic damage was pervasive at Cold Spring Harbor in the 1940s and 1950s due to the presence of Demerec, Max Delbrück, Salvador



"At the Picnic"

Luria, and Barbara McClintock and can be traced to the 1941 Symposium on "Genes and Chromosomes: Structure and Organization," which occurred long before the double helix was revealed. We have come a long way in our understanding since then, and it was about time to correct the long absence of this topic from these Symposia.

The Symposium started with a fascinating first night of introductory talks from Graham Walker, Stephen Elledge, Elizabeth Blackburn, and Fred Alt. In all, the formal scientific program consisted of 67 oral presentations and a record 239 poster presentations, and the meeting attracted 451 participants who packed the Grace Auditorium. I thank Errol Friedberg for agreeing to summarize the meeting and writing such a marvelous and thoughtful summary for the Proceedings, matching the great summaries of previous Symposia.

This meeting was funded in part by the National Cancer Institute, a branch of the National Institutes of Health. In addition, financial help from the Corporate Sponsors, Affiliates, Associates, and Contributors to our meetings program is essential for these symposia to remain a success, and we are most grateful for their continued support. *Corporate Sponsors* include Amgen Inc.; AstraZeneca;

T. Lindahl, M. Kastan, R. Fishel, E. Friedberg

R. Rothstein, T. de Lange

C. Greider, Gwendolyn Marie Comfort

E. Witkin, P. Hanawalt

J. Campbell, R. Kolodner, L. Guarente

B. Stillman, J. Hoeijmakers

Aventis Pharma AG; BASF Bioresearch Corporation; BioVentures Inc.; Bristol-Myers Squibb Company; Chiron Corporation; Chugai Research Institute for Molecular Medicine, Inc.; Diagnostic Products Corporation; DuPont Pharmaceuticals Company; Forest Laboratories; Genentech, Inc.; Genetics Institute; Glaxo Wellcome, Inc.; Hoffmann-La Roche Inc.; Johnson & Johnson; Kyowa Hakko Kogyo Co., Ltd.; Eli Lilly and Company; Merck Research Laboratories; New England BioLabs, Inc.; Novartis Pharma Research; OSI Pharmaceuticals, Inc.; Pall Corporation; Palmetto Partners, Ltd.; Parke-Davis Pharmaceutical Research; PE Biosystems; Pfizer Inc.; Pharmacia & Upjohn, Inc.; Research Genetics, Inc.; Schering-Plough Corporation; and SmithKline Beecham Pharmaceuticals. *Plant Corporate Associates* include Monsanto Company; Novartis Agricultural Discovery; Pioneer Hi-Bred International, Inc.; and Westvaco Corporation. We also thank our *Foundation Associate,* Albert B. Sabin Vaccine Institute, Inc. at Georgetown University; our *Corporate Affiliates,* Affymetrix and Third Wave Technologies, Inc.; and our *Corporate Contributors,* Alexis Corporation, Aquatic Habitats, Applied imaging, BD PharMingen, Biogen, Inc., CuraGen, Digital Gene Technologies, Inc., DoubleTwist, Inc., Dynal, Eppendorf 5 Prime-3 Prime, Gene Machines, Genetix, Ltd., Genomica, Incyte Genomics, InforMax, Lexicon Genetics, Inc., Neomorphic, Promega Corporation, Proteome, Inc., Qiagen, R & D Systems, Sequenom, StressGen Biotechnologies Corp., and ZymoGenetics, Inc.

T. Weinert, G. Wahl, A. Carr, E. Seeberg

Targets and Molecules: The Science of Drug Discovery

March 8–10 107 participants

ARRANGED BY **David Stewart,** Cold Spring Harbor Laboratory
Bruce Stillman, Cold Spring Harbor Laboratory
Jan Witkowski, Cold Spring Harbor Laboratory

This special winter biotechnology conference on Targets and Molecules: The Science of Drug Discovery was one of the scientific celebrations hosted by the Laboratory to welcome in the new millennium. The conference was designed in part to reflect the interest of our industrial supporters, who have supported the Banbury Conference and Grace Meeting programs for a number of years through the invaluable Corporate Sponsor Program. The conference presented some of the most interesting and exciting research in four major areas of drug discovery, specifically cancer, cardiovascular-related diseases, infectious diseases, and neuroscience, together with a short session focused on controversial issues in intellectual property facing the biotech/pharmaceutical industry. In addition, the conference opened and closed with keynote addresses by Peter Colman on novel therapies for influenza, and by John Kozarich on microorganisms and macroorganizations. This meeting was funded in part by the William Theodore Denslow Foundation.

PROGRAM

Introduction
B. Stillman, *Cold Spring Harbor Laboratory*

Keynote Address
P. Colman, *Biomolecular Research Institute, Parkville, Australia*

Cancer: Focus on Angiogenesis
Chairperson: S. Lowe, *Cold Spring Harbor Laboratory*

Round Table Discussion: Patent and Intellectual Property Issues in Genomics, Targets, and Drug Discovery
Moderator: M. Freire, *National Institutes of Health, Bethesda, Maryland*

Infectious Disease Targets
Chairperson: P. Colman, *Biomolecular Research Institute, Parkville, Australia*

Neurodegenerative Diseases: Focus on Alzheimer's Disease
Chairperson: R. Malinow, *Cold Spring Harbor Laboratory*

Neuroscience and Beyond: Targets and Molecules
Chairperson: H. Cline, *Cold Spring Harbor Laboratory*

Closing Address
J.W. Kozarich, *Merck Research Laboratories*

M. Hoffman, D. Stewart

S. Xanthoudakis, L. Van Der Ploeg, J. Kozarich

F. Austin, E. Gimmi

Zebrafish Development and Genetics

April 26–30 467 participants

ARRANGED BY **Michael Brand,** University of Heidelberg, Germany
Marnie Halpern, Carnegie Institution of Washington
John Kuwada, University of Michigan, Ann Arbor
Christine Thisse, IGBMC, France
Leonard Zon, HHMI/Children's Hospital

This fourth meeting on Zebrafish Development and Genetics consisted of more than 350 platform talks and poster presentations and covered many of the most interesting areas of current zebrafish research. Exciting sessions were held on Zebrafish as a Model for Human Disease, Cell Signaling, Early Development, Tissue Patterning, Neural Crest, Organ Patterning, Sensory Organs, Sensory Processing and Behavior, Neurobiology, and Evolution, with additional sessions on Technology and a special Genomics Workshop and Poster session. Harold Varmus, former director of the National Institutes of Health, delivered the keynote address. Progress was reported in using insertional mutagenesis to carry out mutant screens, in the development of transposon-tagging methods, and in genomic analysis of the zebrafish genome. The information presented confirmed the great promise of the zebrafish as a model vertebrate for the study of developmental and physiological processes. The meeting clearly indicated that molecular characterization of genes first identified from zebrafish mutant screens is adding greatly to our knowledge of vertebrate development. More than 30 mutant genes were isolated as candidates, and about 10 positional cloning projects were discussed.

This meeting was funded in part by the National Institute of Child Health and Human Development; National Institute of Neurological Disorders and Stroke; National Institute of Mental Health; National Institute of Diabetes and Digestive and Kidney Diseases; National Institute on Deafness and Other Communication Disorders; National Institute of Environmental Health Sciences; National Institute on Aging; and the National Human Genome Research Institute, branches of the National Institutes of Health; and the National Science Foundation.

M. Brand, J. Kuwada, L. Zon, M. Halpern, C. Thisse

PROGRAM

The Zebrafish as a Model for Human Disease
Chairperson: I. Dawid, *NICHD, National Institutes of Health, Bethesda, Maryland*

Keynote Speaker
H. Varmus, *Memorial Sloan-Kettering Cancer Center*

Cell Signaling I
Chairperson: H. Takeda, *National Institute of Genetics, Mishima, Japan*

Neurobiology I
Chairperson: C. Houart, *University College London, United Kingdom*

Technology I
Chairperson: C. Thisse, *Institute of Genetics and Molecular Cellular Biology, Strasbourg, France*

Evolution
Chairperson: V. Prince, *University of Chicago, Illinois*

Early Development I
Chairperson: D. Kane, *University of Rochester, New York*

Sensory Processing and Behavior
Chairperson: K. Whitlock, *Cornell University, Ithaca, New York*

Technology II
Chairperson: W. Talbot, *Stanford University School of Medicine, California*

Organ Patterning
Chairperson: L. Zon, *Children's Hospital, Boston, Massachusetts*

Neurobiology II
Chairperson: C.-B. Chien, *University of Utah Medical Center, Salt Lake City*

Sensory Organs
Chairperson: M. Brand, *University of Heidelberg, Germany*

Community Meeting
Chairperson: J. Kuwada, *University of Michigan, Ann Arbor*

Early Development II
Chairperson: M. Hammerschmidt, *Max-Planck Institute for Immunobiology, Freiburg, Germany*

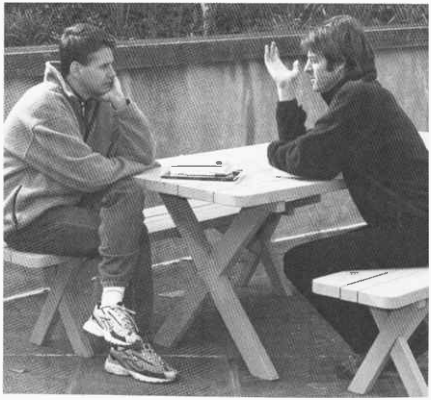
Tissue Patterning
Chairperson: I. Drummond, *Massachusetts General Hospital, Charlestown*

Genomics Workshop
Moderator: S. Johnson, *Washington University School of Medicine, St. Louis, Missouri*

Cell Signaling II
Chairperson: M. Halpern, *Carnegie Institution of Washington, Baltimore, Maryland*

Neural Crest
Chairperson: R. Kelsh, *University of Bath, United Kingdom*

L. Steiner, N. Hopkins, B. Defrich



D. Meyer, W. Talbot

Cancer Development, Diagnosis, and Therapies: Implications for Pancreatic Cancer

May 2 111 participants

ARRANGED BY **Ralph H. Hruban,** Johns Hopkins Medical Institutions
Scott Lowe, Cold Spring Harbor Laboratory

A special 1-day conference on pancreatic cancer was jointly hosted by the Lustgarten Foundation for Pancreatic Cancer Research and Cold Spring Harbor Laboratory. The Lustgarten Foundation is a charitable organization dedicated to improving the diagnosis and treatment of pancreatic cancer. The Foundation has previously awarded several million dollars to support pancreatic cancer research and is currently working with the National Cancer Institute to discuss strategies to improve public awareness and funding of pancreatic cancer. The conference provided an overview of the current status of research in pancreatic cancer and highlighted the use of mouse models, including disruption of TGF-β signaling in mice, novel strategies for generating mouse models of human cancers, and syndrome modeling in mice. The conference also presented novel technologies for identifying cancer genes and for microarray-based tumor diagnosis. Finally, novel anti-angiogenesis therapies were presented and discussed. The conference featured an introduction by James D. Watson and talks by Patrick Brown, Ronald DePinho, Ralph Hruban, Alfred Knudson, Jerry McMahon, Tak Mak, Joan Massague, Harold Varmus, and Michael Wigler.

PROGRAM

Opening Remarks
J.D. Watson, *Cold Spring Harbor Laboratory*

Introduction
R. Hruban, *Johns Hopkins Medical Institutions*

Session I: Technology and Models
Chairperson: S. Lowe, *Cold Spring Harbor Laboratory*

R. DePinho, *Dana Farber Cancer Institute*
H. Varmus, *Memorial Sloan-Kettering Cancer Center*
J. Massague, *Memorial Sloan-Kettering Cancer Center*
P. Brown, *Stanford University Medical Center*

Session II: Targets and Biology
Chairperson: R. Hruban, *Johns Hopkins Medical Institutions*

J. McMahon, *Sugen Inc., South San Francisco, California*
M. Wigler, *Cold Spring Harbor Laboratory*
T. Mak, *Ontario Cancer Institute*
A. Knudson, *Fox Chase Cancer Center*

H. Varmus

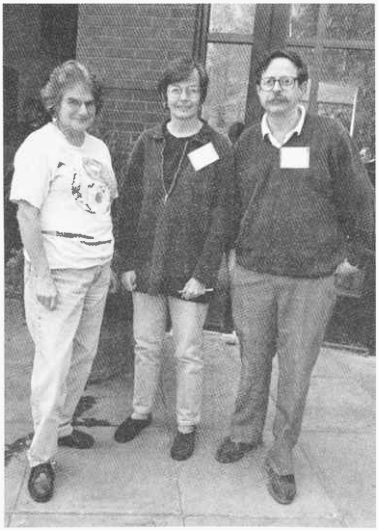
R. Hruban, S. Lowe

Molecular Chaperones and the Heat Shock Response

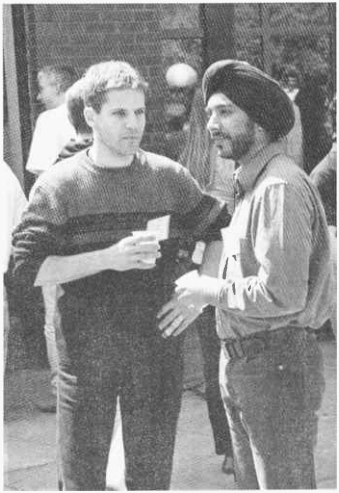
May 3–7 366 participants

ARRANGED BY **Elizabeth Craig,** University of Wisconsin, Madison
Carol Gross, University of California, San Francisco
Arthur Horwich, HHMI/Yale University School of Medicine

The fifth biennial meeting on molecular chaperones and the heat shock response 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 for calnexin/calreticulin. A highlight was a session on quality control in the ER, dealing with chaperone selectivity, the relation of oligosaccharide processing to folding and chaperone recognition, and mechanisms of dislocation of misfolded proteins back to the cytosol for proteasomal degradation. In the area of stress response, new understandings about signaling of ER stress to the cytosol and nucleus were presented. The structure and action of a redox-regulated chaperone, Hsp33, were presented. In the area of protein misfolding in disease, a number of talks dealt with recent observations about presenilin, a membrane protein of the secretory pathway involved in an inherited form of Alzheimer's disease. It was biochemically isolated as part of a large complex, and evidence was presented that it exhibits γ-secretase activity from two *trans*-membrane aspartates. Other talks dealt with polyglutamine repeat disease and with the mechanism of prion formation. Conversion of yeast to a psi-



C. Gross, E. Craig, A. Horwich



H. Scherer, H. Singh-Jasuja

plus state was reported to have been accomplished by physical transfer of in-vitro-converted material into psi-minus cells.

This meeting was funded in part by the National Institute of Neurological Disorders and Stroke, a branch of the National Institutes of Health; the National Science Foundation; the Alzheimer Association; Bayer Corporation; HSP Research Institute; Merck & Co., Inc.; and StressGen Biotechnologies Corporation.

PROGRAM

Diseases of Protein Misfolding
Chairperson: S. Prusiner, *University of California, San Francisco*

Quality Control and Protein Trafficking
Chairperson: A. Helenius, *Swiss Federal Institute of Technology, Zurich*

Cellular Function of Chaperones
Chairperson: U. Hartl, *Max-Planck Institute for Biochemistry, Martinsried, Germany*

Chaperone Function in Disease and Development
Chairperson: S. Radford, *University of Leeds, United Kingdom*

Regulation of the Stress Response
Chairperson: R. Morimoto, *Northwestern University, Evanston, Illinois*

Chaperones and Proteolysis
Chairperson: S. Gottesman, *National Cancer Institute, Bethesda, Maryland*

Chaperone Biochemistry and Protein Folding
Chairperson: D. Agard, *University of California, San Francisco*

L. Bharadwaj, N. Ovsenek, G. Davies, S. Bharadwaj

W.R. Brown, M. Tytell

Genome Sequencing and Biology

May 10–14 513 participants

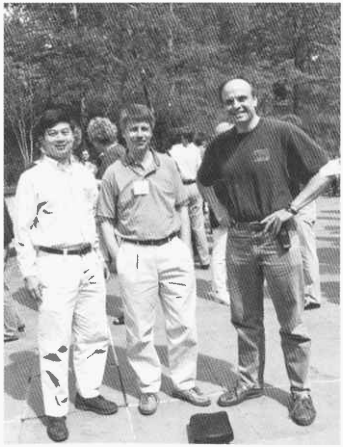
ARRANGED BY **Mark Boguski,** National Center for Biotechnology Information
Stephen Brown, MRC Mouse Genome Centre, United Kingdom
Pui-Yan Kwok, Washington University

This meeting marked the 13th annual gathering of genome scientists in this setting. The past decade or more has seen remarkable progress in the mapping and sequencing of the genomes of many "model organisms," and there is now a strong indication that a "working draft" of the human genome sequence is near completion. Phrases such as "functional genomics" and "post-genome biology" have become common terms. Just over 500 people from around the world attended the meeting, with 298 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 phylogenomics, mapping methods and technologies, functional genomics, computational genomics, and 21st century genetics. This year's poster symposium featured the complete sequencing of human chromosomes 21 and 22 in addition to the announcement by the public human genome project that 80% of the human genome had been sequenced. Once again, projection-style, interactive computer demonstrations in Grace Auditorium effectively highlighted the critical new bioinformatics tools being developed for storing, organizing, and analyzing genomic maps and sequences. There was also an ELSI (Ethical, Legal, and Social Implications) panel discussion, chaired by Charmaine Royal of Howard University, which focused on issues surrounding genetic enhancement.

The major themes of the meeting included the analysis of the complete *Drosophila* sequence and the smallest human chromosomes, with the surprising observation that the number of genes in the human might be as small as 30,000. Specific presentations continued to report major achievements in the sequencing of model organisms and the human, the development of new technologies for genet-

P. Green, E. Green



P.-Y. Kwok, S. Brown, M. Boguski

ic analysis, and the use of microarrays for performing genome analysis. The now traditional Saturday afternoon keynote talk was delivered by Francis Collins, director of the National Human Genome Research Institute and head of the U.S. Human Genome Project.

This meeting was funded in part by the National Human Genome Research Institute, a branch of the National Institutes of Health.

PROGRAM

Polymorphisms I: Discovery/Mapping/Scoring
Chairpersons: A.-C. Syvanen, *Uppsala University, Sweden;* L. Stein, *Cold Spring Harbor Laboratory*

Comparative Genomics I: *Drosophila* and Other Model Organisms
Chairpersons: G. Rubin, *Lawrence Berkeley National Laboratory, California;* C. Venter, *Celera Genomics, Rockville, Maryland*

Bioinformatics I: Database Resources
Chairpersons: S. Sherry, *NCBI, National Institutes of Health, Bethesda, Maryland;* J. Bouck, *Baylor College of Medicine, Houston, Texas*

Comparative Genomics II: Plants
Chairpersons: D. Preuss, *University of Chicago, Illinois;* S. Wessler, *University of Georgia, Athens*

Human Sequencing Poster Symposium
Chairpersons: J. Rogers, *Sanger Centre, Cambridge, United Kingdom;* G. Schuler, *NCBI, National Institutes of Health, Bethesda, Maryland*

Bioinformatics II: Software and Algorithms
Chairpersons: P. Green, *University of Wisconsin, Madison;* P. Rodriguez-Tome, *EMBL-EBI, Cambridge, United Kingdom*

ELSI Panel Discussion: Genetic Enhancement—Ethical, Legal, and Social Points to Consider
Moderator: C. Royal, *Howard University, Washington, D.C.*

Comparative Genomics III: Mouse Genomics/Functional Genomics I—Technologies and Infrastructure
Chairpersons: L. Stubbs, *Lawrence Livermore National Laboratory, California;* V. Cheung, *University of Pennsylvania, Philadelphia*

Polymorphisms II: Applications
Chairpersons: E. Lai, *GlaxoWellcome, Research Triangle Park, North Carolina;* E. Ostrander, *Fred Hutchinson Cancer Research Center, Seattle, Washington*

Bioinformatics III: Expression Technologies
Chairpersons: A. Robinson, *EMBL-EBI, Cambridge, United Kingdom;* D. Gerhold, *Merck & Co., Inc., West Point, Pennsylvania*

Keynote Speaker
F. Collins, *National Human Genome Research Institute*

Functional Genomics II: Applications
Chairpersons: J. Trent, *National Institutes of Health, Bethesda, Maryland;* S. Rastan, *SmithKline Beecham Pharmaceuticals, Harlow, United Kingdom*

B. Denother, L. Flagg, M. Hotic

E. Mardis, D. Brown

The Cell Cycle

May 17–21 353 participants

ARRANGED BY **Fred Cross,** The Rockefeller University
Stephen Elledge, Baylor College of Medicine
J. Wade Harper, Baylor College of Medicine
Jim Roberts, Fred Hutchinson Cancer Center

The sixth biennual Cell Cycle Meeting was held this year at Cold Spring Harbor Laboratory. This conference is internationally recognized for its ability to bring together scientists who study cell cycle regulation in eukaryotes ranging from yeast to humans. The meeting opened with an exciting plenary lecture by Chuck Sherr, who spoke on how cell cycle research was providing new insights into cancer biology as well as suggesting new approaches to cancer therapeutics. Diverse themes from previous years received continued attention: the interaction of cell cycle control with developmental and cancer biology; mechanisms of CDK activation by cyclins and by activating phosphorylation; and the mechanisms of action of cell cycle checkpoints.

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. Neither problem has yet been solved, but there has been considerable progress in understanding both the ORC and MCM complexes and how their activities might be regulated. There was also an extension of previous concepts derived from yeast studies to metazoans in the examination of the coordination between growth and cell cycle progression in mammals and in flies. 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. The influence of mouse genetics becomes stronger each year and will likely become a major component of this meeting in years to come.

S. Elledge, W. Harper, J. Roberts, F. Cross

The cell cycle conference brought together 353 scientists for the presentation of 255 abstracts (74 talks, and 181 posters). 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 and the National Institute on Aging, branches of the National Institutes of Health; and the National Science Foundation.

PROGRAM

CDK Regulation
Chairperson: D. Morgan, *University of California, San Francisco*

Plenary Speaker
C. Sherr, *St. Jude Children's Research Hospital*

Special Lecture
P. Rao, *University of Texas/M.D. Anderson Cancer Center*

Cell Cycle Entry/Exit
Chairperson: C. Lehner, *University of Bayreuth, Germany*

Replication I
Chairperson: T. Orr-Weaver, *Whitehead Institute/MIT, Cambridge, Massachusetts*

Replication II
Chairperson: J. Diffley, *Imperial Cancer Research Fund, South Mimms, United Kingdom*

Mitosis I
Chairperson: A. Amon, *Massachusetts Institute of Technology, Cambridge*

Mitosis II
Chairperson: D. Cleveland, *University of California, San Diego*

Controls
Chairperson: A. Hoyt, *Johns Hopkins University, Baltimore, Maryland*

Development
Chairperson: N. Dyson, *Massachusetts General Hospital Cancer Center, Charlestown*

N. Kato, J. Kato, Y. Arata, K. Tomoda

M. Sanchet, J. Samuel

Retroviruses

May 23–28 441 participants

ARRANGED BY **Jonathan Stoye,** National Institute for Medical Research, United Kingdom
John Young, University of Wisconsin-Madison Medical School

This was the 25th offering of the annual Cold Spring Harbor meeting, which began in 1975 as a meeting on RNA Tumor Viruses and evolved into its current focus on Retroviruses in 1993. The 25th anniversary was celebrated with special nightly keynote addresses by Harold Varmus on Retroviruses and Cancer, John Coffin on Retroviruses and AIDS, and Robin Weiss on Retroviruses in the Lab and in the Field. As always, among those presentations chosen from the openly submitted abstracts, many exciting new findings in diverse areas of retrovirology were presented not only in traditionally strong areas such as viral entry, reverse transcription, and integration, but also on a number of novel topics, including a growing emphasis on understanding retrovirus interactions with host cell factors. Papers illustrating this trend included a description of the application of sophisticated microscopic techniques to examine the association between incoming viruses and microtubules; the identification of localization signals on a number of viral proteins and their interactions with cellular factors; the role of host factors in viral RNA processing and export from the nucleus; and reports of the crucial role for ubiquitination in viral assembly.

PROGRAM

Transcription/Splicing/Translation
Chairpersons: J. Dudley, *University of Texas, Austin;* A. Rice, *Baylor College of Medicine, Houston, Texas*

RNA Export/Packaging
Chairpersons: K. Beemon, *Johns Hopkins University, Baltimore, Maryland;* B. Felber, *NCI-FCRDC, Frederick, Maryland*

Assembly/Budding/Maturation
Chairpersons: E. Hunter, *University of Alabama, Birmingham;* V. Vogt, *Cornell University, Ithaca, New York*

Env/Receptors/Entry
Chairpersons: D. Sanders, *Purdue University, West Lafayette, Indiana;* M. Roth, *Rutgers University, Piscataway, New Jersey*

L. Parent, K. Beemon, C. Hibbert, R. Garbitt

Keynote Address #1
H. Varmus: *Retroviruses and Cancer*

Reverse Transcription
Chairpersons: A. Telesnitsky, *University of Michigan, Ann Arbor;* V. Prasad, *Albert Einstein College of Medicine, Bronx, New York*

Keynote Address #2
J. Coffin: *Retroviruses and AIDS*

Accessory Genes
Chairpersons: E. Freed, *NIAID, National Institutes of Health, Bethesda, Maryland;* B.M. Peterlin, *University of California, San Francisco*

Keynote Address #3
R. Weiss: *Retroviruses in the Lab and the Field*

Vectors/Endogenous Retroviruses
Chairpersons: F.-L. Cosset, *Ecole Normale Superieure, Lyon, France;* S. Russell, *Mayo Foundation, Rochester, Minnesota*

Integration
Chairpersons: A.-M. Skalka, *Fox Chase Cancer Center, Philadelphia, Pennsylvania;* M. Muesing, *The Rockefeller University, New York, New York*

Pathogenesis/Evolution
Chairpersons: K. Radke, *University of California, Davis;* P. Jolicoeur, *Clinical Research Institute of Montreal, Canada*

Antivirals/Vaccines
Chairpersons: S. Hughes, *NCI-FCRDC, Frederick, Maryland;* S. Le Grice, *NCI-FCRDC, Frederick, Maryland*

A. Lever, J. Kaye, S. Griffin

M. Roth, W.-S. Hu

A. Viejo-Bordella, B. Blot, V. Blot

New York Structural Biology Group

August 2 238 participants

ARRANGED BY **Barry Honig,** Columbia University, New York
Leemor Joshua-Tor, Cold Spring Harbor Laboratory
John Kuriyan, The Rockefeller University

The summer meeting of the New York Structural Biology Discussion Group was the third 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 240 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.

Financial support was provided by Hampton Research Inc., Molecular Structure Corporation, Bruker AXS Inc., Protein Solutions Inc., Pfizer Inc., Hoffmann-La Roche Inc., and Merck Research Laboratories.

PROGRAM

Session I

Chairperson: B. Honig, *Columbia University Medical School*

J. Becker, *Merck Research Laboratories*
T. Gaasterland, *The Rockefeller University*
D. Engelman, *Yale University*
E. Gouaux, *Columbia University Medical School*
D. Patel, *Memorial Sloan-Kettering Institute*

Session II

Chairperson: L. Shapiro, *Mount Sinai School of Medicine*

A. Palmer, *Columbia University Medical School*
V. Rath, *Pfizer Inc.*
T. Lazaridis, *City College New York*
L. Joshua-Tor, *Cold Spring Harbor Laboratory*

Session II

Chairperson: A. Neuwald, *Cold Spring Harbor Laboratory*

D. Raleigh, *SUNY Stony Brook*
Y.-M. Chook, *The Rockefeller University*
J. Pandit, *Pfizer*

Session III

Chairperson: S. Roderick, *Albert Einstein College of Medicine*

S. Burley, *The Rockefeller University*
D. Cowburn, *The Rockefeller University*
P. Fitzgerald, *Merck*
B. Honig, *Columbia University*

L. Joshua-Tor, L. Shapiro

B. Honig, A. Palmer, A.M. Pyle

S. Burley, L. Shapiro, C. Ojek

Cancer Genetics and Tumor Suppressor Genes

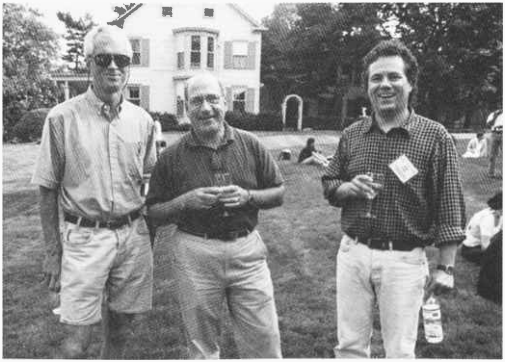
August 16–20 327 participants

ARRANGED BY **Terri Grodzicker,** Cold Spring Harbor Laboratory
Doug Hanahan, University of California, San Francisco
David Livingston, Dana Farber Cancer Institute
Scott Lowe, Cold Spring Harbor Laboratory
Bruce Ponder, University of Cambridge
Carol Prives, Columbia University

The third Cancer Genetics and Tumor Suppressor Genes meeting allowed scientists in different disciplines to discuss their latest results on different aspects of cancer cell biology and to have extensive crosstalk concerning ideas and methodologies. The conference was attended by more than 325 scientists meeting to present both talks and posters. Launched by a keynote address by Arnold Levine, President of The Rockefeller University, the meeting covered an extensive range of work: from location and nature of mutations in different tumors to properties of the p53 tumor suppressor gene. Many talks dealt with growth control of cancer cells, and areas that received much attention included control of the cell cycle, apoptosis, signaling transduction pathways, DNA repair, and transcriptional regulation of and by tumor suppressor genes. Animal models have provided much information about tumorigenesis, and more than one session dealt with knock-out and transgenic mouse models. An emphasis of many talks this year dealt with signaling to and regulation of tumor suppressor gene activity. This meeting continued to be enthusiastically supported and all sessions found the lecture and poster halls packed with extensive discussions and exchanges of information. The meeting again featured a special session called "NCI Listens," chaired by Tyler Jacks. NCI staff and scientists who served on institute panels answered questions from the audience regarding NCI grants, policy, and future directions.

T. de Lange

This meeting was funded in part by the National Cancer Institute, a branch of the National Institutes of Health.



D. Hanahan, D. Livingston, S. Lowe

PROGRAM

Mouse Models I
Chairpersons: L. Strong, *University of Texas/M.D. Anderson Cancer Center, Houston;* D. Hanahan, *University of California, San Francisco*

Keynote Address
A. Levine, *The Rockefeller University, New York, New York*

Proliferation and DNA Damage Responses
Chairpersons: R. Fishel, *Thomas Jefferson University, Philadelphia, Pennsylvania;* T. de Lange, *The Rockefeller University, New York, New York*

Mortality and Apoptosis
Chairpersons: G. Evans, *University of California, San Francisco;* T. Jacks, *Massachusetts Institute of Technology, Cambridge*

Regulation of Tumor Suppressor Gene Activity
Chairpersons: C. Sherr, *St. Jude Children's Research Hospital, Memphis, Tennessee;* M. Oren, *Weizmann Institute, Rehovot, Israel*

"NCI Listens"
Chairpersons: T.E. Jacks, *Massachusetts Institute of Technology;* D.S. Singer, *NCI, National Institutes of Health, Bethesda, Maryland*

Cancer Genetics/Genomics
Chairpersons: D. Housman, *Massachusetts Institute of Technology, Cambridge;* B. Ponder, *University of Cambridge, United Kingdom*

Signal Transduction/Animal Models II
Chairpersons: G. Vande Woude, *Van Andel Research Institute, Grand Rapids, Michigan;* A. Berns, *The Netherlands Cancer Institute, Amsterdam*

Gene Regulation
Chairpersons: E. Harlow, *Massachusetts General Hospital, Charlestown;* J. Brugge, *Harvard Medical School, Boston, Massachusetts*

C. Prives' lab at the wine & cheese party

M. Green, M. Green, L. Gann

R. Nahta, A. Reddy

Molecular Genetics of Bacteria and Phages

August 22–27 219 participants

ARRANGED BY **Tania Baker**, Massachusetts Institute of Technology
Alan D. Grossman, Massachusetts Institute of Technology
Thomas Silhavy, Princeton University

The Molecular Genetics of Bacteria and Phages meeting continued to include exciting and high-quality research in prokaryotic molecular biology. Initiated as a forum for bacteriophage λ and T4 genetics, it expanded to include other phages and then the molecular genetics of their bacterial host, *Escherichia coli*. Now, many other bacteria and even archaebacterial phages are discussed, providing insights into previously unanswerable questions. Surprising results were reported on the localization and rapid movement of proteins involved in regulating cell division. Most remarkable is the movement of two proteins from one cell pole to the other, on the time scale of approximately 20 seconds. There continued to be strong representation from the bacterial transcription field, with several interesting sessions on aspects of the structure and function of the transcription machinery and accessory proteins. Several talks explored the interactions between pathogens and hosts and described mechanisms controlling gene expression in pathogens. There were several presentations on genome sequence analysis and using whole-genome sequences to explore evolutionary and functional relationships and to study gene expression.

This meeting was funded in part by the National Science Foundation.

K. Schnetz, J. Sayers, G. Koudelka

T. Baker, A. Grossman, T. Silhavy

PROGRAM

Cell Surfaces and Secretion
Chairperson: H. Shuman, *Columbia University, New York, New York*

RNA Polymerase Structure, Function
Chairperson: R. Ebright, *Howard Hughes Medical Institute, Waksman Institute, Piscataway, New Jersey*

Cell Biology and Chromosome Partitioning
Chairperson: P. de Boer, *Case Western Reserve University School of Medicine, Cleveland, Ohio*

Transcription Activators and Repressors
Chairperson: J. Roberts, *Cornell University, Ithaca, New York*

Replication, Recombination, Transposition
Chairperson: R. Weisberg, *NICHD, National Institutes of Health, Bethesda, Maryland*

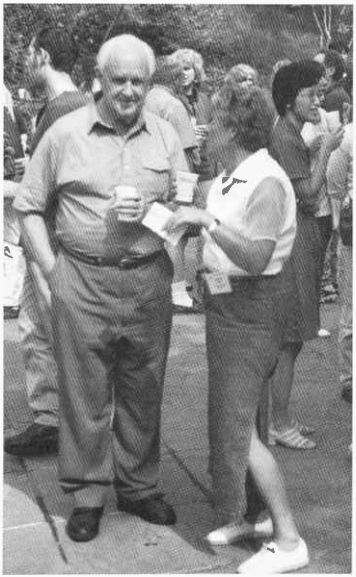
Growth and Environmental Stress
Chairperson: G. Storz, *NICHD, National Institutes of Health, Bethesda, Maryland*

Pathogens and Symbionts
Chairperson: V. Miller, *Washington University School of Medicine, St. Louis, Missouri*

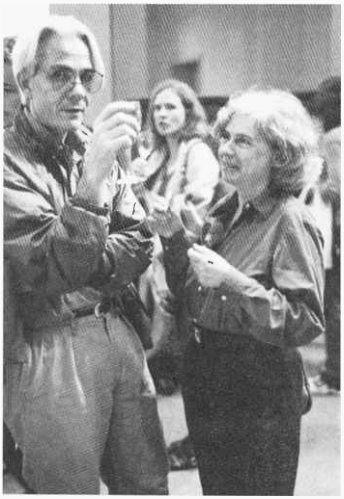
Transcription Elongation and Termination
Chairperson: R. Landick, *University of Wisconsin, Madison*

Quorum Sensing and Signaling
Chairperson: B. Bassler, *Princeton University, New Jersey*

Posttranscriptional Regulation
Chairperson: C. Squires, *Tufts University School of Medicine, Boston, Massachusetts*



W. Szybalski, J. Wild



J. Roberts, S. Gottesman

Mouse Molecular Genetics

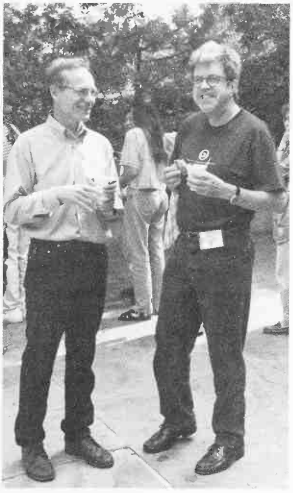
August 30–September 3 367 participants

ARRANGED BY **Richard Behringer,** The University of Texas/M.D. Anderson Cancer Center
Carmen Birchmeier, Max Delbruck Center, Germany
Maja Bucan, University of Pennsylvania
Richard Harvey, The Victor Chang Cardiac Research Institute, Australia

Held every other year at Cold Spring Harbor Laboratory, this meeting attracted more than 350 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 sleep patterns of mice.

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. In an attempt to highlight some of the contemporary approaches, two workshops on Comparative Sequence Analysis (Eric Green, discussion leader) and Conditional Genetics (Richard Behringer and Andras Nagy, discussion leaders) were arranged where several groups who are doing mutagenesis in the mouse came and presented their approaches. Although concurrent with the poster sessions, these workshops were very well attended.

The mouse meeting has evolved over the years to have a stronger genetic and genomics emphasis, and this year invited presentations included detailed presentations on the state of the mouse EST database, the importance of genetic background, etc. The meeting exemplified some of the special



E. Rubin, W. Birchmeier

S. Sharan, R. Behringer

K. Frazer, M. Bucan

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.

This meeting was funded in part by the National Institute of Child Health and Human Development; the National Human Genome Research Institute; the National Cancer Institute; the National Institute of Mental Health; the National Institute on Deafness and Other Communication Disorders; the National Institute of Dental and Craniofacial Research; and the National Institute of Arthritis and Musculoskeletal and Skin Diseases, branches of the National Institutes of Health.

PROGRAM

Genetics
Chairperson: M. Justice, *Baylor College of Medicine, Houston, Texas*

Genomics
Chairperson: E. Rubin, *Lawrence Berkeley National Laboratory, California*

Workshop on Comparative Sequence Analysis
Chairperson: E. Green, *NHGRI, National Institutes of Health, Bethesda, Maryland*

Stem Cells and Regeneration
Chairperson: J. Rossant, *Samuel Lunenfeld Research Institute, Toronto, Canada*

Signals and Receptors
Chairperson: P. Soriano, *Fred Hutchinson Cancer Research Center, Seattle, Washington*

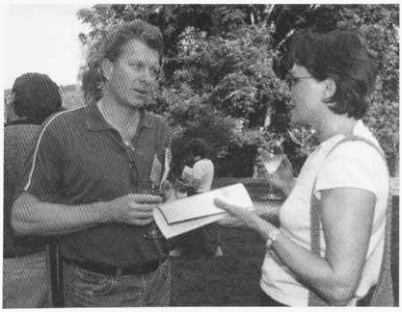
Workshop on Conditional Genetics
Chairpersons: R. Behringer, *University of Texas/M.D. Anderson Cancer Center, Houston;* A. Nagy, *Samuel Lunenfeld Research Institute, Toronto, Canada*

Models of Human Disease
Chairperson: M. Meisler, *University of Michigan, Ann Arbor*

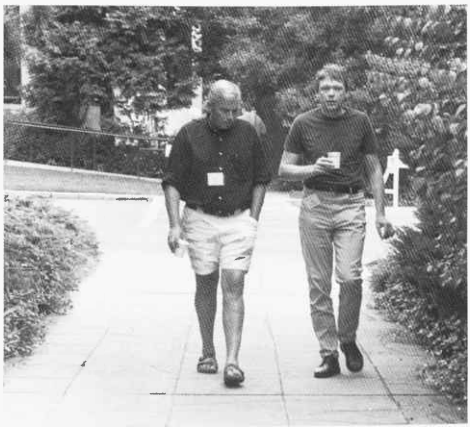
Organogenesis
Chairperson: R. Harvey, *Victor Chang Cardiac Research Institute, Darlinghurst, Australia*

Patterning
Chairperson: E. Lacy, *Memorial Sloan-Kettering Cancer Center, New York, New York*

Neural Development
Chairperson: S. Pfaff, *The Salk Institute, La Jolla, California*



R. Harvey, V. Papaioannou



S. O'Gorman, R. Kühn

Translational Control

September 6–10 469 participants

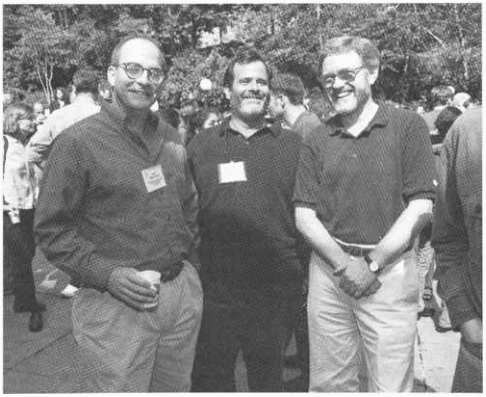
ARRANGED BY **Alan Hinnebusch,** NICHD, National Institutes of Health
Michael Mathews, New Jersey Medical School, University of Medicine and Dentistry
Stuart Peltz, Robert Wood Johnson Medical School, University of Medicine and Dentistry New Jersey

The 2000 Translational Control meeting attracted more than 450 participants from around the world and included eight platform and three poster sessions that covered a record 403 abstracts.

The session on Translation Mechanisms began with the plenary lecture by Harry Noller on the striking X-ray crystal structure of the 70S ribosome in complexes with mRNA and tRNA fragments bound to the A, P, and E sites. Other highlights included cryo-EM analysis of EF-G-dependent tRNA translocation on the ribosome (Frank lab), the co-crystal structure of the elongation factor eEF1A:eEF1B complex (Kinzy lab), and the structure of ribosome release factor RRF (Kaji lab). Provocative evidence for translation in the nucleus was reported by Peter Cook.

In the second session, cell-cycle-regulated IRESs in the mRNAs for ODC, c-Myc (Sonenberg lab), and p58 PITSLRE kinase, and another IRES activated by amino acid starvation (Hatzoglou), were described. Jacquemin-Sablon reported that Unr protein is required in vivo for certain IRESs, and ITAF45 (aka murine proliferation-associated protein or Mpp1) was identified as a *trans*-acting factor required for a particular picornavirus IRES (Hellen lab). Intriguing results suggested that cellular IRESs are composed of short independently functioning modules that base pair with 18S rRNA (Edelman).

In the mRNA Turnover session, The Wilusz' and Peltz' labs described a decapping activity in HeLa extracts stimulated by AU-rich elements (AREs) and the analysis of decapping and ARE-mediated decay in yeast cell-free systems. Joan Steitz' lab reported that human homologs of yeast UPF2 or UPF3 are sufficient to promote nonsense-mediated mRNA decay (NMD) when tethered 3′ to a stop codon, and biochemical evidence that NMD requires splicing-dependent remodeling of mRNP was provided by Maquat and Moore. The Schoenberg lab reported that vigilin binding can selectively block cleavage of vitellogenin, but not albumin, mRNA by PMR-1 endonuclease, and Schneider's group found that ubiquitination of p37-AUF1 and proteosome activity are determinants of ARE-mRNA turnover.



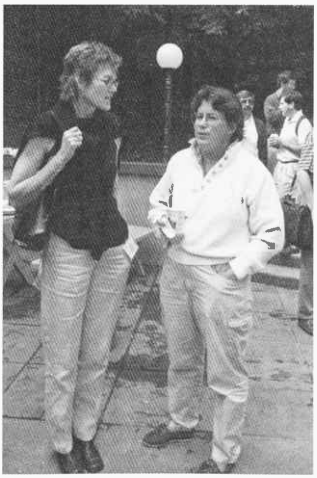
A. Hinnebusch, S. Peltz, M. Mathews

The Initiation session highlighted the role of Hsp27 in blocking translation during heat shock by dissociating eIF4F (Schneider) and discovery of a novel PABP-interacting protein (Paip2) that inhibits translation (Sonenberg). The eukaryotic IF2 homolog, eIF5B, was implicated in subunit joining (Hellen and Pestova and Dever labs) and was shown to interact specifically with eIF1A (Hinnebusch and Dever labs). Mutational analysis of eIF5 by the Maitra and Proud labs suggests that it may function as a classical GTPase-activating protein (GAP) for eIF2, and the catalytic center in eIF2B (the GEF for eIF2) was localized to the carboxyl terminus of its ε-subunit (Pavitt). Finally, Pestova reported that 43S initiation complexes can scan to a start codon in unstructured mRNA without ATP or the eIF4 group of factors.

In the session on Regulatory Elements, Gallie reported that HSP101 is a light-responsive regulator of ferredoxin mRNA translation and stability. Mayfield showed that a chloroplast PAB stimulates translation and likely directs localization of mRNAs to the proper site of photosystem II assembly in chloroplasts. Expanded CUG repeats in the 3′ UTR of the myotonin protein kinase gene were found to activate the eIF2α kinase PKR and inhibit translation (Mathews). Genetic evidence was presented that S6 kinase mediates the amino acid control of TOP mRNA translation (Meyuhas), and Calkoven reported that PKR and mTOR control the ratio of C/EBP isoform expression through an uORF. Finally, new strategies were described to identify novel proteins that can regulate translation or stability of mRNAs when tethered to the 3′ UTR (Fields and Wickens), or to identify all of the in vivo mRNA targets of a given RNA-binding protein (Keene).

The session on Phospho-Modulators from the Wek, Hinnebusch, Kaufman, and Ron labs showed that the eIF2α kinases GCN2 and PEK/PERK are activated separately by amino acid limitation and ER-stress, that uncharged tRNA binding activates GCN2 by conformational rearrangement, that the ER-lumenal domain of PERK mediates activation by unfolded proteins, and that eIF2α phosphorylation stimulates translation of uORF-containing mRNAs in mammalian cells, as in yeast. The heme-regulated eIF2α kinase was shown to be important for controlling red blood cell size in mice (Chen). The eIF2α kinase PKR was found to have a novel function in splicing (Kaempfer), and the requirement for the dsRNA-binding domain for activation of PKR was satisfied with a heterologous dimerization domain (Dever).

A. Page, A. Murariu



L. Maquat, M. Moore

The session on Development Controls included a report by Richter et al. that phosphorylation of CPEB by kinase Egf2 is crucial for cytoplasmic polyadenylation in *Xenopus* oocytes, and Wormington showed that ARE elements enhance mRNA deadenylation by poly(A) ribonuclease. Hentze et al. found that an hnRNP K+E1 repressor inhibits 15-LOX mRNA translation from the 3′UTR by blocking ribosomal subunit joining at the start codon. It was reported that Nanos and Pumilio mediate poly(A) tail removal from hunchback mRNA via 3′UTR elements in addition to regulating translation (Lehmann). Pumilio seems to regulate bicoid mRNA translation by interacting with something besides Nanos (Gottlieb), and data from Gavis indicate that translational repression of Nanos mRNA by a 3′ element occurs at the elongation phase of translation. Wickens reported that Pumilio family members in *C. elegans* are involved in multiple aspects of germ-line differentiation through varied interactions with Nanos and CPEB homologs.

In the session on Viral Strategies, Poncet et al. reported that rotaviral NSP3 protein stimulates translation from the 3′ end via interactions with eIF4G and that the activator of reinitiation from CaMV may function by interacting with eIF3 and a ribosomal protein (Hohn lab). It was shown that adenovirus 100K protein stimulates shunting on late mRNAs dependent on its tyrosine phosphorylation (Schneider). The Wickner lab presented evidence that poly(A) is required in yeast primarily to counteract regulatory proteins that inhibit translation of nonpolyadenylated viral mRNAs. Striking results from Sarnow et al. indicated that cricket paralysis virus IRES directs translation initiation without an AUG codon, initiator tRNA, or initiation factors. Antagonism of the antiviral function of PKR in phosphorylating eIF2α was analyzed for two proteins encoded by herpesvirus (Mohr), the E2 (Taylor et al.), and NS5A (Katze) proteins of hepatitis C virus, and the E3L and K3L proteins of vaccinia virus (Jacobs).

PROGRAM

Translation Mechanisms
Chairperson: T.G. Kinzy, *UMDNJ-Robert Wood Johnson Medical School, Piscataway*

Plenary Lecture
Chairperson: H.F. Noller, *University of California, Santa Cruz*

IRESs
Chairperson: C.U.T. Hellen, *SUNY Health Science Center, Brooklyn*

mRNA Turnover
Chairperson: J. Wilusz, *UMDNJ-New Jersey Medical School, Newark*

Initiation
Chairperson: P. Linder, *University of Geneva, Switzerland*

Regulatory Elements
Chairperson: M. Sachs, *Oregon Graduate Institute, Portland*

Phospho-Modulators
Chairperson: R. Wek, *Indiana University School of Medicine, Indianapolis*

Developmental Controls
Chairperson: R. Lehmann, *Skirball Institute, New York University School of Medicine, New York*

Viral Strategies
Chairperson: T. Hohn, *Friedrich Miescher Institut, Basel, Switzerland*

M. Ptushkina, P. Linder

Dynamic Organization of Nuclear Function

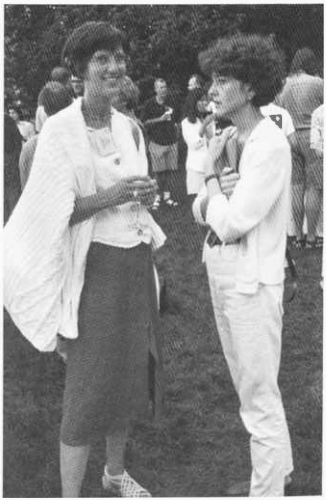
September 13–17 284 participants

ARRANGED BY **Thomas Cremer,** Ludwig-Maximilians University, Germany
Robert D. Goldman, Northwestern University
Pamela Silver, Dana Farber Cancer Institute
David L. Spector, Cold Spring Harbor Laboratory

The Dynamic Organization of Nuclear Function meeting was a successful gathering of researchers from a variety of different fields to discuss the relationship between nuclear structure and function. In the opening session devoted to diseases, H.T. Orr discussed spinocerebellar ataxia type 1. This autosomal dominant neurodegenerative disease results in the localization of a mutant protein ataxin-1 to the nucleus; transgenic mice expressing the mutant protein lacking a nuclear localization signal no longer develop nuclear inclusions or ataxia, suggesting that the localization of this protein is important to disease. X-linked Emery-Dreyfuss muscular dystrophy is also associated with a protein mislocalization. In this case, of emerin, a lamin-binding protein normally found associated with the nuclear membrane that relocalizes to the endoplasmic reticulum (K.L. Wilson). Other talks on myotonic dystrophy (T.A. Cooper), spinal muscular atrophy (L. Pellizzoni), various leukemias (E. Columbo), and other syndromes (Treacher Collins, U.T. Meier), Werner and Bloom (N. Neff, H. Yan) all highlighted the close association of nuclear structure to disease.

Numerous talks demonstrated the use of fluorescence recovery after photobleaching (FRAP) to examine the dynamics of GFP-tagged nuclear proteins. G. Hager used glucocorticoid receptor (GR) tagged with GFP and a cell line carrying a tandem array of GR-binding elements to monitor stable binding in living cells. FRAP showed that the hormone-occupied receptor exchanged rapidly between chromatin and the nucleoplasm, demonstrating that the interaction of regulatory proteins with target sites in chromatin is much more dynamic than previously believed. T. Misteli measured the residence time of histone H1-GFP on chromatin; trichostatin A, an inhibitor of histone deacetylase and an inducer of chromatin remodeling, decreased the residence time. D. Hoogstraten examined the relationship between transcription and the nucleotide

R. Goldman, P. Silver, D. Spector, T. Cremer



C. Vourc'h, E. Heard

excision repair of lesions in DNA induced by UV light. UV-irradiation induced a transient and dose-dependent immobilization of several components involved in XPA which presumably reflected the binding to a lesion. S. Huang examined the dynamics of several GFP-tagged nucleolar components (including UBF, TBP, nucleolin, and fibrillarin) and found that the high mobility of UBF was not reduced when transcription ceased during mitosis.

A variety of other approaches were also used to monitor nuclear dynamics. For example, A. Belmont fused the GFP-*lac* repressor to the wild-type estrogen receptor and expressed this construct in cells bearing a tandem array of *lac* operator elements embedded in heterochromatin; the array now marked by GFP unfolded, and estrogen addition partially recondensed it within 30 minutes. T. Pederson tracked ribosome movements. Oligonucleotides complementary to rRNA and tagged with a caged fluorescein were microinjected into cells where they concentrated in nucleoli. Upon uncaging, the hybridized oligonucleotide could be followed as it moved to the cytoplasm with kinetics that were consistent with diffusion.

Several studies advanced our understanding of structural proteins in the nucleus. T.P. Spann reported that lamins may have an important structural role during transcription. Microinjecting lamin A bearing an amino-terminal deletion reduced the incorporation of Br-UTP into nucleoplasmic RNA but had no effect on RNA polymerase I activity. Actin was identified on the Balbiani ring pre-mRNP particles, found in Chironomus, where it interacts with an hnRNP protein (P. Percipalle). A possible partner of actin, a form of myosin 1 with an amino-terminal extension of 16 residues, was also identified; significantly, myosin with this extension localized close to RNA polymerase II in nuclei, and antibodies directed against it blocked transcription in vitro (P. de Lanerolle).

PROGRAM

Nuclear Structure and Disease
Chairperson: H. Orr, University of Minnesota, Minneapolis

Dynamic Properties of the Nuclear Envelope/Lamina
Chairperson: K. Wilson, *Johns Hopkins University School of Medicine, Baltimore, Maryland*

Nuclear Transport
Chairperson: M. Rout, *The Rockefeller University, New York, New York*

Genome Organization
Chairperson: A. Belmont, *University of Illinois, Urbana*

RNA Processing
Chairperson: T. Pederson, *University of Massachusetts Medical School, Worcester*

Nucleoli and Other Inclusions
Chairperson: J. Gall, *Carnegie Institution of Washington, Baltimore, Maryland*

Nuclear Assembly/Disassembly
Chairperson: D. Cleveland, *University of California, San Diego*

S. Polo, E. Fabre, B. Arcangioli

Axon Guidance and Neural Plasticity

September 20–24 389 participants

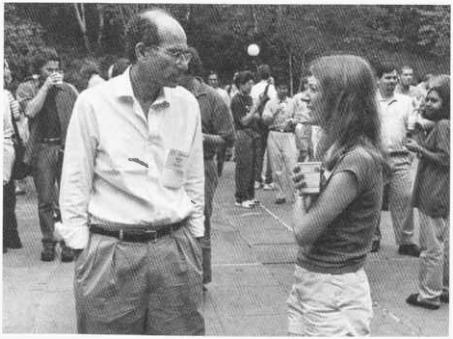
ARRANGED BY **Tobias Bonhoeffer,** Max-Planck Institute of Neurobiology, Germany
Marc Tessier-Lavigne, HHMI/University of California, San Francisco
Larry Zipursky, HHMI/University of California, San Francisco

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 bienni-

M. Tessier-Lavigne, L. Zipursky, T. Bonhoeffer



R. Axel, C. Bargmann

al CSH conference series on "Axon Guidance and Developmental Plasticity of the Nervous System" was initiated in 1998. This year, the second of these meetings was held in September 2000, and 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 meeting, the response of the field to this conference was one of overwhelming enthusiasm. There were 365 registrants, 231 of whom submitted abstracts. 64 abstracts were selected for talks, in eight sessions. Senior researchers, starting assistant professors, postdoctoral fellows, and graduate students were are well represented. All 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 a keynote addresses. The first was by Dr. Tim Mitchison of Harvard Medical School, and focused on basic mechanisms controlling the cytoskeleton. The second was by Dr. Patricia Kuhl of the University of Washington and focused on mechanisms involved in language acquisition. The meeting provided an important clearing house for ideas and approaches and helped scientists in the field get the most up-to-date information, as well as enabling them to meet, to network, and to establish collaborations. 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; and the National Science Foundation.

PROGRAM

Plasticity I
Chairpersons: C. Shatz, *Harvard Medical School, Boston, Massachusetts;* H. Cline, *Cold Spring Harbor Laboratory*

Guidance I: Ephrins and Netrins
Chairpersons: C. Goodman, *University of California, Berkeley;* C. Holt, *University of Cambridge, United Kingdom*

Special Lecture I
T. Mitchison, *Harvard Medical School, Boston, Massachusetts*

Cell Migration
Chairpersons: M.B. Hatten, *The Rockefeller University, New York, New York;* T. Curran, *St. Jude Children's Research Hospital, Memphis, Tennessee*

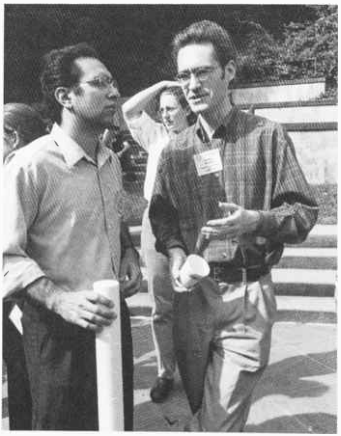
Guidance II: Semaphorins and Slits
Chairpersons: M.-M. Poo, *University of California, San Diego;* J. Flanagan, *Harvard Medical School, Boston, Massachusetts*
Targeting and Synaptogenesis
Chairpersons: J. Sanes, *Washington University School of Medicine, St. Louis, Missouri;* M. Takeichi, *Kyoto University, Japan*

Guidance III
Chairpersons: R. Axel, *Columbia University, New York, New York;* F. Bonhoeffer, *Max-Planck Institut fur Entwicklungsbiologie, Tübingen, Germany*

Plasticity II
Chairpersons: M. Bear, *Brown University, Providence, Rhode Island;* A. Doupe, *University of California, San Francisco*

Special Lecture II
P. Kuhl, *University of Washington, Seattle*

Guidance IV
Chairpersons: J. Raper, *University of Pennsylvania School of Medicine, Philadelphia;* C. Bargmann, *University of California, San Francisco*



D. Chklovskii, G. Goodhill

Gene Therapy

September 25–29 236 participants

ARRANGED BY **Jeffrey Leiden,** Abbott Laboratories
Margaret Liu, Lafayette, California
Gary Nabel, National Institutes of Health
James Wilson, University of Pennsylvania

The Gene Therapy meeting was the fifth in the series of biyearly meetings. There has been an explosion of interest and progress in the field and a growing conviction that truly beneficial clinical applications of gene transfer technologies will soon appear. Of the many meetings on gene therapy, the Cold Spring Harbor meeting remains the only one with an abstract-driven format and therefore continues to be the most accessible to the broad gene therapy community, including students, fellows, and junior and senior investigators. Several sessions focused on developments in vector design and regulation of gene expression, and one session examined emerging technologies including stem cells and tissue engineering. A number of sessions addressed the immune system and disease models including hemophilia, hematopoietic and muscle disorders, cancer, AIDS and infectious diseases, and neurological, metabolic, and cardiovascular disorders. Although results and progress in a number of ongoing clinical trials were presented, the program of the meeting emphasized the molecular genetics and cell biology issues underlying approaches to human gene therapy. A special panel discussion was convened to discuss ethical issues in the design of clinical trials and the practice of data safety monitoring.

The meeting was supported in part by a generous grant from the National Institute of Child Health and Human Development, a branch of the National Institutes of Health.

J. Wilson, M. Liu

E. Feudner, M. Slack

PROGRAM

Disease Models I: Hematopoietic, Hemophilia, Muscle Disorders
Chairperson: K. High, *University of Pennsylvania School of Medicine, Philadelphia*

Gene Therapy for AIDS and Vaccination Approaches to Infectious Diseases
Chairpersons: M. Liu, *Lafayette, California;* G. Nabel, *National Institutes of Health, Bethesda, Maryland*

Immunity and Gene Transfer
Chairperson: J. Leiden, *Abbott Laboratories, Abbott Park, Illinois*

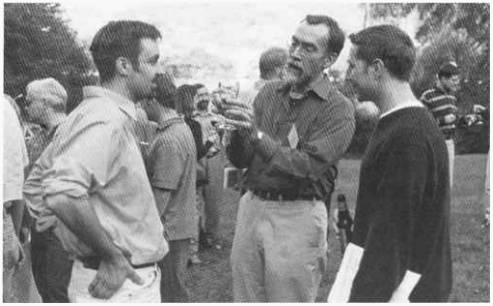
Regulation of Gene Expresssion
Chairperson: H. Bujard, *ZMBH, University Heidelberg, Germany*

Panel Discussion: Clinical Trial Design, Data Safety Monitoring, and Bioethics

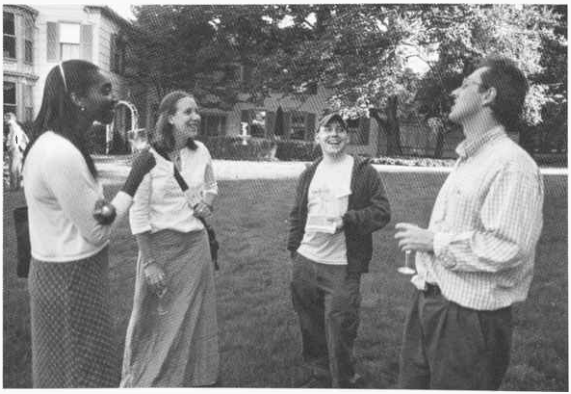
Genetic Approaches and Immunotherapy for Cancer and Angiogenesis
Chairperson: J. Folkman, *Children's Hospital/Harvard Medical School, Boston, Massachusetts*

Viral Vector Development and Regulation of Gene Expression
Chairperson: I. Verma, *Salk Institute, San Diego, California*
New Technology, Stem Cells, and Tissue Engineering
Chairperson: J. Wilson, *University of Pennsylvania, Philadelphia*

Disease Models II: Neurological, Metabolic, and Cardiovascular
Chairperson: T. Friedmann, *University of California, San Diego*



W. Wybranietz, D. Henderson, J. Bramson



"Gene Therapy—Surely you're joking"

Germ Cells

October 5–8 204 participants

ARRANGED BY **Geraldine Seydoux,** Johns Hopkins University School of Medicine, Baltimore, Maryland
Chris Wylie, Children's Hospital Research Foundation, Cincinnati, Ohio

An essential requirement for sexual reproduction is the germ line—the immortal cell lineage that generates the male and female gametes. During 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. The second Cold Spring Harbor Germ Cells meeting was organized with the goal of bringing together researchers who study germ line development in both vertebrate and invertebrate systems, as well as plants, so that recent exciting advances in one field could cross-fertilize the other. During the course of the meeting, researchers showed how *nanos*-related genes regulate early germ cell development in organisms as diverse as *Drosophila, Caenorhabditis elegans, Xenopus*, zebrafish, and planarians. The *piwi* gene family is another family with evolutionarily conserved roles: A mouse homolog was shown at the meeting to be essential for spermatogenesis. A final highlight of the meeting was the unveiling of the sequence of the human Y chromosome, whose repetitive structure appears to be at the root of many male infertility syndromes. The next Germ Cells meeting will be held in the fall of 2002, with David Page and Ruth Lehmann serving as the organizers.

C. Wylie, G. Seydoux

This meeting was funded in part by the National Institute of Child Health and Human Development, a branch of the National Institutes of Health; the National Science Foundation; Lalor; and the March of Dimes.

PROGRAM

The Early Germ Line
Chairpersons: S. Strome, *Indiana University, Bloomington;* B. Hogan, *Vanderbilt University Medical Center, Nashville, Tennessee*

Regulation of Germ Cell Gene Expression
Chairpersons: T. Schedl, *Washington University School of Medicine, St. Louis, Missouri;* S. Tilghman, Princeton University, New Jersey

Oogenesis and Egg Polarity
Chairpersons: D. St. Johnson, *Wellcome/CRC Institute, Cambridge, United Kingdom;* C. Wylie, *University of Minnesota Medical School, Minneapolis*

Sperm Differentiation
Chairpersons: S. Wassarman, *University of California, San Diego;* R. Braun, *University of Washington School of Medicine, Seattle*

Fertilization and Gamete Function
Chairpersons: B. Wakimoto, *University of Washington, Seattle;* P. Wassarman, *Mt. Sinai School of Medicine, New York, New York*

Meiosis
Chairpersons: T. Orr-Weaver, *Massachusetts Institute of Technology, Cambridge;* S. Hawley, *University of California, Davis*

P. Wassarman, A. Mahowald

U. Grossniklaus, D. Stewart

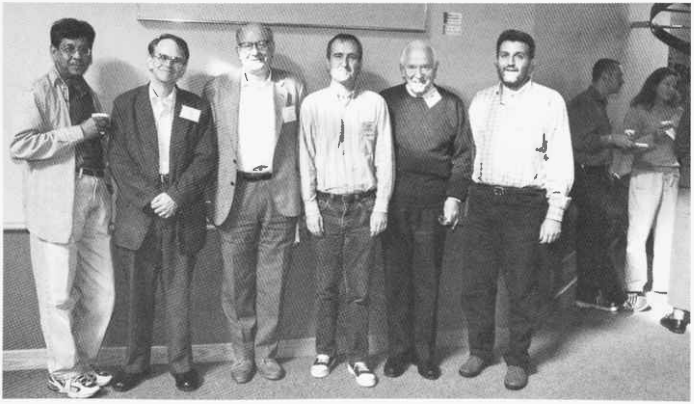
Cold Spring Harbor Millennium Meeting on Human Origins and Disease

October 25–29 167 participants

ARRANGED BY **Michael Brown,** Emory University School of Medicine
Aravinda Chakravarti, Case Western Reserve University
Luigi Cavalli-Sforza, Stanford University
Colin Renfrew, University of Cambridge
Christopher Stringer, Natural History Museum, London
Antonio Torroni, University of Rome "La Sapienza"
Douglas Wallace, Emory University School of Medicine

The third in a series of Cold Spring Harbor meetings focused on the evolution of modern human beings and brought together fossil, linguistic, and substantial amounts of molecular genetic information in an attempt to achieve a new synthesis in our understanding of human origins. New paleontological evidence was presented that supports a recent "out-of Africa" origin for modern *Homo sapiens*, implying that modern humans arose once in Africa between 100,000 and 150,000 years before present (YBP), migrated out-of-Africa in perhaps two migrations, and replaced contemporaneous *Homo erectus* and Neanderthal individuals, further supported by the recent comparison of Neanderthal and modern mitochondrial (mt) DNA sequences which revealed a striking molecular discontinuity between archaic and modern humans.

Molecular anthropological studies of maternally inherited mtDNA and paternally inherited Y chromosomes were presented, indicating that Africa harbors both the greatest genetic diversity and the oldest lineages, with the two complementary approaches yielding a remarkable consensus that modern humans arose in Africa about 143–144,000 YBP. Molecular analyses of autosomal and X chromosome loci variation have proved more cumbersome, but work presented at the meeting clearly indicates the African populations as being the oldest and the Native Americans, the youngest. However, in contrast to mtDNA and Y chromosome studies, most of the genetic variation of the autosomes and X chromosome is shared across all continents, and the coalescence time for the variation is much older. This discrepancy probably reflects the higher effective population size and recombination of the autosomes as opposed to the mtDNA and the Y chromosome. Even so, there is considerable information to be obtained from analysis of chor-



A. Chakravarti, D. Wallace, C. Renfrew, C. Stringer, L. Cavalli-Sforza, A. Torroni

mosomal loci, particularly using linkage disequilibrium coupled with high-throughput chromosomal sequencing and single nucleotide polymorphism (SNP) analysis. Another promising source of historical information within the autosomes are transposable elements: SINES and LINES. Analysis of recent *Alu* SINE repeats is beginning to shed additional light on the radiation of higher primates and the recent dispersal of modern humans.

Besides being used to address global questions of human origins, molecular anthropology has permitted novel approaches to the study of local population issues. These types of studies confirm that considerable historical and migratory information can be deduced from our genes. What are needed now are new sources of data and new analytical tools. High-throughput genomic sequencing, rapid genotyping of polymorphic loci, and more reliable mathematical methods for analysis will be required to accurately extract the information. Not only will such studies tell us much about human history, they will provide vital data on continent and population-specific variation that will be important for localizing and cloning common disease genes.

PROGRAM

Overview of Human Origins
D.C. Wallace, *Emory University School of Medicine, Atlanta, Georgia*

Global
Chairperson: L. Cavalli-Sforza, *Stanford University, Stanford*

Global I
Chairperson: P. Underhill, *Stanford University, Stanford*

Global II
Chairperson: C. Stringer, *Natural History Museum, London*

Africa I
Chairperson: K. Kidd, *Yale University, Connecticut*

Africa II
Chairperson: C. Renfrew, *University of Cambridge, United Kingdom*

Asia I
Chairperson: M. Hammer, *University of Arizona, Tucson*

Asia II
Chairperson: J. Friedman, *The Rockefeller University, New York*

Middle East–India
Chairperson: L. Jorde, *University of Utah, Salt Lake City*

Europe I
Chairperson: M. Brown, *Emory University School of Medicine, Atlanta, Georgia*

Europe II
Chairperson: A. Torroni, *University of Rome "La Sapienza," Italy*

Americas
Chairperson: J. Renfrew, *University of Cambridge, United Kingdom*

Gene and Environment–Common Disease
Chairperson: J. Felsenstein, *University of Washington, Seattle*

Analysis of Chromosomal Variation I
Chairperson: A. Clark, *Pennsylvania State University, Philadelphia*

Analysis of Chromosomal Variation II
Chairperson: A. Chakravarti, *Case Western Reserve University, Cleveland, Ohio*

General Lecture I
Chairperson: T. Jenkins, *S. Africa Institute of Medical Research, Johannesburg*

Lines and Sines
Chairperson: M.-C. King, *University of Washington, Seattle*

Evolutionary Genetics of Common Disease
Chairperson: A. Motulsky, *University of Washington, Seattle*

J.D. Watson, P. Tobias

J. Witkowski, N. Wade, M.-C. King

S. Patel, S. McBrearty

WINTER BIOTECHNOLOGY CONFERENCE

Therapeutic Opportunities in Neurodegenerative Diseases

November 30–December 3 126 participants

ARRANGED BY **Sam Gandy,** New York 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 a 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 heretability or identifiable genetic mutations, it has been possible during 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, fruitfly, 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 beta 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 Meetings instituted in December 2000 with the explicit goal of facilitating the translation of "breakthrough" science into effective medicines.

S. Gandy, M. MacDonald, H. Levine III

J. Saffell, C. Masters

For the 2000 meeting, seven 3- or 4-hour platform sessions were organized around common technological themes. Chairpersons, invited speakers, and speakers selected from submitted abstracts were equally drawn from the academic and pharma sectors. Although the discussion of new, unpublished data was emphasized, the group was tolerant of a wide range of intellectual property conventions. Poster 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 in the 2000 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, mitochondrial function and dysfunction, programmed cell death, neurotrophins, and metabolic and hormonal influences on disease protein metabolism in cell culture and living animal models, as well as in human clinical trials. Diseases considered at the 2000 meeting included Alzheimer's, Huntington's, spinocerebellar atrophies, ALS, Parkinson's, tauopathies, and synucleinopathies.

PROGRAM

Aggregation and Anti-Aggregation
Chairperson: S. Priola, *Rocky Mountain Laboratories, NIAID, National Institutes of Health, Hamilton, Montana*

Enzymology and Pharmacology of Alzheimer and Parkinson Diseases
Chairperson: S. Sinha, *Elan Pharmaceuticals, South San Francisco, California*

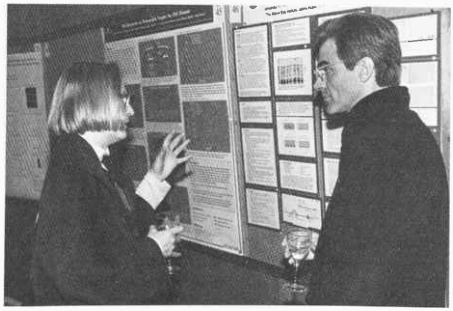
Mechanisms of Neurodegeneration and Cell Death
Chairperson: D. Choi, *Washington University School of Medicine, St. Louis, Missouri*

Molecular Pathology and Pharmacology: Clinical Correlations
Chairperson: M. Sano, *Columbia University College of Physicians & Surgeons, New York, New York*

Infection, Inflammation, and Immunization: Pathogenesis and Therapy
Chairperson: N. Mazarakis, *Oxford BioMedica (UK) Ltd., United Kingdom*

Genomic Approaches to Elucidation of Pathogenesis and to Tailoring Therapy
Chairpersons: A. Califano, *First Genetic Trust, Inc.;* R. Sherrington, *Pfizer Global Research and Development, Alameda, California*

Polyglutamine Diseases, Tauopathies, and Synucleinopathies
Chairperson: H. Zoghbi, *Baylor College of Medicine, Houston, Texas*



P. Radel, S. McKinnon

D. Westaway, P. Mathews

Arabidopsis Genomics

December 7–10 150 participants

ARRANGED BY **Rob Martienssen,** Cold Spring Harbor Laboratory
Michael Bevan, John Innes Center, United Kingdom

This meeting served as the third in a series of biotechnology symposia aimed at bringing together those groups sequencing and analyzing the genome of *Arabidopsis thaliana* with those attempting to determine the biological function of plant genes and other chromosomal sequences. The meeting just preceded the December 14th publication in the journal *Nature* of the complete *A. thaliana* genome by a consortium of international groups, many of whom were able to present their latest data and interpretation at the meeting. The meeting included an opening plenary talk on the completed *Drosophila melanogaster* genome by Michael Ashburner, and a tribute by Ron Davis to DeLill Nasser, who together with colleagues at the National Science Foundation spear-headed the international sequencing effort in *Arabidopsis* (sadly, DeLill could not be at the meeting and passed away a few weeks later). Mike Snyder gave a fascinating keynote talk on new tools for functional genomics in yeast. In all, the meeting included two keynotes, 39 oral presentations, a functional genomics workshop, 43 posters, and closing remarks by Steve Tanksley. The auspicious timing of this meeting served to underscore the celebration for many of those involved in the international sequencing effort. The American Society of Plant Physiology generously supported a champagne reception on December 9th to celebrate completion of the first plant genome.

D. Preuss, M. Dilworth, R. Martienssen, E. Meyerowitz, J.D. Watson, H.-W. Mewes, W.R. McCombie, M. Bevan

PROGRAM

Genome Analysis
Chairperson: E. Meyerowitz, *California Institute of Technology, Pasadena*

Plenary Speaker
M. Ashburner, *EMBL-EBI, Wellcome Trust Genome Campus, Hinxton, United Kindgom*

Functional Genomics I
Chairperson: M. Bevan, *John Innes Centre, Norwich, United Kingdom*

Plenary Speaker
M. Snyder, *Yale University, New Haven, Connecticut*

Functional Genomics Workshop and Coordination Meeting
Chairperson: R. Martienssen, *Cold Spring Harbor Laboratory*

Functional Genomics II
Chairperson: M. Sussman, *University of Wisconsin, Madison*

New Technologies
Chairperson: N. Fedoroff, *Pennsylvania State University, University Park*

Genome Organization and Evolution
Chairperson: E. Richards, *Washington University, St. Louis, Missouri*

New Biological Insights From Genomics
Chairperson: G. Jürgens, *University of Tübingen, Germany*

M. Salanoubat, M. Bevan, S. Rounsley

V. Colot, E. Heard, M. Ashburner

N. Fedoroff, T. Mitchell-Olds, M. Sussman

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.

Advanced Genome Sequence Analysis

March 15–28

INSTRUCTORS **S. Clifton,** Ph.D., Washington University School of Medicine, St. Louis, Missouri
E. Mardis, Ph.D., Washington University School of Medicine, St. Louis, Missouri
W.R. McCombie, Ph.D., Cold Spring Harbor Laboratory
J. McPherson, Ph.D., Washington University School of Medicine, St. Louis, Missouri

ASSISTANTS

M. de la Bastide, Cold Spring Harbor Laboratory
J. Eldred, Washington University School of Medicine, St. Louis, Missouri
M. Fitzgerald, Genome Therapeutics Corporation, Waltham, Massachusetts
E. Huang, Cold Spring Harbor Laboratory
D. Johnson, Washington University School of Medicine, St. Louis, Missouri
J. Kramer, Washington University School of Medicine, St. Louis, Missouri
M. Nhan, Washington University School of Medicine, St. Louis, Missouri
A. O'Shaughnessy, Cold Spring Harbor Laboratory
R. Preston, Cold Spring Harbor Laboratory
M. Rodriguez, Cold Spring Harbor Laboratory
R. Shah, Cold Spring Harbor Laboratory
L. Spiegel, Cold Spring Harbor Laboratory

This course focused on obtaining and analyzing genomic DNA sequence data. It also focused on large-scale sequencing, presenting students with important information on the technical and project management aspects of genomic DNA sequencing projects as well as the computational analysis of the sequence. This was achieved by carrying out a large-scale sequencing project during the class, with a special emphasis on the technical nuances of large-scale sequencing. Increases in sequencing efficiency now permit us to carry out the production phase of the student's sequencing project in less time. As a result, the course increased its emphasis on two areas: the finishing process and the analysis of DNA sequences (both computational and experimental). Last year's course included a module on sequence-based polymorphism analysis. This year, the course expanded the polymorphism analysis to include sequencing of regions from multiple individuals and additional SNP analysis. This curriculum allowed students to learn advanced techniques and principles of genomic DNA sequence analysis, from data generation to analysis of sequence variations in populations.

PARTICIPANTS

Brown, D., B.S., M.S., Research Genetics, Huntsville, Alabama
Chan, D., B.S., M.Phil., Prince of Wale's Hospital (CUHK), Hong Kong
Dubbelde, C., B.S., HHMI at Washington, St. Louis, Missouri
Eliana, G.-S., B.S., CIAT, Columbia
Garrigues, C., B.S., Molecular Dynamics, Sunnyvale, California
Hauser, L., B.S., Ph.D., University of Tennessee/Oak Ridge National Laboratory, Tennessee
Lee, Y.-S., B.A., Ph.D., College of Agriculture & Life Sciences, Kangwon National University, South Korea
Liu, Y., B.M., M.S., Defence Medical Research Institute, Singapore
Lohi, H., M.Sc., Ph.D., University of Helinski, Finland
Nguyen, K., B.A., M.S., Tularik Inc., Greenlawn, New York
Ohmen, J., B.S., Ph.D., Parke-Davis Laboratory for Molecular Genetics, Alameda, California
Pennacchio, L., B.A., Ph.D., Lawrence Berkeley National Laboratory, Berkeley, California
Struyf, F., M.D., Northwestern University Medical School, Chicago, Illinois
Wiencis, A., B.S., M.A., Aventis Pharmaceuticals, Cambridge, Massachusetts
Wilson, B., B.S., Ph.D., Jackson State University, Jackson, Mississippi
Wing, R., A.B., Ph.D., Clemson University Genomics Institute, Clemson, South Carolina

SEMINARS

Green, E., NHGRI/National Institutes of Health, Bethesda, Maryland: Mapping and sequencing a human chromosome: How and why.
Hadd, A., Molecular Dynamics, Sunnyvale, California: NanoPrep sequencing projects. Capillary electrophoresis: Fundamental principles.
Hobert, O., Columbia University, New York, New York: Using the genome sequence to study neural development in *C. elegans*.
Johnston, R., Incyte Microarray System, Fremont, California: cDNA microarray technology.
Kaul, S., The Institute for Genomic Research, Rockville, Maryland: *Arabidopsis* genome.
Korf, I., Washington University School of Medicine, St. Louis, Missouri: Human analysis.
Nickerson, D., Washington University School of Medicine, St. Louis, Missouri: SNPs in candidate genes.
Watson, J.D., Cold Spring Harbor Laboratory: Thoughts on DNA.
Wilson, R., Washington University School of Medicine, St. Louis, Missouri: Sequencing the human genome.

Protein Purification and Characterization

April 5–18

INSTRUCTORS
R. Burgess, University of Wisconsin, Madison
A. Courey, University of California, Los Angeles
S.-H. Lin, University of Texas, Houston
S. Mische, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, Connecticut

ASSISTANTS:
L. Anthony, University of Wisconsin, Madison
V. Bhaskar, University of California, Los Angeles
F. Gharahdaghi, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, Connecticut
E. Han, University of Texas, Houston
D. Meagher, The Rockefeller University, New York, New York
D. Phan, University of Texas, Houston
N. Thompson, University of Wisconsin, Madison

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 a discussion session every evening. Each student became familiar with each of the major techniques in protein purification by actually performing four separate isolations including (1) a regulatory protein from

muscle tissue, (2) a sequence-specific DNA-binding protein, (3) a recombinant protein overexpressed in *E. coli*, and (4) a membrane-bound receptor. A variety of bulk fractionation, electrophoretic, and chromatographic techniques were used, including precipitation by salts, pH, and ionic polymers; ion exchange, gel filtration, hydrophobic interaction, and reversed-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 including 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

Birrell, G., B.S., Ph.D., Stanford University, California
Bonnal, S., B.S., Ph.D., National Institute of Health and Medical Research, France
Duggan, L., B.S., Ph.D., Wistar Institute, Philadelphia, Pennsylvania
Esumi, N., M.D., Ph.D., Johns Hopkins University School of Medicine, Baltimore, Maryland
Gutstein, H., A.B., M.D., University of Texas/M.D. Anderson Cancer Center, Houston
Li, R., B.S., Ph.D., Children's Research Institute, Washington, D.C.
Lopato, S., B.S., Ph.D., University of Vienna, Austria
Manogaran, A., B.S., Ph.D., Marquette University, Milwaukee, Wisconsin
Pollak, E., A.B., M.D., Children's Hospital of Philadelphia, Pennsylvania
Ray, A., M.S., Ph.D., Cleveland Clinic Foundation, Ohio
Reddi, P., B.S., Ph.D., University of Virginia, Charlottesville
Reichert, A., Ph.D., Max-Planck Institute for Evolution/Anthropology, Germany
Struffi, P., M.S., University of Michigan, East Lansing
Tiscornia, G., B.S., Ph.D., University of Wisconsin, Madison
Ximenez-Fyvie, L., D.D.S., D.M.S., National University of Mexico, Mexico
Zhang, H., B.M., Ph.D., Brigham and Women's Hospital, Boston, Massachusetts

SEMINARS

Burgess, R., University of Wisconsin, Madison: Overview of protein purification, immunoaffinity purfication. Biochemical studies of RNA polymerase/Sigma factor interactions.
Courey, A., University of California, Los Angeles: Protein-protein interactions in dorsal/ventral pattern formation in *Drosophila*.
Guidotti, G., Harvard University, Cambridge, Massachusetts: Membrane proteins and extracellular ATP.
Joshua-Tor, L., Cold Spring Harbor Laboratory: Proteins in 3-D.
Kern, D., Brandeis University, Waltham, Massachusetts: Not just structures: NMR studies of regulatory proteins in action.
Lin, S.-H., M.D. Anderson Cancer Center, Houston, Texas: C-CAM: A novel tumor suppressor with anti-angiogenesis activity.
Mische, S., Boehringer Ingelheim Pharmaceuticals, Inc. Ridgefield, Connecticut: Microanalytical protein preparation and characterization.
Severinov, K., Waksman Institute, Rutgers, Piscataway, New Jersey: Protein-protein interactions in bacterial transcription.

Cell and Developmental Biology of *Xenopus*

April 8–18

INSTRUCTORS **P. Krieg,** University of Arizona, Tucson
S. Moody, George Washington University Medical Center, Washington, D.C.

ASSISTANTS **O. Cleaver,** Harvard University, Cambridge, Massachusetts
K. Kenyon, George Washington University, Washington, D.C.
P. Pandur, University of Ulm, Germany
A. Zorn, University of Cambridge/Wellcome CRC Institute, Cambridge, United Kingdom

The frog *Xenopus* is an important vertebrate model for studies of maternal factors, 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 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) preparation of transgenic embryos; and (8) use of *Xenopus tropicalis* for genetic analyses. This course was suited for both investigators who had no previous experience with *Xenopus* and those who had worked with *Xenopus* and wished to learn new and cutting-edge techniques. All participants had current training in molecular biology and some knowledge of developmental biology.

PARTICIPANTS

Bosetti, A., B.S., San Raffaele Scientific Institute, Milano, Italy
Boyer, B., B.S., M.D., Ph.D., Centre National de la Recherche Scientifique, Orsay, France
Drawbridge, J., B.S., Ph.D., Rider University, Lawrenceville, New Jersey
Hamilton, F., B.Sc., University of Dundee, United Kingdom
Holley, C., B.S., Duke University, Durham, North Carolina
Kim, S.W., B.S., M.S., University of Texas/M.D. Anderson Cancer Center, Houston
Luo, X., B.S., Ph.D., University of North Carolina, Chapel Hill
Marchant, J., B.A., M.A., Ph.D., University of California, Irvine
Mullor, J., B.S., Ph.D., Skirball Institute, New York University Medical Center, New York, New York
Ogawa, M., B.M., Keio University, School of Medicine, Tokyo, Japan
Otero, L., B.A., D.Phil., University of Cambridge, United Kingdom
Park, E.K., B.S., Ph.D., National Cancer Institute/Frederick Cancer Research and Development Center, Maryland
Peng, Y., M.D., Ph.D., National Cancer Institute, Frederick, Maryland
Seville, R., B.Sc., University of Warwick, Coventry, United Kingdom
Vernon, A., B.S., University of Cambridge, United Kingdom
Warkman, A., B.Sc., University of Western Ontario, Canada

SEMINARS

Amaya, E., Cambridge University, United Kingdom: Introduction to transgenic techniques.
Cleaver, O., Harvard Medical School, Cambridge, Massachusetts: Pancreas development.
DeSimone, D., University of Virginia, Charlottesville: Regulation of cell adhesion and morphogenesis in *Xenopus*.
Grainger, R., University of Virginia, Charlottesville: *Xenopus tropicalis:* A new model for vertebrate developmental genetics.
Gurdon, J., Cambridge University, United Kingdom: Mechanisms of perception of morphogen concentration.
Heasman, J., University of Minnesota, Minneapolis: Maternal control of tissue specification.
Keller, R., University of Virginia, Charlottesville: Early embryonic anatomy and morphogenesis of *Xenopus*.
King, M.L., University of Miami, Florida: Role of localized maternal RNAs in *Xenopus* development.
Krieg, P., University of Arizona, Tucson: Cardiovascular development.
Moody, S., George Washington University Medical Center, Washington, D.C.: Introduction to early embryology. The competence to form retina and amacrine cells.
Zorn, A., Wellcome Trust, Cambridge, United Kingdom: Regulation of endoderm specification.

Genetic-Epidemiological Studies of Complex Diseases

June 7–13

INSTRUCTORS **E. Squires-Wheeler,** Columbia University College of Physicians & Surgeons, New York, New York
N. Risch, Stanford University, California

This lecture course considered the many difficulties in studying the genetic basis of complex disorders, such as diabetes, cardiovascular disease, cancer, Alzheimer's disease, schizophrenia, and epilepsy, with a particular emphasis on neuropsychiatric conditions. We discussed genetic-epidemiologic study designs, including family, twin, and adoption studies, as well as mode of inheritance analyses, and their role in setting the framework for understanding the genetic and nongenetic components of a disease. A major focus was the identification of specific gene effects using both linkage and association analyses. We discussed the efficiency and robustness of different designs for such analysis. We also considered how evidence from genetic-epidemiologic studies informs both the design and interpretation of molecular genetic studies. Recent discoveries of genes for both Mendelian and non-Mendelian diseases guided the discussion of the various methodologic issues.

PARTICIPANTS

August, P., B.A., M.D., Cornell Medical College, New York, New York
Bai, Y., M.B., Ph.D., National Cancer Institute/NIH, Bethesda, Maryland
Bergson, C., B.S., Ph.D., Medical College of Georgia, Augusta
Diaz, G., B.A., Ph.D., Mount Sinai School of Medicine, New York, New York

Hall, M., B.Sc., Ph.D., Kings College, London, United Kingdom
Heng, C.-K., B.S., Ph.D., National University of Singapore, Singapore
Hsu, I., B.S., Ph.D., National Tsing Hua University, Hsinchu, Taiwan, Republic of China
Hwa, K., B.S., Ph.D., Academia Sinica, Taipei, Taiwan, Republic of China
Johansson, C., M.S., Karolinska Institutet, Stockholm, Sweden
Lou, W.Y.W., B.S., Ph.D., Mount Sinai School of Medicine, New York, New York
Loy, C., M.B., B.S., M.Med., University of Sydney/Garvin Institute, Sydney, Australia
Malhotra, A., B.A., Ph.D., Hillside Hospital, Glen Oaks, New York
Marler, J., A.B., M.D., National Institute of Neurological Communicative Disorders and Stroke, Bethesda, Maryland
Millwood, I., B.A., Ph.D., Imperial College of Medicine, Middlesex, United Kingdom
Odeberg, J., M.S., Ph.D., Royal Institute of Technology, Stockholm, Sweden
Peissel, B., M.D., Ph.D., National Cancer Institute, Milan, Italy
Pennesi, G., M.D., Ph.D., National Institutes of Health, Bethesda, Maryland
Rana, B., B.A., Ph.D., University of California, San Diego, La Jolla
Scheinman, S., A.B., M.D., SUNY Health Science Center, Syracuse, New York
Seielstad, M., B.S., Ph.D., Harvard School of Public Health, Boston, Massachusetts
Van Belzen, M., M.Sc., University Medical Center Utrecht, The Netherlands
Wager-Smith, K., B.S., M.A., The Scripps Research Institute, La Jolla, California
Zhang, K., B.S., Ph.D., Johns Hopkins University, Baltimore, Maryland

SEMINARS

Bressman, S., Beth Israel, New York, New York: Dystonia.
Kardia, S., University of Michigan, Ann Arbor: Candidate genes in cardiovascular disease.
Keats, B., Louisiana State University Medical Center, New Orleans: Mendelian principles and linkage analysis I. Genetic disorders in the arcadian population.
Merikangas, K., Yale University School of Medicine, New Haven, Connecticut: Further issues in genetic epidemiology.
Meyer, J., Millenium Pharmaceuticals, Cambridge, Massachusetts: Biometrical genetics.
Myers, R., Stanford University School of Medicine, California: Molecular technology and the genome project.
Ott, J., Columbia University College of Physicians & Surgeons, New York, New York: Linkage analysis II.
Ottman, R., Columbia University School of Public Health, New York, New York: First principles of genetic epidemiology.
Risch, N., Stanford University, California: Autism.
Sandkuijl, L., Leiden University, Delft, The Netherlands: Biopolar disorder.
Spielman, R., University of Pennsylvania School of Medicine, Philadelphia: Polycystic ovary syndrome.
Witte, J., Case Western Reserve University, Cleveland, Ohio: Family and non-family-based linkage disequilibrium studies.

Advanced Bacterial Genetics

June 7–27

INSTRUCTORS **B. Bassler,** Princeton University, New Jersey
C. Manoil, University of Washington, Seattle
J. Slauch, University of Illinois, Urbana-Champaign

ASSISTANTS **C. Ellermeier,** University of Illinois, Urbana-Champaign
M. Miller, Princeton University, New Jersey
E. Round, University of Washington, Seattle

The laboratory course presented logic and methods used in the genetic dissection of complex biological processes in bacteria. The methods presented included mutagenesis using transposons, mutator strains, and chemical and physical mutagens; mapping mutations using genetic and physical techniques; generation and analysis of gene fusions; molecular cloning; polymerase chain reaction; Southern blot analysis; epitope insertion mutagenesis; and site-directed mutagenesis. A key component of the course was the use of sophisticated genetic methods in the analysis of pathogenic and "undomesticated" bacteria. Invited lecturers described the use of genetic approaches to study biological processes in a variety of bacteria.

PARTICIPANTS

Backhed, F., M.S., Karolinska Institutet, Stockholm, Sweden
Bakshi, S., B.Sc., M.Sc., University of Oxford, United Kingdom
Georgellis, D., B.S., Ph.D., Harvard Medical School, Boston, Massachusetts
Gueneau de Novoa, P., M.Sc., Ph.D., Instituto Venezolano de Investigaciones Cientifica, Altos de Pipe-Miranda, Venezuela
Kotton, Camille, B.A., M.D., Massachusetts General Hospital, Boston
Levy, D., B.A., Ph.D., U.S. Food and Drug Administration, Washington, D.C.
Madsen, E., B.A., Ph.D., Cornell University, Ithaca, New York
Ohl, M., B.S., M.D., University of Washington, Seattle
Rosen, H., A.B., M.D., University of Washington, Seattle
Sambandamurthy, V., M.Sc., Ph.D., Albert Einstein College of Medicine, Bronx, New York
Stockbauer, K., B.A., University of California, Los Angeles
Sturgill-Koszyck, S., B.S., Ph.D., University of Michigan, Ann Arbor
Thomsen, L., M.S., The Royal Veterinary and Agricultural University, Copenhagen, Denmark
Ting, C., B.A., Ph.D., Massachusetts Institute of Technology, Cambridge
Todd, W., B.A., Ph.D., LSU Agricultural Center and School of Veterinary Medicine, Baton Rouge, Louisiana
Vanrompay, D., Ph.D., University of Gent, Belgium

SEMINARS

Grossman, A., Massachusetts Institute of Technology, Cambridge: Control of development by replication initiation proteins in *Bacillus subtilis*.
Harriman, P., National Science Foundation, Arlington, Virginia: Microbiology and genetics at the NSF.
Hughes, K., University of Washington, Seattle: Translation: The grand unifying theory in type III secretion.
Jacobs, W., Albert Einstein College of Medicine, Bronx, New York: The power of bacterial genetics: A 1914-D penny, lotto, tuberculosis control, and beyond.
Kaplan, H., University of Texas Medical School, Houston: Sensing and integration of multiple signals during *Myxococcus* multicellular development.
Lory, S., University of Washington, Seattle: DNA microarrays as tools for studying host-pathogen interactions.
Metcalf, W., University of Illinois, Urbana: Genetic analysis of methanogenesis in *Methanosarcina* species.
Miller, J., University of California, Los Angeles: Signal transduction during the *Bordetella* infectious cycle.
Pogliano, K., University of California, San Diego, La Jolla: Moving and fusing membranes during *Bacillus subtilis* sporulation.
Roth, J., University of Utah, Salt Lake City: Adapative mutability: Fact or artifact.

Molecular Embryology of the Mouse

June 7–27

INSTRUCTORS	**A. Nagy,** Mount Sinai Hospital, Toronto, Ontario, Canada **P. Tam,** Children's Medical Research Institute, Wentworthville, Australia
CO-INSTRUCTORS	**S.-L. Ang,** Institut de Genetique et de Biologie Moleculaire et Cellulaire, Strasbourg, France **A. Gossler,** Institut fur Molekularbiologie, Hannover, Germany
ASSISTANTS	**B. Davidson,** Children's Medical Research Institute, Wentworthville, Australia **M. Gertsenstein,** Samuel Lunenfeld Research Institute, Toronto, Ontario, Canada

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 that were covered in the practicals included 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. The participating speakers were Siew-Lan Ang, Marisa Bartolomei, Richard Behringer, Charles Emerson, Achim Gossler, Brigid Hogan, Nancy Jenkins, Tom Jessell, Alexandra Joyner, Monica Justice, Robin Lovell-Badge, Terry Magnuson, Anne McLaren, Peter Mombaerts, Andras Nagy, Lee Niswander, Ginny Papaioannou, Janet Rossant, Austin Smith, Davor Solter, Phil Soriano, Patrick Tam, and David Wilkinson.

PARTICIPANTS

Bartsch, D., B.S., Ph.D., Columbia University, New York, New York
Buchner, G., B.Sc., Guy's Hospital, London, United Kingdom
Chinoy, M., B.S., Ph.D., Pennsylvania State University, Hershey
Hirschi, K., B.S., Ph.D., Baylor College of Medicine, Houston, Texas
Lopez-Guisa, J., B.S., Ph.D., University of Washington School of Medicine and Children's Hospital, Seattle
Lykke-Andersen, K., B.A., M.S., Yale University, New Haven, Connecticut
Milewski, R., M.D., Ph.D., Yale University, New Haven, Connecticut
Porse, B., M.S., Ph.D., University Hospital of Copenhagen, Denmark
Tang, D., B.S., Ph.D., University College London, United Kingdom
Van Hengel, J., M.S., Ph.D., University of Ghent, Belgium
Wang, B., B.S., Ph.D., Johns Hopkins University School of Medicine, Baltimore, Maryland
Yang-Yen, H.-F., B.S., Ph.D., Academia Sinica, Taiwan, Republic of China
You, Y., B.S., Ph.D., Oak Ridge National Laboratory, Tennessee
Zarach, J., B.S., Johns Hopkins University, Baltimore, Maryland

SEMINARS

Ang, S.-I., Institut de Genetique et de Biologie Moleculaire et Cellulaire, C.U. de Strasbourg, France: Head formation.
Bartolomei, M., University of Pennsylvania School Medicine, Philadelphia: Genomic imprinting.
Behringer, R., University of Texas/M.D. Anderson Cancer Center, Houston: Transgenesis. Reproductive organs.
Emerson, C., University of Pennsylvania School of Medicine, Philadelphia: Signaling and transcriptional mechanisms controlling skeletal myogenesis.
Gossler, A., Institut fur Molekularbiologie, Hannover, Germany: Somitogenesis.
Hogan, B., Vanderbilt University Medical Center/HHMI, Nashville, Tennessee: BMP signaling, Forkhead genes, and morphogenesis.
Jenkins, N., NCI-Frederick Cancer Research and Development Center, Maryland: Mouse genetics.
Jessell, T., Columbia University, New York, New York: Regionalization and cell-type specification.
Joyner, A., New York Medical Center/Skirball Institute of Biology, New York, New York: Brain patterning.
Justice, M., Baylor College of Medicine, Houston, Texas: ENU mutagenesis.
Lovell-Badge, R., MRC National Institute for Medical Research, London, United Kingom: Mouse models and experimental manipulation. Sox genes and cell fate decision. Sex determination.
Magnuson, T., Case Western Reserve University, Cleveland, Ohio: Genetic analysis of development.
McLaren, A., Wellcome/CRC Institute, Cambridge, United Kindom: Germ cells.
Mombaerts, P., The Rockefeller University, New York, New York: Analysis of olfactory function. Cloning by nuclear transfer.
Nagy, A., Mount Sinai Hospital, Toronto, Ontario, Canada: ES cells and the biology of chimeras. Gene targeting: Strategy and technology.
Niswander, L., Memorial Sloan-Kettering Cancer Center, New York, New York: Fgf signaling in vertebrate and invertebrate development.
Papaioannou, G., Columbia University College of Physicians & Surgeons, New York, New York: T-box genes.
Rossant, J., Mount Sinai Hospital, Toronto, Canada: Extraembryonic tissues.
Smith, A., University of Edinburgh, United Kingdom: Pluripotency and differentiation of embryonic stem cells.
Solter, D., Max-Planck Institut fur Immunbiologie, Freiburg, Germany: Fertilization and preimplantation development.
Soriano, P., Fred Hutchinson Cancer Research Center, Seattle, Washington: PDGF signaling. Gene trap.
Tam, P., Children's Medical Research Institute, Wentworthville, Australia: Body patterning and early organogenesis.
Wilkinson, D., MRC National Institute for Medical Research, London, United Kingdom: In situ hybridization and Eph signaling in patterning.

Physiological Approaches to Ion Channel Biology

June 7–27

INSTRUCTORS	**T. Otis,** University of California, Los Angeles **A. Ribera,** University of Colorado, Denver **L. Trussell,** Oregon Health Sciences Center, Portland
CO-INSTRUCTORS	**L. Blair,** Brown University, Providence, Rhode Island **G. Borst,** Universiteit van Amsterdam, The Netherlands
ASSISTANTS	**S. Brenowitz,**, Oregon Health Sciences Center, Portland **A. Linares,** University of Colorado, Denver **V. Maubecin,** Oregon Health Sciences Center, Portland

This intensive laboratory/lecture course introduced students to electrophysiological approaches for the study of ion channels in their native environments. Hands-on exercises included patch-clamp recording of ion channel activity in acutely isolated or cultured cells or neurons in brain slice preparations.

Different recording configurations (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 were related to information previously gained from molecular cloning and heterologous expression of ion channel genes. Areas of particular interest included channels that (1) were activated by the neurotransmitter at central and peripheral synapses, (2) interacted with other channels to shape the response properties of neurons, (3) respond to neuromodulators with changes in functional properties, and (4) were 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. Guest speakers included Bruce Bean, Leslie Blair, Gerard Borst, Sascha DuLac, Paul Fuchs, Elisabeth Glowtzki, Ruth Heidelberger, John Huguenard, Robert Malinow, Isabel Mintz, Indira Raman, and Jim Surmeier.

PARTICIPANTS

Desai, R., M.Sc., Ph.D., Weizmann Institute of Science, Rehovot, Israel
Fellin, T., Ph.D., University of Padua, Italy
Hardelin, J.-P., M.D., Pasteur Institute, Paris, France
Khan, F., F.Sc., M.D., Virginia Commonwealth University, Richmond
Kim, G., B.S., M.S., University of Pittsburgh School of Medicine, Pennsylvania
Klug, A., M.S., University of Texas, Austin
Leguen, I., B.Sc., Ph.D., Institut Nat'l de Recherche Agronomique, Rennes, France
Li, Y., B.S., M.S., Ph.D., University of Illinois, Urbana-Champaign
Nitabach, M., B.A., Ph.D., New York University, New York, New York
Olofsdotter, K., M.S., Ph.D., Lund University, Sweden
Song, I., B.S., M.S., Postech, Pohang, Republic of Korea
Weissmann, A., B.A., Oregon Health Sciences University, Portland

SEMINARS

Bean, B., Harvard Medical School, Boston, Massachusetts: Ionic mechanisms of spontaneous activity of central neurons II, or spikes and the single channel.
Blair, L., Brown University, Providence, Rhode Island: Rapid modulation of calcium channels by tyrosine phosphorylation.
Borst, G., Universiteit van Amsterdam, The Netherlands: Physiology of a presynaptic terminal in the CNS, I. Physiology of a presynaptic terminal in the CNS, II.
DuLac, S., The Salk Institute, La Jolla, California: Cellular mechanisms of eye movement control.
Fuchs, P., Johns Hopkins University Medical School, Baltimore, Maryland: Hair cell physiology. A molecular mechanism for electrical tuning. Efferent control of the cochlea. Cholinergic inhibition of the cells.
Heidelberger, R., University of Texas/Houston Medical School, Houston: Synaptic transmission in the retina.
Huguenard, J., Stanford University Medical School, Stanford, California: Differential ion channel expression in neuronal subclasses: Cellular and circuit consequences.
Malinow, R., Cold Spring Harbor Laboratory: Probing synaptic transmission and plasticity in hippocampal slices using acute recombinant expression techniques.
Mintz, I., Boston University Medical Center, Massachusetts: Patch-clamp and amperometric studies of dopamine dendritic release in the substantia nigra.
Otis, T., University of California, Los Angeles: Control of metabotropic glutamate receptor signaling at the parallel fiber synapse by a colocalized postsynaptic glutamate transporter.
Raman, I., Northwestern University, Evanston, Illinois: Ionic mechanisms of spontaneous activity of central neurons I.
Ribera, A., University of Colorado, Denver: Ion channel function in zebrafish behavioral mutants.
Surmeier, J., Northwestern University Medical School, Chicago, Illinois: Dopaminergic modulation of ion channels.
Trussell, L., Oregon Health Sciences University, Portland: Gating kinetics of glutamate receptor channels and synaptic function.

Computational Neuroscience: Vision

June 15–28

INSTRUCTORS **E.J. Chichilnisky,** The Salk Institute, La Jolla, California
P. Glimcher, New York University, New York, New York
E. Simoncelli, New York University, New York, New York

ASSISTANTS **J. Gardner,** University of California, San Francisco
G. Horwitz, The Salk Institute, La Jolla, California

Computational approaches to neuroscience have produced important advances in our understanding of neural processing. Prominent successes have come in areas where strong inputs from neurobiological, behavioral, and computational investigation can interact. The theme of the course was that an understanding of the computational problems, the constraints on solutions to these problems, and the range of possible solutions can help guide research in neuroscience. Through a combination of lectures and hands-on experience in a computer laboratory, this intensive course examined color vision, spatial pattern analysis, motion analysis, oculomotor function, attention, and decision making. Lecturers were Edward Adelson, David Brainard, Marisa Carrasco, Eduardo Chichilnisky, Sascha Du Lac, Wilson Geisler, Paul Glimcher, David Heeger, J. Anthony Movshon, Andrew Parker, Fred Rieke, Michael Shadlen, Maggie Shiffrar, Eero Simoncelli, Larry Snyder, and Stefan Treue.

PARTICIPANTS

Bayer, H., B.A., Ph.D., New York University, New York, New York
Blaser, E., B.A., Ph.D., Rutgers University, Piscataway, New Jersey
Bredfeldt, C., B.A., M.A., University of California, Los Angeles
Churchland, A., B.A., Ph.D., University of California, San Francisco
David, S., B.A., University of California, Berkeley
DiCarlo, J., B.S., M.D., Ph.D., HHMI/Baylor College of Medicine, Houston, Texas
Grossman, E., B.A., Ph.D., Vanderbilt University, Nashville, Tennessee
Huk, A., B.A., Stanford University, California
Lesmes, L., B.S., University of Southern California, Los Angeles
Lipson, M., B.Sc., Ph.D., University of Oxford, United Kingdom
Muller, J., B.S., Ph.D., Stanford University, California
Murray, R., B.A., M.Phil., University of Toronto, Canada
Panerai, F., B.S., M.S., Ph.D., CNRS/College de France, Paris, France
Pillow, J., B.A., New York University, New York, New York
Ress, D., B.S., Ph.D., Stanford University, California
Rowe, M., B.S.E., Ph.D., University of California, Santa Barbara
Saenz, M., B.S., The Salk Institute, La Jolla, California
Schwartz, O., B.S., M.S., New York University, New York, New York
Sharon, D., B.S., M.S., Weizmann Institute, Rehovot, Israel
Uzzell, V., B.A., Ph.D., University of California, San Diego
Wolfe, U., B.A., Ph.D., New York University, New York, New York
Yang, T., B.S., Baylor College of Medicine, Houston, Texas
Zenger, B., M.Sc., Ph.D., Stanford University, California
Zhao, R., B.S., M.S., Harvard Medical School, Cambridge, Massachusetts

SEMINARS

Adelson, E., Massachusetts Institute of Technology, Cambridge: Elements of vision/lightness.
Brainard, D., University of California, Santa Barbara: Color matching, cones, and context effects.
Carrasco, M., New York University, New York, New York: Attention psychophysics.
Chichilnisky, E., The Salk Institute, La Jolla, California: Color context effects/modeling. Retinal ganglion cell physiology and white noise analysis.
Du Lac, S., The Salk Institute, La Jolla, California: VOR/Saccades.
Geisler, W., University of Texas, Austin: Pattern psychophysics.
Glimcher, P., New York University, New York, New York: Eye movement. Parietal cortex.
Heeger, D., Stanford University, California: V1 modeling. fMRI studies of pattern vision.
Movshon, J.A., New York University, New York, New York: LGN/V1 physiology. MT physiology.
Parker, A., University of Oxford, United Kingdom: MT and perception.
Rieke, F., University of Washington, Seattle: Rods and photon detection. RGC spike coding/information theory.
Shadlen, M., University of Washington, Seattle: Frontal eye fields.
Shiffrar, M., Rutgers University, Newark, New Jersey: Motion psychophysics.
Simoncelli, E., New York University, New York, New York: Statistical properties of visual images. Motion modeling.
Snyder, L., Washington University School of Medicine, St. Louis, Missouri: Motor vs. decision.
Treue, S., University of Tubingen, Tubingen, Germany: Attention physiology.

Making and Using DNA Microarrays

June 21–27

INSTRUCTORS **J. DeRisi,** University of California, San Francisco
V. Iyer, Stanford University Medical Center, California

ASSISTANTS **A. Alizadeh,** Stanford University, California
H. Bennett, University of California, San Francisco
J. Boldrick, Stanford University, California
M. Diehn, Stanford University, California
C. Seidel, University of California, Berkeley

A DNA microarray is a simple, inexpensive, and versatile tool for experimental explorations of genome structure, gene expression programs, gene function, and cell and organismal biology. In this hands-on course, students were guided through the process of building a robot for printing DNA microarrays, preparing DNA samples and slides to be used for printing microarrays, printing DNA microarrays, designing and conducting experiments for analysis by DNA microarray hybridization, data analysis, display, and interpretation. Experimental applications covered in the course included systematic studies of global gene expression programs, inferring gene function using microarrays, and genotyping and measuring changes in gene copy number. Students who completed this course were able to set up their own independent facility for printing and experimental use of DNA microarrays. Guest lecturers presented the state of the art in new technology, experimental applications, and interpretation of large genomic data sets.

PARTICIPANTS

Allingham-Hawkins, D., B.Sc., Ph.D., North York General Hospital, Toronto, Canada
Antoniou, E., B.S., Ph.D., University of Missouri, Columbia
Arctander, P., M.Sc., Ph.D., University of Copenhagen, Denmark
Borsani, G., B.S., Ph.D., Telethon Institute of Genetics and Medicine, Milano, Italy
Caudy, M., B.S., Ph.D., Cornell Medical College at Burke Research Institute, White Plains, New York
Dressman, H., B.S., Ph.D., Duke University Medical Center, Durham, North Carolina
Ferrari, S., Ph.D.. University of Brescia, Italy
Hensey, C., B.Sc., Ph.D., University College Dublin, Ireland
Huang, S., B.S., Ph.D., Memorial Sloan-Kettering Cancer Center, New York, New York
Hughes, K., B.A., Ph.D., Arizona State University, Phoenix
Kamdar, S., B.S., The Jackson Laboratory, Bar Harbor, Maine
Mardis, E., B.S., Ph.D., Washington University School of Medicine, St. Louis, Missouri
Myers, G., B.Sc., Ph.D., Walter & Eliza Hall Institute of Medical Research, Melbourne, Australia
Rocap, G., Ph.D., Massachusetts Institute of Technology, Cambridge
Satagopan, J., B.S., Ph.D., Memorial Sloan-Kettering Cancer Center, New York, New York
Stetson, D., B.S., M.S., Massachusetts General Hospital, Boston
Zhou, Y., Ph.D., University of British Columbia, Vancouver, Canada

SEMINARS

Alizadeh, A., Stanford University, California: Exploring expression variations in normal and malignant immune cells.
Basarsky, T., Axon Instruments, Inc., Foster City, California: Image analysis.
Boldrick, J., Stanford University, California: Genomic responses to infection.
Brown, P.O., Stanford University, California: The living genome.
DeRisi, J., University of California, San Francisco: Introduction to the microarrays course.
Diehn, M., Stanford University, California: Identifying membrane and secreted proteins using DNA microarrays. Introduction to S.O.U.R.C.E.
Haab, B., Van Andel Research Institute, Grand Rapids, Michigan: Protein arrays.
Iyer, V., Stanford University, California: Mapping protein/DNA interactions at the genomic level.
Kim, S., Stanford University, California: Whole genome *C. elegans* expression analysis.
Seidel, C., University of California, Berkeley: Introduction to LINUX and PERL.

Arabidopsis Molecular Genetics

June 30–July 20

INSTRUCTORS **K. Barton,** University of Wisconsin, Madison
J. Bowman, University of California, Davis
U. Grossniklaus, University of Zurich, Switzerland

ASSISTANTS **R. Joy,** University of Wisconsin, Madison
D. Page, University of Zurich, Switzerland
F. Rodriguez, University of Wisconsin, Madison

This course provided an intensive overview of topics in plant growth, physiology, and development, focusing on molecular genetic approaches to understand 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; gene dosage effects and epigenetic phenomena; cell biology (including calcium signaling, cell polarity, and centromere behavior); plant development (including development of flowers, roots, meristems, and leaves, male and female gametophytes, and embryos); floral induction; synthesis, function, and perception of hormones; bioinformatics tools available to the *Arabidopsis* community; and the potential of the *Arabidopsis* genome project for accelerating *Arabidopsis* research. Speakers provided overviews of their fields, followed by in-depth discussions of their own work. The laboratory sessions provided an introduction to important techniques currently used in *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 isolation and detection; mass spectrometry, gene chip technology, QTL analysis, and techniques commonly used in genetic and physical mapping, and map-based cloning. The course also included several short workshops on important themes in genetics. Speakers included Bonnie Bartel, Kathy Barton, David Baulcombe, Jim Birchler, John Bowman, Barbara Butler, Kelly Dawe, Caroline Dean, Nancy Dengler, Ueli Grossniklaus, Peter Hepler, David Jackson, Jonathan Jones, Rich Jorgensen, Nancy Kerk, Joe Kieber, Maarten Koornneef, Rob Last, Rob Martienssen, Peter McCourt, Scott Peck, Tom Petersen, Bob Pruitt, Katrina Ramonell, Eric Schaller, Ben Scheres, Sidney Shaw, David Spector, and Shu Hsing Wu.

PARTICIPANTS

Allen, G., B.Sc., Ph.D., University of California, San Diego
Coleman, C., B.S., Ph.D., Brigham Young University, Provo, Utah
Colot, V., Ph.D., John Innes Centre, Paris, France
Foreman, J., B.Sc., Ph.D., University of California, Davis
Friesner, J., B.S., University of California, Davis
Haswell, E., B.S., Ph.D., University of California, San Francisco
Hoening, T., B.A., University of Wisconsin, Madison
Kaur, J., B.Sc., M.Sc., Centre for Cellular and Molecular Biology, Hyderabad, India
Kanaoka, M., B.S., Ph.D., Kyoto University, Japan
Kwon, M., B.S., M.S., Friedrich Miescher Institute, Basel, Switzerland
McGinnis, K., B.S., Ph.D., Washington State University, Pullman
Neuman, D., M.A., Ph.D., University of Nevada, Las Vegas
Reyes, J., B.A., Ph.D., Consejo Superior de Investigaciones Cientificas, Sevilla, Spain
Rolland, F., M.S., Ph.D., University of Leuven, Belgium
Sera, T., B.S., Ph.D., Novartis Agricultural Discovery Institute, Inc., San Diego, California
Vandenabeele, S., B.S., Ph.D., Flanders Interuniversity Institute of Biotechnology, Gent, Belgium

SEMINARS

Bartel, B., Rice University, Houston, Texas: Synthesis and metabolism of auxin in *Arabidopsis*.
Barton, K., University of Wisconsin, Madison: Formation of the shoot apical meristem during embryogenesis.
Baulcombe, D., Sainsbury Laboratory, Norwich, United Kingdom: Gene silencing.
Birchler, J., University of Missouri, Columbia: Effects of varying gene dosage: Aneuploidy and polyploidy in maize. Gene silencing in *Drosophila*.
Bowman, J., University of California, Davis: Floral development.
Butler, B., Genetics Computer Group, Madison, Wisconsin: Bioinformatics for *Arabidopsis* molecular geneticists.
Dawe, K., University of Georgia, Athens: Meiosis and centromere function in plants.
Dean, C., John Innes Centre, Norwich, United Kingdom: Induction of flowering in *Arabidopsis*.
Dengler, N., University of Toronto, Canada: The anatomy of *Arabidopsis*.
Grossniklaus, U., University of Zurich, Switzerland: Female gametophyte development.
Hepler, P., University of Massachusetts, Amherst: Calcium in plant signaling.
Jackson, D., Cold Spring Harbor Laboratory: The shoot apical meristem and protein movement.
Jones, J., Sainsbury Laboratory, Norwich, United Kingdom: Plant pathogen interactions.
Jorgensen, R., University of California, Davis: Epigenetic mechanisms in plants.
Kerk, N., Yale University, New Haven, Connecticut: Root meristem initiation and organization.
Kieber, J., University of North Carolina, Chapel Hill: Molecular genetics of cytokinins.
Koornneef, M., Wageningen Agricultural University, Wageningen, The Netherlands: Analysis of quantitative traits.
Last, R., Cereon Genomics LLC, Cambridge, Massachusetts: Biochemical genetics.
Martienssen, R., Cold Spring Harbor Laboratory: Functional genomics in *Arabidopsis*.
McCourt, P., University of Toronto, Canada: Abscisic acid in *Arabidopsis* growth and development.
Peck, S., Friedrich Miescher Institute, Switzerland: Use of mass spectrometry in *Arabidopsis*.
Petersen, T., Iowa State University, Ames: Transposable elements.
Pruitt, B., Harvard University, Cambridge, Massachusetts: Fertilization and the development of the male gametophyte.
Ramonell, K., Carnegie Institution of Washington, Stanford, California: Gene chip technology.
Schaller, E., University of New Hampshire, Durham: The biochemistry and molecular biology of ethylene action.
Scheres, B., Utrecht University, The Netherlands: Cell interactions in the root apical meristem.
Shaw, S., Stanford University, California: Development of cellular polarity in plants.
Spector, D., Cold Spring Harbor Laboratory: Microscopy.
Sussex, I., Yale University, New Haven, Connecticut: An introduction to plant morphological diversity.
Wu, S.-H., Carnegie Institution of Washington, Stanford, California: Gene chip technology.

Molecular Cloning of Neural Genes

June 30–July 20

INSTRUCTORS	**J. Boulter,** University of California, Los Angeles **C. DuLac,** Harvard University, Cambridge, Massachusetts **C. Lai,** The Scripps Research Institute, La Jolla, California **D. Lavery,** Glaxo Wellcome Experimental Research, Lausanne, Switzerland
CO-INSTRUCTOR	**M. Schwab,** The Scripps Research Institute, La Jolla, California
ASSISTANTS	**E. Fung,** The Scripps Research Institute, La Jolla, California **H. Kong,** New York University Medical Center, New York, New York **J. Rihel,** Harvard University, Boston, Massachusetts **M. Samson,** Harvard Medical School/HHMI, Boston, Massachusetts **T. Taketani,** The Scripps Research Institute, La Jolla, California **I. Tietjen,** Harvard University, Boston, Massachusetts **W. Walwyn,** University of California, Los Angeles

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, aquisition of cell identity, and axon guidance in the developing nervous system. The laboratory portion of the course began with instruction 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)$^+$ RNA 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 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

Adams, N., B.Sc. D.phil., The Rockfeller University, New York, New York
Cantrell, Angela, B.S., Ph.D., University of Tennessee, Memphis
Fernandes, J., B.Sc., Ph.D., Miami University, Oxford, Ohio
Kaplin, A., B.S., Ph.D., Johns Hopkins University School of Medicine, Baltimore, Maryland
Leamey, C., B.Sc., Ph.D., Sydney University, Australia
Mermelstein, P., B.S., Ph.D., Stanford University, California
Raman, C., B.S., Ph.D., University of California, Irvine
Rodriguez, C., B.S., Ph.D., Harvard Medical School, Boston, Massachusetts
Shu, T.Z., M.D., Ph.D., University of Maryland, Baltimore
Stewart, A., B.A., Ph.D., George Washington University, Washington, D.C.
Stotz, S., B.Sc., M.Sc., University of Calgary, Canada
Taylor, J., B.Sc., Ph.D., University of Oxford, United Kingdom
Unguez, G., B.S., Ph.D., New Mexico State University, Las Cruces
Weiss, J., B.S., Ph.D., University of California, Irvine
Williams, S., B.A., Ph.D., Columbia University, New York, New York
Zaki, P., B.S., University of California, Los Angeles

SEMINARS

Barres, B., Stanford Medical School, California: Neuron-glial interactions in the developing central nervous system.
Bettler, B., Novartis AG, Basel, Switzerland: Molecular insights into GABA, receptor physiology.
Darnell, R., The Rockefeller University, New York, New York: Molecular cloning of neural genes in pursuit of brain function: Nature's autoimmune approach and paraneoplastic neuronal degenerations.
Dulac, C., Harvard University, Cambridge, Massachusetts: Sensory coding of pheromone signals in mammals.
Julius, D., University of California, San Francisco: From peppers to pain: The molecular biology of nociception.
Lemke, G., Salk Institute, La Jolla, California: Genetic studies of the role of EphA receptors in topographic mapping.
Sheng, M., Massachusetts General Hospital/HHMI, Boston: Molecular organization of the postsynaptic specialization.
Silva, A., University of Calfornia Medical Center, Los Angeles: You have cloned a neuronal gene: Now what?
Walsh, C., Beth Israel Deaconess Medical Center/Harvard Medical School, Boston, Massachusetts: Mining the human genome for genes that regulate cerebral cortical development.
Weinmaster, G., University of California School of Medicine, Los Angeles: *Notch* signal transduction in mammalian cells.

Neurobiology of *Drosophila*

June 30–July 20

INSTRUCTORS **K. Broadie,** University of Utah, Salt Lake City
S. DeBelle, University of Nevada, Las Vegas
G. Tear, King's College, London, United Kingdom

ASSISTANTS **B. Butland,** King's College, London, United Kingdom
M. Ginsburg, University of Nevada, Las Vegas
J. Rohrbough, University of Utah, Salt Lake City

This laboratory/lecture course was intended for researchers at all levels, from beginning graduate students through established primary investigators, who want to use *Drosophila* as an experimental system for nervous system investigation. The 3-week course was divided into the study of development, physiology/function, and behavior. Daily seminars introduced the students to a variety of research topics and developed those topics by including recent experimental contributions and outstanding questions in the field. Guest lecturers brought original preparations for viewing and discussion and direct laboratory exercises and experiments in their area of interest. The course provided students with hands-on experience using a variety of experimental preparations that are used in the investigation of current neurobiological questions. The lectures and laboratories focused on both the development of the nervous system and its role in controlling larval and adult behaviors. In addition to an exposure to the molecular genetic approaches available in *Drosophila*, students learned a variety of techniques, including embryo in situ hybridization, labeling of identified neurons, electrophysiological recording from nerves and muscles, and the analysis of larval and adult behavior. Collectively, the course provided a comprehensive and practical introduction to modern experimental methods for studying the *Drosophila*

nervous system. The specific topics from this year's course included neurogenesis, axon pathfinding, synaptogenesis, membrane excitability, synaptic function and plasticity, photoreception, mechanoreception, neural circuits, biological rhythms, courtship, learning and memory, and locomotor and flight behaviors. Lecturers included Robert Anholt, Vanessa Auld, Kendal Broadie, Steven De Belle, Barry Ganetzky, Ralph Greenspan, Volker Hartenstein, Charlotte Helfrich-Forster, Kei Ito, Maurice Kernan, Rod Murphey, Kevin O'Dell, Diane O'Dowd, William Pak, Andreas Prokop, Dave Shepherd, Jim Skeath, Roland Strauss, Guy Tear, and Tim Tully.

PARTICIPANTS

Ceriani, M., B.S., Ph.D., Scripps Research Institute, La Jolla, California
Griffiths, R., B.Sc., University of Cambridge, United Kingdom
Keller, A., M.S., Universitat Wuerzburg, Germany
Latham, K., B.S., Ph.D., Oregon State University, Corvallis
Mao, T., B.S., Ph.D., Johns Hopkins University, Baltimore, Maryland
Newport, J., B.A., Ph.D., University of California, San Diego
Nino, M., M.D., M.Sc., Baylor College of Medicine, Houston, Texas
Nordberg, E., B.S., University of Arizona, Tucson
Perazzona, B., B.S., Ph.D., Baylor College of Medicine, Houston, Texas
Trotta, N., B.A., Lafayette College, Eastern, Pennsylvania
Verstreken, P., B.S., M.S., Baylor College of Medicine, Houston, Texas
Zlatic, M., B.A., M.S., University of Cambridge, United Kingdom

SEMINARS

Anholt, R., North Carolina State University, Raleigh: Odor recognition and olfactory behavior in *Drosophila melanogaster*.
Auld, V., University of British Columbia, Vancouver, Canada: Role of glia in neurodevelopment.
Broadie, K., University of Utah, Salt Lake City: Synaptic development, function, and plasticity.
De Belle, S., University of Nevada, Las Vegas: Neuroanatomy of memory in *Drosophila*.
Ganetzky, B., University of Wisconsin, Madison: Genetic investigation of ion channels.
Greenspan, R.J., Neuroscience Institute, San Diego, California: Fly sleep. Molecular genetics of natural variation in behavior.
Hartenstein, V., University of California, Los Angeles: Introduction to the embryo and patterning.
Helfrich-Forster, C., Universitat Tubingen, Germany: Circadian rhythms in *Drosophila:* From molecules to neurons.
Ito, K., National Institute for Basic Biology, Aichi, Japan: Neurogenesis of the larva and adult.
Kernan, M., SUNY, Stony Brook, New York: Mechanoreception and hearing in *Drosophila*.
Murphey, R., University of Massachusetts, Amherst: Analysis of *Drosophila* neural circuits.
O'Dell, K.M.C., University of Glasgow, United Kingdom: Courtship and its neurbiological basis in *Drosophila*.
O'Dowd, D., University of California, Irvine: Ion channels and *Drosophila* neuron culture.
Pak, W., Purdue University, West Lafayette, Indiana: Mechanisms of phototransduction.
Prokop, A., Johannes Gutenberg-Universitaet Mainz, Germany: Lineage tracing and the development of identity.
Shepherd, D., University of Southampton, United Kingdom: Axonogenesis in the adult.
Skeath, J., Washington University School of Medicine, St. Louis, Missouri: Early neurogenesis.
Strauss, R., Julius-Maximillians-Universitaet Wurzburg, Germany: Central processing of motor behavior in *Drosophila*.
Tear, G., King's College London School of Medicine, London, United Kingdom: Axon guidance in the embryo/larva.
Tully, T., Cold Spring Harbor Laboratory: Genes, genes, and more genes for memory.

Brain Development and Function

July 6–19

INSTRUCTORS **R. McKay,** National Institutes of Health, Bethesda, Maryland
M. Posner, Cornell Medical College, New York, New York

In this advanced lecture and discussion course on the development and function of the nervous system, 17 participants with diverse geographical and intellectual backgrounds met for 2 weeks with leading neuroscience researchers. The lectures provided both a comprehensive introduction and a detailed presentation of current research. There were many opportunities for interaction. This was an unusual opportunity to review contemporary ideas about the brain with leading scientists. The speakers included Richard Aslin, Steve Burden, Susan Carey, Pietro De Camilli, Howard Eichenbaum, Charles Gilbert, Steve Hillyard, Mary Kennedy, Jeff Lichtman, Roberto Malinow, Bruce McEwen, Ron McKay, Michael Meaney, Raja Parasuraman, Michael Posner, Marcus Raichle, Murray Sherman, David Silbersweig, James Swanson, Kathleen Thomas, Tim Tully, and Mike Young.

The specific topics covered included neurogenetics, stem cell differentiation, cell and gene therapy, axon guidance, synapse formation, synaptic plasticity, hippocampal function, neuroimaging methods, human brain development, and aging. The goal was to provide a critical view of the different levels of knowledge that are required for an integrated understanding of the brain.

PARTICIPANTS

Agerman, K., M.S., Karolinska Institutet, Stockholm, Sweden
Cecchi, C., Ph.D., DIBIT, San Raffaele Institute, Milan, Italy
Hoeppner, D., B.S., Ph.D., Cold Spring Harbor Laboratory
Hsieh, J., B.S., Carnegie Institution of Washington, Baltimore, Maryland
Logvinova, A., B.S., M.D., Buck Center for Research in Aging, Novato, California
Munno, D., B.A., University of Calgary, Alberta, Canada
Pedersen, K., M.Sc., Karolinska Institutet, Stockholm, Sweden
Ramanan, N., B.S., M.S., National University of Singapore, Singapore
Rodriguez, J., B.A., Ph.D., Centre Nat'l de la Recherche Scientifique, Gif-sur-Yvette, France
Ryu, S., B.A., Ph.D., University of California, Berkeley
Saleh, M.-C., B.S., Institut Pasteur, Paris, France
Trus, M., B.A., Ph.D., Hebrew University, Jerusalem, Israel
Velasco, M., B.S., Ph.D., Universidad Nacional Autonoma de Mexico, Mexico City
Verheijen, M., B.S., Ph.D., The Salk Institute, La Jolla, California
Wolf, E., M.D., Ph.D., Karolinska Institutet, Stockholm, Sweden
Yeo, E., B.A., B.S., Institute of Molecular and Cell Biology, Singapore

SEMINARS

Aslin, R., University of Rochester, New York: Development of visually guided behavior.
Burden, S., New York University Medical Center, New York, New York: The neuromuscular junction, the classic synapse.
Carey, S., New York University, New York, New York: The development of the infant mind.
De Camilli, P., Yale University, New Haven, Connecticut: The cell biology of synapses.
Eichenbaum, H., Boston University, Massachusetts: Mid temporal mechanisms in memory and amnesia.
Gilbert, C., The Rockefeller University, New York, New York: Structure of the visual cortex.
Hillyard, S., University of California, San Diego, La Jolla: Electrical and magnetic imaging.
Kennedy, M., California Institute of Technology, Pasadena: Proteon complexes and synaptic function.
Lichtman, J., Washington University, St. Louis, Missouri: Competition is all at the NMJ.
Malinow, R., Cold Spring Harbor Laboratory: Hippocampal synaptic properties.
McEwen, B., The Rockefeller University, New York, New York: Stress mechanisms.
McKay, R., National Institutes of Health, Bethesda, Maryland: Reconstructing the brain.
Meaney, M., McGill University, Montreal, Quebec, Canada: Development of stress mechanisms.
Parasuraman, R., The Catholic University of America, Washington, D.C.: Aging and dementia.
Posner, M., Cornell Medical College, New York, New York: Attentional networks and higher mental processes.
Raichle, M., Washington University School of Medicine, St. Louis, Missouri: Neuroimaging history and current state.
Sherman, M., SUNY, Stony Brook, New York: Interactions between thalamus and cortex in the visual system.
Silbersweig, D., Cornell University, New York, New York: Imaging mechanisms in schizophrenia.
Swanson, J., Cornell Medical College, New York, New York: Attention deficit disorder: Genes and neurosystems.
Thomas, K., Cornell University, New York, New York: Development of implicit memory in humans.
Tully, T., Cold Spring Harbor Laboratory: The genetics of learning in flies and mice.
Young, M., The Rockefeller University, New York, New York: Molecules and rythmns.

Mouse Behavioral Analysis

July 7–20

INSTRUCTORS **M. Fanselow,** University of California, Los Angeles
M. Gallagher, Johns Hopkins University, Baltimore, Maryland
M. Mayford, University of California, San Diego, La Jolla
A. Silva, University of California Medical Center, Los Angeles

ASSISTANTS **S. Anagnostaras,** University of California School of Medicine, Los Angeles
R. Costa, University of California, Los Angeles
R. Bourtchuladze, Columbia University, New York, New York

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. In addition, there were demonstrations of several aspects of in vitro electrophysiology (fields and whole-cell recordings of synaptic plasticity).

PARTICIPANTS

Boerkoel, C., B.A., Ph.D., Baylor College of Medicine, Houston, Texas
Fischbach, K., B.A., Ph.D., University of Texas, San Antonio
Halldner, L., M.D., Karolinska Institutet, Stockholm, Sweden
Israely, I., B.S., Ph.D., University of California, Los Angeles
Liljelund, P., B.Sc., Ph.D., Aston University, Birmingham, United Kingdom
McGlone, J., B.S., Ph.D., Texas Technology University, Lubbock
McHugh, T., B.A., Massachusetts Institute of Technology, Cambridge
Peters, M., M.Sc., Ph.D., University College London, United Kingdom
Shumyatsky, G., M.S., Ph.D., Columbia University College of Physicians & Surgeons, New York, New York
Speca, D., B.A., Ph.D., University of California, Berkeley
Van Herpen, E., M.Sc., Erasmus University, Rotterdam, The Netherlands
Vogt, T., M.S., Ph.D., Princeton University, New Jersey

SEMINARS

Amaral, D., University of California, Davis: Functional neuroanatomy of the hippocampal formation.
Chapman, P., Memorial Sloan-Kettering Cancer Center, New York, New York: Animal models of Alzheimer's.
Eichenbaum, H., Boston University, Massachusetts: Mapping brain function in cognitive neuroscience: From humans to animal models.
Fanselow, M., University of California, Los Angeles: Animal behavior from an ethological and evolutionary view.
Holland, P., Duke University, Durham, North Carolina: Learning processes and behavioral paradigms.
Laroche, S., CNRS-Universite de Paris-Sud, Orsay, France: LTP and learning in the hippocampus and neocortex.
Lione, L., University Forvie Site, Cambridge, United Kingdom: Motor and sensorimotor functions in rodents.
Mainen, Z., Cold Spring Harbor Laboratory: Neural plasticity in mammalian brain.
Mauk, M., University of Texas Medical School, Houston: Cerebellar learning.
Mayford, M., University of California, San Diego: Inducible genetic approaches to learning and memory.
Montague, R., Baylor College of Medicine, Houston, Texas: Computational models of brain function.
Muller, B., SUNY Health Science Center, Brooklyn, New York: Place field recording in the mouse.
Silva, A., University of California Medical Center, Los Angeles: Genetics and the study of cellular neurosystems.
Tonegawa, S., Massachusetts Institute of Technology, Cambridge: Genetics of plasticity.

Molecular Mechanisms of Human Neurological Diseases

July 21–27

INSTRUCTORS **A. Aguzzi,** University Hospital of Zurich, Switzerland
S. Gandy, New York University, Orangeburg, New York
J. Hardy, Mayo Clinic Jacksonville, Florida

How and why do neurons die in specific human neurological disorders? What are the molecular and biochemical manifestations of specific genetic lesions in neurodegenerative disorders? Do different pathological deaths share common mechanisms? What practical treatments are 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 Alzheimer's disease, Parkinson's disease, frontotemporal dementia, amyotrophic lateral sclerosis, prion diseases, and polyglutamine repeat disorders. Taking advantage of small class size and extensive discussion, invited faculty lecturers examined critical issues in their areas of expertise. Overviews were provided and course participants did not have to be familiar with neurological diseases. 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. Participating lecturers were A. Aguzzi, D. Borchelt, D. Cleveland, S. Gandy, J. Ghiso, R. Glockshuber, J. Hardy, D. Holtzman, E. Koo, V. Lee, M. MacDonald, J. Morris, H. Orr, D. Tagle, R. Tanzi, and J. Trojanowski.

PARTICIPANTS

Behrens, A., B.S., Ph.D., Institute of Neuropathology, Zurich, Switzerland
Costa, J., B.S., Ph.D., Instituto de Tecnologia, Oeiras, Portugal
Coulombe, N., B.S., M.S., Merck Forsst & Co., Quebec, Canada
Gao, W., M.D., Ph.D., Veteran Affairs, Beford, Massachusetts
Haberman, R., B.S., Ph.D., University of North Carolina, Chapel Hill
Holm, P., B.S., M.S., Karolinska Institutet, Stockholm, Sweden
Johansson, C., B.A., D.D.S., Karolinska Institutet, Stockholm, Sweden
Jonsson, A., B.Sc., M.Sc., Umea University Hospital, Sweden
Lashuel, H., B.S., Ph.D., The Picower Institute for Medical Research, Manhasset, New York
Lau, L.-F., M.phil., Ph.D., Pfizer, Inc., Groton, Connecticut
Lawson, V., B.S., Ph.D., NIAID/National Institutes of Health, Hamilton, Montana
Liscic, R., B.S., Ph.D., Institut for Medical Research, Zagreb, Croatia
Liu, X.F., B.S., Ph.D., AstraZeneca, Worcester, Massachusetts
Marler, J., A.B., M.D., NINDS/National Institutes of Health, Bethesda, Maryland
Momma, S., M.S., Karolinska Institutet, Stockholm, Sweden
Mullen, C., B.S., Biogen, Inc., Cambridge, Massachusetts
Murphy, D., B.A., Ph.D., NINDS/National Institutes of Health, Rockville, Maryland
Neocleous, V., B.S., Ph.D., Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus
Rosso, S., M.D., University Hospital Rotterdam, Dijkzigt, The Netherlands
Sandhu, J., B.Sc., Ph.D., National Research Council of Canada, Ottawa
Schenone, A., M.D., University of Genova, Italy
Srivatsan Ayyangar, M., B.S., Ph.D., University of Kentucky College of Medicine, Lexington
Stanford, P., B.Sc., Ph.D., Garvan Institute of Medical Research, Sydney, Australia
Tseng, H.-C., B.S., Ph.D., Iowa State University, Ames

SEMINARS

Aguzzi, A., University Hospital of Zurich, Switzerland: Immunopathology of prion disease.
Ahlijanian, M., Pfizer, Inc., Groton, Connecticut: cdk5 in tauopathies.
Bates, G., Guy's, King's and Thomas's School of Medicine, King's College, London, United Kingdom: Trinucleotide repeat diseases.
Eidelberg, D., North Shore University Hospital, Manhasset, New York: Clinical and structural basis of Parkinson's disease.
Friedlander, R., Harvard Medical School, Charlestown, Massachusetts: Programmed cell death in neurodegenerative disease.
Gandy, S., New York University, Orangeburg, New York: Molecular and regulatory bases for Aβ generation.
Ghiso, J., New York University Medical Center, New York, New York: Amyloidosis and familial British dementia.
Hardy, J., Mayo Clinic, Jacksonville, Florida: Overview of neurogenetic basis of neurodegenerative disease.
Holtzman, D., Washington University School of Medicine, St. Louis, Missouri: Role of apoE in Alzheimer's disease.
Hutton, M., Mayo Clinic, Jacksonville, Florida: Genetics and transgenesis of tauopathies.
Lee, V., University of Pennsylvania School of Medicine, Philadelphia: Molecular pathology and biochemistry of tauopathies.
Li-Huei, T., Harvard Medical School/HHMI, Boston, Massachusetts: Role of CDK5 in neurodegeneration.
Lingappa, V., University of California, San Francisco: Cell and structural biology of prion disease.
Morris, J., Washington University, St. Louis, Missouri: Clinicopathological correlations in cognitive impairment and Alzheimer's disease.
Price, D., Johns Hopkins University, Baltimore, Maryland: Overview of molecular neuropathology of neurodegeneration.
Selkoe, D., Harvard Medical School, Boston, Massachusetts: Genotype-phenotype relationships in Alzheimer's disease; the biology of the presenilins/gamma secretases.
Trojanowski, J., University of Pennsylvania School of Medicine, Philadelphia: Synucleinopathies.
Vassar, R., Amgen, Inc., Thousand Oaks, California: BACE and b-secretase processing of APP: Therapeutic implications for Alzheimer's disease.

C. elegans

July 25–August 14

INSTRUCTORS
M. Hengartner, Cold Spring Harbor Laboratory
E. Jorgensen, University of Utah, Salt Lake City
R. Korswagen, University Hospital Utrecht, The Netherlands
R. Plasterk, Netherlands Cancer Institute, Amsterdam, The Netherlands

ASSISTANTS
M. Hammarlund, University of Utah, Salt Lake City
S. Milstein, Cold Spring Harbor Laboratory
S. Tharin, Cold Spring Harbor Laboratory

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 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

Boulton, S., B.Sc., Ph.D., Massachusetts General Hospital Cancer Center, Charlestown
Drescher, R., M.S., Aventis Research & Technologies, Frankfurt, Germany
Fingerle-Rowson, G., M.D., The Picower Institute for Medical Research, Manhasset, New York
Haklai Topper, L., B.Sc., M.Sc., The Weizmann Institute of Science, Rehovot, Israel
Hudson, M., B.Sc., Ph.D., Oregon Health Sciences University, Portland
Kinnunen, T., M.Sc., Ph.D., University of Birmingham, United Kingdom
Martinez-Torres, A., B.S., M.S., University of California, Irvine
McNew, J., B.S., Ph.D., Memorial Sloan-Kettering Cancer Center, New York, New York
Palmitessa, A., B.S., Ph.D., Thomas Jefferson University, Philadelphia, Pennsylvania
Pasierbek, P., B.S., M.S., University of Vienna, Austria
Rolls, M., B.S., Harvard Medical School, Boston, Massachusetts
Scott, J., B.S., Ph.D., University of Hawaii, Hilo
Siomos, M., B.Sc., Ph.D., Institute of Molecular Pathology, Vienna, Austria
Taschner, P., M.Sc., Ph.D., Leiden University, The Netherlands
Van Berkel, W., B.Sc., M.Sc., Utrecht University, The Netherlands
Walhout, A., Ph.D., Dana Farber Cancer Institute, Boston, Massachusetts

SEMINARS

Ahringer, J., University of Cambridge, United Kingdom: Embryonic development; RNAi.
Avery, L., University of Texas Southwestern Medical Center, Dallas: Nematode excitable cell function.
Barstead, R., Washington University School of Medicine, St. Louis, Missouri: Muscle biology; gene knockouts.
Chisholm, A., University of California, Santa Cruz: Genetics of *C. elegans* morphogenesis.
Emmons, S., Albert Einstein College of Medicine, Bronx, New York: The male.
Fitch, D., Wayne State University School of Medicine, Detroit, Michigan: Evolution of nematodes.
Hengartner, M., Cold Spring Harbor Laboratory: Programmed cell death; suppression genetics.
Hodgkin, J., University of Cambridge, United Kingdom: Sex determination and bacterial infection in *C. elegans*.
Jorgensen, E., University of Utah, Salt Lake City: Synaptic transmission forward genetics.
Korswagen, R., University Hospital Utrecht, The Netherlands: Wnt pathways.
Plasterk, R., Netherlands Cancer Institute, Amsterdam, The Netherlands: *mut-7* and regulation of transposition of transposons.
Stein, L., Cold Spring Harbor Laboratory: The *C. elegans* genome ACeDB.
Sternberg, P., California Institute of Technology, Pasadena: Development of the vulva.
Thomas, J., University of Washington, Seattle: Defecation and Cam kinase.

Eukaryotic Gene Expression

July 25–August 14

INSTRUCTORS
B. Dynlacht, Harvard University, Cambridge, Massachusetts
G. Gill, Harvard Medical School, Boston, Massachusetts
J. Goodrich, University of Colorado, Boulder
J. Lees, Massachusetts Institute of Technology, Cambridge

ASSISTANTS
T. Bolger, Harvard Medical School, Boston, Massachusetts
H. Ferguson, University of Colorado, Boulder
J. Rayman, Harvard University, Cambridge, Massachusetts
J. Trimarchi, Massachusetts Institute of Technology, Cambridge

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 foot-

printing assays. These assays were used to study protein-DNA interactions in crude extracts and using recombinant proteins. Both coimmunoprecipitation and yeast two-hybrid methods were employed to investigate protein-protein interactions with the general transcription machinery. During 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. The speakers included David Allis, Laura Attardi, Keith Blackwell, Steve Buratowski, Steve Burley, Ken Burtis, Mike Carey, Brian Dynlacht, Grace Gill, Jim Goodrich, Caroline Hill, Kathy Jones, Leemor Joshua-Tor, Jackie Lees, Bob Roeder, Dimitris Thanos, Marc Timmers, and Jerry Workman.

PARTICIPANTS

Asp, P., B.S., M.S., Massachusetts Institute of Technology, Cambridge
Borrego, F., B.S., Ph.D., NIAID/National Institutes of Health, Rockville, Maryland
Dangond, F., B.A., M.D., Brigham and Women's Hospital, Boston, Massachusetts
Diehl, S., B.S., University of Vermont, Burlington
Fitzpatrick, G., B.S., Roswell Park Cancer Institute, Buffalo, New York
Giagtzoglou, N., B.S., M.S., University of Crete, Heraklion, Greece
Hansson, A., B.S., M.S., Lund University, Malmo, Sweden
Haronen, R., B.S., M.S., University of Helsinki, Finland
Jones, G., B.S., Ph.D., University of Kentucky, Lexington
Kim, D., B.S., Ph.D., Chicago Medical School, North Chicago, Illinois
Knight, J., B.A., Oxford University, United Kingdom
Maestro, R., B.S., Ph.D., Centro di Riferimento Oncologico, Aviano, Italy
Millevoi, S., B.S., Ph.D., Centre National de la Recherche Scientifique, Toulouse, France
Petersen, R., B.S., M.S., University of Illinois, Urbana-Champaign
Srivastava, M., B.Sc., Ph.D., NICHD/National Institutes of Health, Bethesda, Maryland
Yang, T., M.S., Ph.D., Ohio State University, Columbus

SEMINARS

Allis, D., University of Virginia, Charlottesville: Linking histone modifications to gene activity and more.
Attardi, L., Massachusetts Institute of Technology, Cambridge: Identification of apoptosis-specific p53 target genes.
Blackwell, K., Harvard Medical School, Boston, Massachusetts: Mechanisms of developmental gene regulation in *C. elegans*.
Buratowski, S., Harvard Medical School, Boston, Massachusetts: Connecting transcription to chromatin and mRNA processing.
Burley, S., The Rockefeller University, New York, New York: Structural biology of eukaryotic transcriptional regulation.
Carey, M., University of California School of Medicine, Los Angeles: Enhanceosome assembly and function.
Dynlacht, B., Harvard University, Cambridge, Massachusetts: Transcriptional regulation during the mammalian cell cycle.
Gill, G., Harvard Medical School, Boston, Massachusetts: Transcriptional regulation in the nervous system.
Goodrich, J., University of Colorado, Boulder: Mechanisms of human RNA polymerase II transcription.
Hill, C., Imperial Cancer Research Fund, London, United Kingdom: TGF-β signaling and regulation of transcription.
Jones, K., The Salk Institute for Biological Studies, La Jolla, California: Regulation of HIV transcription.
Joshua-Tor, L., Cold Spring Harbor Laboratory: Transcription factors in 3D.
Lees, J., Massachusetts Institute of Technology, Cambridge: The in vivo of the E2F transcription factors.
Powers, T., University of California, Davis: Exploring gene expression during nitrogen utilization in yeast.
Roeder, R., The Rockefeller University, New York, New York: Role of general and gene-specific coactivators in transcriptional regulation.
Thanos, D., Columbia University, New York, New York: Activation and attenuation of transcription by the IFN-β enhanceosome.
Timmers, M., Utrecht University, The Netherlands: Basal transcription by RNA polymerase II.
Workman, J., Pennsylvania State University, University Park: Transcription regulation by SWI/SNF and HAT complexes.

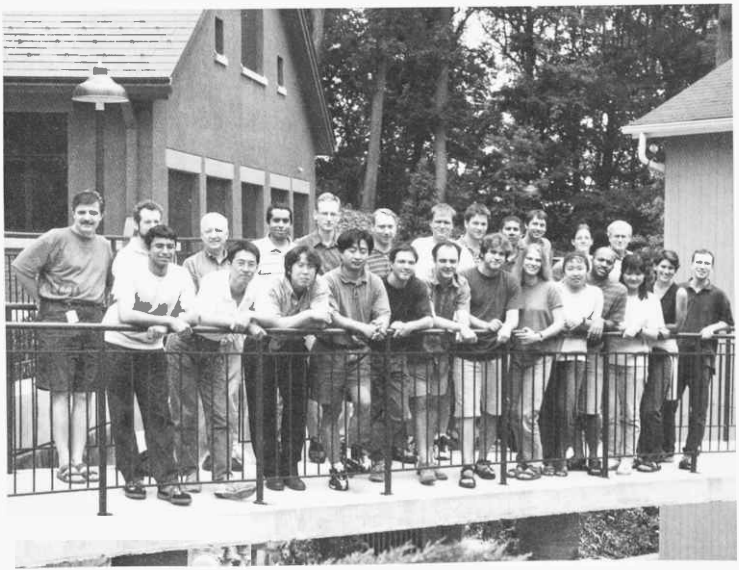
Imaging Structure and Function in the Nervous System

July 25–August 14

INSTRUCTORS **K. Delaney,** Simon Fraser University, Burnaby, Canada
V. Murthy, Harvard University, Cambridge, Massachusetts
K. Svoboda, Cold Spring Harbor Laboratory

ASSISTANTS **J. Dantzker,** The Salk Institute, La Jolla, California
B. Hall, Simon Fraser University, Burnaby, Canada
Z. Li, Harvard University, Cambridge, Massachusetts
M. Maravall, Cold Spring Harbor Laboratory

Advances in light microscopy and digital image processing and the development of a variety of powerful fluorescent probes present expanding opportunities for investigating the nervous system, from synaptic spines to networks in the brain. This intensive laboratory and lecture course provided participants with the theoretical and practical knowledge to utilize emerging imaging technologies. The primary emphasis of the course was on vital light microscopy. Students learned the principles of light microscopy, as well as use of different types of electronic cameras, laser-scanning systems, functional fluorophores, delivery techniques, and digital image-processing software. In addition to transmitted light microscopy for viewing cellular motility, the course examined a variety of molecular probes of cell function, including calcium-sensitive dyes, voltage-sensitive dyes, photo-activated ("caged") com-



pounds, and exocytosis tracers. Issues arising in the combination of imaging with electrophysiological methods were covered. Particular weight was given to multiphoton laser scanning microscopy and to newly available biological fluorophores, especially green fluorescent protein (GFP) and its variants. A spectrum of neural and cell biological systems was used, including living animals, brain slices, and cultured cells. Applicants had a strong background in the neurosciences and in cell biology. Lecturers included W. Almers, C. Aoki, William Betz, Holly Cline, Jami Dantzker, Richard Day, Kerry Delaney, Winfried Denk, Joseph Fetcho, Paul Forscher, J. Fujimoto, Amiram Grinvald, Ernst Keller, Delaney Kerry, Fred Lanni, Lew Loew, Tobias Meyer, B. Moomaw, Venkatesh Murthy, M. Nonet, Tim Ryan, Bernado Sabatini, Karel Svoboda, Roger Tsien, Samuel Wang, and Shimon Weiss.

PARTICIPANTS

Castonguay, A., B.S., Ph.D., Université de Montréal, Quebec, Canada
Das, T., B.A., University of Cambridge, United Kingdom
Golding, N., B.S., Ph.D., Northwestern University, Evanston, Illinois
Lezcano, N., M.A., Ph.D., Medical College of Georgia, Augusta
Louissaint, Jr., A., B.A., M.A., Weill Medical College of Cornell University, New York, New York
Prescott, S., B.Sc., M.D., McGill University, Montreal, Quebec, Canada
Ratte, S., B.Sc., Ph.D., University of Birmingham, United Kingdom
Scheiffele, P., Ph.D., University of California, Berkeley
Snyder, E., B.A., Ph.D., Brown University, Providence, Rhode Island
Tao, H., B.S., M.S., Ohio University, Athens
Wang, C.-T., B.S., M.S., University of Wisconsin, Madison
Zelles, T., M.D., Ph.D., Institute of Experimental Medicine, Hungarian Academy of Scicience, Budapest, Hungary

SEMINARS

Almers, W., Vollum Institute, Portland, Oregon: Vesicle imaging, TIRF.
Aoki, C., New York University, New York, New York: Electron microscopy. Applications of electron microscopy.
Betz, W., University of Colorado, Denver: FM-143 imaging.
Cline, H., Cold Spring Harbor Laboratory: In vivo imaging of development in *Xenopus*.
Dantzker, J., Salk Institute, La Jolla, California: Uncaging to study neural circuits.
Day, R., University of Virginia, Charlottesville: Basics of GFP. FRET microscopy.
Delaney, K., Simon Fraser University, Burnaby, Canada: Ca^{2+} imaging applications.
Denk, W., Max-Planck Institute for Medical Research, Heidelberg, Germany: Two-photon 1. Two-photon 2. Two-photon applications.
Fetcho, J., SUNY, Stony Brook, New York: In vivo imaging in zebrafish.
Forscher, P., Yale University, New Haven, Connecticut: Phase and DIC microscopy. Video DIC and time-lapse.
Fujimoto, J., Massachusetts Institute of Technology, Cambridge: Optical coherence tomography. Applications of optical coherence tomography.
Grinvald, A., Weizmann Institute of Science, Rehovot, Israel: Intrinsic signal and voltage sensitive dyes in vivo.
Keller, E., Carl Zeiss, Inc., Thornwood, New York: Basics of microscopy; image formation. Basics on microscopy.
Kerry, D., Simon Fraser University, Burnaby, Canada: Ca^{2+} imaging.
Lanni, F., Carnegie-Mellon University, Pittsburgh, Pennsylvania: Fluorescence. Fluorescence microscopy.
Loew, L., University of Connecticut Health Center, Farmington: Voltage sensitive dyes. Voltage-sensitive dyes and harmonic imaging.
Meyer, T., Duke University Medical Center, Durham, North Carolina: Imaging intracellular signaling using GFP.
Moomaw, B., Hamamatsu Photonic Systems, Bridgewater, New Jersey: CCD cameras.
Murthy, V., Harvard University, Cambridge, Massachusetts: Imaging secretion.
Nonet, M., Washington University, St. Louis, Missouri: Imaging synaptogenesis in *C. elegans* using GFP.
Ryan, T., Cornell University Medical College, New York, New York: Vesicle imaging, Phluorins.
Sabatini, B., Cold Spring Harbor Laboratory: Quantitative two-photon imaging in small compartments.
Svoboda, K., Cold Spring Harbor Laboratory: Basic optics. In vivo imaging in mammalian neocortex.
Tsien, R., University of California, San Diego, La Jolla: Functional probes 1. Functional probes 2. Functional imaging.
Wang, S., Princeton University, New Jersey: Uncaging. Cages.
Weiss, S., Lawrence Berkeley National Laboratory, Berkeley, California: Q-dots; single molecules; near-field.

Yeast Genetics

July 25–August 14

INSTRUCTORS **D. Burke,** University of Virginia, Charlottesville
O. Cohen-Fix, National Institutes of Health, Bethesda, Maryland
D. Dawson, Tufts University, Boston, Massachusetts
T. Stearns, Stanford University, California

ASSISTANTS **Z. Hiloti,** National Institutes of Health, Bethesda, Maryland
R. Kamieniecki, Tufts University, Boston, Massachusetts
M. McCleland, University of Virginia, Charlottesville

This was a modern, state-of-the-art laboratory course designed to teach the students the full repertoire of genetic approaches needed to dissect complex problems in the yeast *Saccharomyces cerevisiae*. Combinations of classical genetic approaches were emphasized, including the isolation and characterization of mutants, tetrad analysis, complementation, and mitotic recombination. Molecular genetic techniques were emphasized, including various types of yeast transformations, gene replacement with plasmids and PCR, construction and analysis of gene fusions, and generation of mutations in cloned genes. Students used classical and molecular approaches to gain experience in identifying and interpreting various kinds of genetic interactions including suppression and synthetic lethality. Students were immersed in yeast genomics and performed and interpreted experiments with DNA arrays. Students gained first-hand experience in modern cytological approaches such as epitope tagging and imaging yeast cells using indirect immunofluorescence, GFP-protein fusions, and a variety of fluores-

cent 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. Participating speakers included J. Konopka, A. Amon, A. Hopper, B. Futcher, M. Lichten, T. Weinert, D. Roof, P. Meluh, D. Pellman, G. Fink, M. Snyder, V. Lundbald, J. Boeke, and M. Winey.

PARTICIPANTS

Aguilaniu, H., M.S., Ph.D., Chalmers University, Gothenburg, Sweden
Boutonnet, C., B.S., Ph.D., INSERM, Toulouse, France
Castelli, L., B.A., Commonwealth and Scientific Industrial Research Organization, Parkville, Australia
Chang, W.-H., M.S., Ph.D., Stanford University School of Medicine, California
Debburman, S., B.A., Ph.D., Kalamazoo College, Michigan
Fehrenbacher, K., B.S., Columbia University, New York, New York
Goddard, M., B.Sc., Ph.D., University of London, Ascot, United Kingdom
Gunjan, A., B.S., Ph.D., Imperial Cancer Research Fund, Herts, United Kingdom
Hoss, M., Ph.D., Institut Suisse de Recherche Experimental sur la Cancer, Epalinges, Switzerland
Jiang, M., B.S., Ph.D., University of California, Los Angeles
Lewis, M., B.A., Ph.D., University of Texas, Austin
Lin, W., D.E.A., Institut Curie, Paris, France
Luke, B., B.Sc., M.S., Queen's University, Kingston, Canada
McCarty, G., B.S., University of Maryland, Baltimore
Moreira dos Santos, M.M., B.S., M.S., Technical University of Denmark, Denmark
Tyler, J., B.Sc., Ph.D., University of California, San Diego, La Jolla

SEMINARS

Amon, A., Massachusetts Institute of Technology, Cambridge: Exit from mitosis in yeast.
Biggins, S., Fred Hutchinson Cancer Research Institute, Seattle, Washington: Ipl1 and chromosome segregation.
Cyert, M., Stanford University, California: Calcium regulation in yeast.
Fox, T., Cornell University, Ithaca, New York: Mitochondrial genetics and biogenesis.
Hollingsworth, N., SUNY, Stony Brook, New York: Protein-protein interactions essential for meiosis in yeast.
Kamakaka, R., National Institute of Child Health and Human Development, Bethesda, Maryland: Sir1 and transcriptional silencing at HMR.
Klar, A., National Cancer Institute, Frederick, Maryland: Mating-type switching in yeast.
Lew, D., Duke University, Durham, North Carolina: The morphogenesis checkpoint.
Pringle, J., University of North Carolina, Chapel Hill: On selecting an axis of polarization: Bud-site section in *S. cerevisiae*.
Sclafani, R., University of Colorado Health Sciences, Denver: Using yeast genetics to study DNA replication.
Shaw, J., University of Utah, Salt Lake City: Fission and fusion among yeast mitochondria.
Smith, M., University of Virginia, Charlottesville: Novel histones, novel functions.

Yeast Genetics Course Millennium Reunion

August 11–13

ARRANGED BY **Dan Burke,** University of Virginia, Charlottesville
David Stewart, Cold Spring Harbor Laboratory

The Cold Spring Harbor Yeast Genetics course began in 1970 and was taught for a total of 17 years by Gerald Fink and Fred Sherman. Successive cadres of instructors have taken up the challenge to teach the yeast course every summer since then. To celebrate 30 years of yeast genetics, a millennium celebration was held on the last weekend of the 2000 yeast genetics course. Invitations were issued to former instructors and past students still active in the field of yeast genetics and biology. The weekend featured a fascinating combination of scientific stories past and present, with ample time for reminiscences of every kind, and provided an opportunity for old friends and colleagues to catch up (and run in the famous Plate Race together). It also afforded the students in the 2000 course a chance to meet many of the famous alumni from the course who have contributed so much over the years to the field of yeast genetics and biology. Reunion participants included:

Alison Adams
David Botstein
James Broach
Judith Campbell
Scott Clark
Nancy Du
Nicholas Edgington
Francisco Ferrezuelo
Gerald Fink
Rosalie Fink
Brigitte Gallwitz
Dieter Gallwitz
Ira Herskowitz
Jim Hicks
Philip Hieter
Jun Horiuchi
Alexander Johnson
Chris Kaiser
Amar Klar
Christopher Lawrence
Susan Michaelis
Thomas Petes
Peter Philippsen
Mark Rose
Elena Rustchenko
Randy Schekman
Arndt Schmitz
Fred Sherman
Pamela Silver
Gerry Smith
George Sprague
Jeremy Thorner
Reed Wickner

The reunion, held at Bush Auditorium, featured the following program:

Friday August 11

8:00 pm *Opening Remarks:* James Watson
Introduction: Terri Grodzicker
Fred Sherman, Gerry Fink

Saturday August 12

9:00 am *Session Chair:* Tim Stearns
David Botstein, Ira Herskowitz,
Thomas Petes

11.00 am *Session Chair:* Orna Cohen-Fix
Jeremy Thorner, Peter Phillipsen

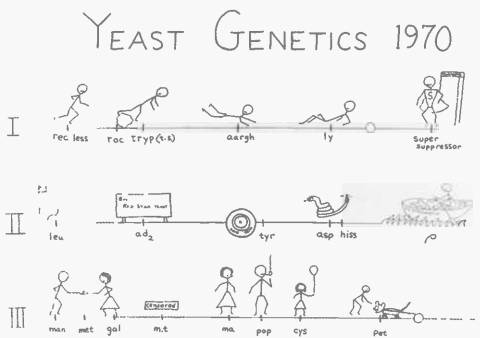
1:30 pm *Session Chair:* Dean Dawson
Amar Klar, Pam Silver, Chris Kaiser

3:30 pm *Session Chair:* Dan Burke
Phil Hieter, Mark Rose

4.30 pm *Closing Remarks:* Bruce Stillman

5:00 pm Plate Race

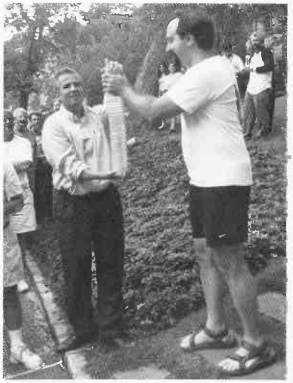
6:00 pm Cocktails & Banquet



J. Hicks, J.D. Watson, A. Kiar, J. Strathern

G. Fink, T. Grodzicker

S. Michaelis, I. Herskowitz, P. Silver



"The veteran's get ready"
C. Kaiser, T. Stearns

Start of the race (veteran's team led by Mark Rose on the right)

Advanced *Drosophila* Genetics

July 31–August 13

INSTRUCTORS **M. Ashburner,** EMBL-EBI, Cambridge, United Kingdom
S. Hawley, University of California, Davis

This intensive seminar course provided an introduction to the theory and practice of methods used to manipulate the *Drosophila* genome. It was suitable for graduate students and researchers, with some experience with *Drosophila*, who were interested in expanding their knowledge of the wide range of genetic techniques now available for use with this organism. Topics included chromosome mechanics, the design and execution of genetic screens, and the use of transposable elements as genetic tools. This year was the year of the fly genome, and its impact on genetic analysis was a particular focus of the course.

PARTICIPANTS

Barcelo, H., B.S., Ph.D., North Dakota State University, Fargo
Champion, M., B.S., Ph.D., University of California, Davis
Gallio, M., M.S., Karolinska Institutet, Huddinge, Sweden
Gilbert, M., B.S., Ph.D., SUNY, Stony Brook, New York
Haywood, A., B.Sc., M.Sc., Memorial University of New Foundland, New Zealand
Hendricks, J., B.S., Ph.D., University of Pennsylvania, Philadelphia
Jones, C., B.S., Emory University, Atlanta, Georgia
Lau, G., B.S., Ph.D., Massachusetts General Hospital, Boston
Paddy, M., B.S., Ph.D., University of California, Davis
Rattner, B., B.S., Ph.D., Tufts University, Medford, Massachusetts
Ribeiro, C., dipl. Phil. II, Biozentrum der Universitaet Basel, Switzerland

Serpe, M., M.S., Ph.D., University of Minnesota, Minneapolis
Shilkova, O., B.S., M.S., St. Petersburg State University, Russia
Singh, S., M.Sc., Ph.D., SUNY, Buffalo, New York
Ting, C.-T., B.S., Ph.D., University of Chicago, Illinois
Walker, D., B.A., Ph.D., University of Utah, Salt Lake City
Weber, F., B.S., Ph.D., The Scripps Research Institute, San Diego, California
Wolf-Watz, H., M.S., Ph.D., University of Stockholm, Sweden
Yildiz, O., B.S., University of North Carolina, Chapel Hill

SEMINARS

Ashburner, M., EMBL-EBI, Cambridge, United Kingdom: Introduction to *Drosophila* biology, phylogeny. FlyBase: Where to find information. Chromosomes, cytogenetics, chromosome aberrations, mapping (genetic and cytogenetic).
Ashburner, M., EMBL-EBI, Cambridge, United Kingdom, and S.R. Hawley, University of California, Davis: Summing up.
Burtis, K., University of California, Davis: DNA microarrays in *Drosophila*.
Cline, T., University of California, Berkeley: Alternative to brute force: Selective and sensitized genetic screens and their use in the genetic dissection of *Drosophila* sex determination.
Golic, K., University of Utah, Seattle, Washington: Mosaic systems; FRT/FLP.
Hawley, S.R., University of California, Davis: Genetics of meiosis; methods of study and exploitation.
Heberlein, U., University of California, San Francisco: Neurogenetics and behavior.
Karpen, G., The Salk Institute, La Jolla, California: Heterochromatin, centromeres, and telomeres.
Lehmann, R., Skirball Institute, HHMI, New York, New York: Genetic screens for mutations affecting embryogenesis (including maternal effect mutations): Methods for their analysis.
Rørth, P., Carnegie Institute of Washington, Baltimore, Maryland: P-element technologies/gain-of-function genetics.
Rubin, G., HHMI/University of California, Berkeley: The fly genome.
Sullivan, B., University of California, San Francisco: Cell biology.

Bioinformatics: Writing Software for Genome Research

October 11–24

INSTRUCTORS **S. Rozen,** Whitehead Institute for Biomedical Research, Cambridge, Massachusetts
L. Stein, Cold Spring Harbor Laboratory

ASSISTANTS **R. Halgren,** Michigan State University, East Lansing
J. Kissinger, University of Pennsylvania, Philadelphia
C. Mungall, Berkeley *Drosophila* Genome Project, Berkeley, California

The desktop computer is rapidly becoming an indispensable tool in the biologist's tool chest. The success of the Human Genome Project has 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 2-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 posed problems using their own data and worked with each other and the faculty to solve them.

PARTICIPANTS

Buck, G., B.S., Ph.D., Virginia Commonwealth University, Richmond
Burnside, J., B.S., Ph.D., University of Delaware, Newark
Ebersberger, I., B.S., Max-Planck Institute for Evolutionary Anthropology, Leipzig, Germany
Eichler, E., B.S., Ph.D., Case Western Reserve University, Cleveland, Ohio
Farrer, M., B.S., Ph.D., Mayo Clinic, Jacksonville, Florida
Ferrezuelo, F., B.S., Ph.D., Cold Spring Harbor Laboratory
Gerton, J., B.A., Ph.D., University of California, San Francisco
Gunsalus, K., B.A., Ph.D., Cornell University, Ithaca, New York
Haqq, C., B.S., Ph.D., University of California, San Francisco
Hazard, S., B.S., M.S., Ph.D., Medical University of South Carolina, Charleston
Li, M., B.S., Ph.D., Bristol Myers Squibb, Princeton, New Jersey
Martell, R., B.A., M.B.A., National Institutes of Health, Bethesda, Maryland
Mayer, L., B.S., Ph.D., Centers for Disease Control, Atlanta, Georgia
McPherson, J., B.Sc., Ph.D., Washington University, St. Louis, Missouri
Nickerson, E., B.S., Ph.D., Baylor College of Medicine, Houston, Texas
Reboul, J., M.S., Ph.D., Dana Farber Cancer Institute, Boston, Massachusetts
Rowen, L., B.A., Ph.D., Institute for Systems Biology, Seattle, Washington
Rutherford, R., B.A., Ph.D., University of California/HHMI, San Francisco
Scherer, S., B.A., Ph.D., Baylor College of Medicine, Houston, Texas
Shoulders, C., B.A., D.Phil., MRC, Imperial College School of Medicine, London, United Kingdom
True, H., B.S., Ph.D., University of Chicago, Illinois
Ware, D., B.S., Ohio State University ABRC, Columbus
West, J., B.S., Cold Spring Harbor Laboratory
Whetzel, T., B.S., Ph.D., University of Pennsylvania School of Medicine, Philadelphia
Yang, U.-C., B.S., Ph.D., National Yang-Ming University, Taiwan

SEMINARS

Bader, J., CuraGen Corporation, New Haven, Connecticut: Analysis of expression data.
Birney, E., EMBL-EBI, Hinxton, United Kingdom: Writing perl modules. The ensemble genome database.
Burge, C., Massachusetts Institute of Technology, Cambridge: Computational gene finding.
Chervitz, S., Neomorphics, Inc., Berkeley, California: Writing perl modules. The ensemble genome databases.
Hughey, R., University of California, Santa Cruz: HMMs.
Marth, G., National Center for Biotechnology Information, Bethesda, Maryland: Sequence analysis pipeline: Lashing everything together with perl.
Pearson, B., University of Virginia, Charlottesville: Sequence similarity.
Peitzsch, R., Pfizer, Inc., Groton, Connecticut: SQL. SQL/Databases.
Rebhan, M., AstraZeneca, Inc., Cambridge, Massachusetts: Web usability.
Slonim, D., Whitehead Institute, Cambridge, Massachusetts: Analysis of expression data.
Young, P., SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania: Gene expression databases.

Gene Isolation: Advanced Methods in Positional Cloning

October 11–24

INSTRUCTORS **C. Amemiya,** Boston University School of Medicine, Massachusetts
H. Jacob, Medical College of Wisconsin, Milwaukee
G. Silverman, Children's Hospital/Harvard Medical School, Boston, Massachusetts
F. Spencer, Johns Hopkins University School of Medicine, Baltimore, Maryland

ASSISTANTS **D. Askew,** Harvard Medical School, Boston, Massachusetts
L. Roden, University of Warwick, Coventry, United Kingdom
S. Twigger, Medical College of Wisconsin, Milwaukee

Classical positional cloning strategies have relied on the use of genetic mapping studies to localize the gene and physical mapping reagents to clone the candidates. The completion of major sequencing efforts promised to alter this paradigm by providing the identity and syntenic relationships of all the genes. This laboratory-based course was designed to assist in the physical isolation of novel genes, including well-mapped disease-related genes and QTLs, using all available assets including physical mapping tools and sequenced genomes. Principal procedures included physical mapping by contig construction using YACs and BACs, STS-content mapping, DNA fingerprinting, pulsed-field gel electrophoresis, and end-rescue. Methods for converting YAC contigs to sequence-ready BAC contigs were also illustrated. Procedures using the yeast as a host organism for YAC analysis and manipulation were covered. These techniques included YAC transfer between strains to facilitate purification of pure clone DNA, and fragmentation and retrofitting strategies for modifying clones prior to their introduction into cell lines or ES cells. Modification of BACs by recombination cloning methodologies were also emphasized. Methods used for the cloning of genes from large cloned DNA segments such as

cDNA selection or exon trapping were compared to gene-identification computer algorithms used for searching genomic DNA sequence databases. Bioinformatics sessions emphasizing end-user applications, comparative genomic analysis, and database mining were incorporated into multiple problem solving sessions. Recombinational cloning of disease-related loci using BACs and YACs were used as a means to circumvent conventional library construction and cloning techniques. Studies in mutational analysis were also included. The laboratory-based component was supplemented by lectures from prominent investigators who had pioneered the development and application of techniques utilized in the course. All participants presented a synopsis of their work. These brief presentations served as a means to discuss positional cloning strategies in the context of real research projects. Due to conservation of genomic sequence across a diversity of species, investigators engaged in projects outside mainstream sequencing projects were also encouraged to apply.

PARTICIPANTS

Conway, G., B.S., Ph.D., NASA Ames Research Center, Moffett Field, California
Culp, D., B.S., Ph.D., University of Rochester, New York
Doucet, J., B.S., Ph.D., Nichols State University, Thibodaux, California
D'Souza, J., M.Sc., Ph.D., IMA, National University of Singapore, Singapore
Geiger, H., B.S., Ph.D., University of Kentucky, Lexington
Hasham, S., M.S., B.S., University of Texas, Houston
Johannesson, M., B.S., M.S., Lund University, Sweden
Knapik, E., M.D., GSF-National Research Center, Munich, Germany
Li, W.-D., M.D., Ph.D., University of Pennsylvania, Philadelphia
Magnusson, V., B.S., Ph.D., Uppsala University, Sweden
Pereira, L.F., B.S., Ph.D., Londrina, Brazil
Riba, L., B.S., M.S., Natinonal University of Mexico, Mexico
Silahtaroglu, A., M.Sc., Ph.D., University of Copenhagen, Denmark
Vanavichit, A., M.S., Ph.D., Kasetsart University, Nakorn Pathom, Thailand
Von Tell, D., M.S., Ph.D., Karolinska Institutet, Stockholm, Sweden

SEMINARS

Amemiya, C., Boston University, Massachusetts: Applications of large-insert cloning for studies on the evolution and development of the vertebrate immune system and of the HOX gene system.
Baxevanis, A., National Institutes of Health, Bethesda, Maryland: Bioinformatics II: Gene prediction. Bioinformatics III: Protein prediction.
Birren, B., Whitehead Center for Genome Research, MIT, Cambridge, Massachusetts: From genome to clones and BAC again.
Dewar, K., Whitehead Center for Genome Research, MIT, Cambridge, Massachusetts: Comparative genomic sequencing.
Green, E., National Institutes of Health, Bethesda, Maryland: Mapping and sequencing mammalian chromosomes: How and why?
Hengartner, M., Cold Spring Harbor Laboratory: Positional cloning in *C. elegans*.
Jacobs, H., Medical College of Wisconsin, Milwaukee: From QTL to gene using comparative genomics, bioinformatics, and SNPS I. From QTL to gene using comparative genomics, bioinformatics, and SNPS II.
Kwiatkowski, D., Harvard Medical School, Brigham and Women's Hospital, Boston, Massachusetts: Methods for mutations/SNP detections: HD Gels, SSCP, DGGE, DHPLC.
Matise, T., Rutgers University, Piscataway, New Jersey: Bioinformatics I: Mapping.
Meltzer, P., National Institutes of Health, Bethesda, Maryland: Gene expression profiling with cDNA microarrays.
Reeves, R., Johns Hopkins University School of Medicine, Baltimore, Maryland: Understanding Down syndrome as a (VERY) complex genetic disorder.
Roden, L., University of Warwick, Coventry, United Kingdom: Circadian rhythms: Photoperiodism and the search for "clock" genes in *Arabidopsis*.
Roe, B., University of Oklahoma, Norman: The Human Genome Project: So many bases, so little time—A view from the trenches.
Rubin, E., University of California, Berkeley: Sifting sequence for function: Exploiting the mouse.
Satish, P., Johnson & Johnson, Skillman, New Jersey: cDNA selection I. cDNA selection II.
Silverman, G., Harvard Medical School, Children's Hospital, Boston, Massachusetts: Delineation of the serpin superfamily by comparative genomics.
Spencer, F., Johns Hopkins University School of Medicine, Baltimore, Maryland: Chromosome segregation in yeast.
Stewart, F., EMBL, Heidelberg, Germany: ET cloning.
Trask, B., Fred Hutchinson Cancer Research Center, Seattle, Washington: Fluorescence in situ hybridization (FISH) and genome analysis.
Twigger, S., Medical College of Wisconsin, Milwaukee: Bioinformatics: It's not just for geeks. Practical ways to start using bioinformatics in the lab.
Williams, S., Smith College, Southampton, Massachusetts: Filarial genome project.

Macromolecular Crystallography

October 11–24

INSTRUCTORS **W. Furey,** V.A. Medical Center, Pittsburgh, Pennsylvania
G. Gilliland, National Institute of Standards & Technology, Rockville, Maryland
A. McPherson, University of California, Irvine
J. Pflugrath, Molecular Structure Corporation, The Woodlands, Texas

ASSISTANT **S. Chu,** National Institute of Standards & Technology, Rockville, Maryland

Crystallography and X-ray diffraction yielded a wealth of structural information unobtainable through other methods. This intensived laboratory/computational course focused on the major techniques used to determine the three-dimensional structures of macromolecules. It was designed for scientists with a working knowledge of protein structure and function, but who are new to macromolecular crystallography. Topics covered included 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 dispersion phase determination, solvent flattening, molecular replacement and averaging, electron density interpretation, structure refinement, 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.

Participating lecturers were Paul Adams, William Furey, Gary Gilliland, Wayne Hendrickson, Andrew Howard, Li-Wei Hung, Morten Kjeldgaard, Alexander McPherson, Jorge Navaza, Anastassis Perrakis, James Pflugrath, David Richardson, Jane Richardson, Robert Sweet, and Dale Tronrud.

PARTICIPANTS

Bezprozvanny, L., M.S., Ph.D., University of Texas Southwestern Medical Center, Dallas
Campos, A., B.S., Ph.D., University of Illinois, Chicago
Chaves, S., B.S., Ph.D., The Rockefeller University, New York, New York
Cullen, D., B.S., Ph.D., Connecticut College, New London
Gariani, T., B.Sc., Ph.D., Umea University, Sweden
Ghirlanda, G., Ph.D., University of Pennsylvania, Philadelphia
Mancini, E., M.S., Ph.D., European Molecular Biology Laboratory, Heidelberg, Germany
Margulies, D., Ph.D., National Institutes of Health, Bethesda, Maryland
Maynes, J., B.Sc., Ph.D., University of Alberta, Edmonton, Canada
Morin, P., B.S., Ph.D., Dupont Pharmaceuticals, Wilmington, Delaware
Nollert, P., Ph.D., University of California, San Francisco
Nymalm, Y., M.S., Abo Akademi University, Turku, Finland
Plotnikov, A., M.S., Ph.D., New York University School of Medicine, New York, New York
Sickmier, A., B.S., Ph.D., St. Jude Children's Research Hospital, Memphis, Tennessee
Stine, J., B.A., Ph.D., University of Texas Health Science Center, San Antonio
Weeks, K., B.A., Ph.D., University of North Carolina, Chapel Hill

SEMINARS

Furey, W., V.A. Medical Center, Pittsburgh, Pennsylvania: Direct methods and many site Se-Met MAD problems: An example.
Gilliland, G., National Institute of Standards & Technology, Gaithersburg, Maryland: Structural genomics: High-throughput crystallization.
Joshua-Tor, L., Cold Spring Harbor Laboratory: Origin recognition by a replication initiation factor: The DNA-binding domain of E1 from papillomarvirus.
Kjeldgaard, M., University of Aarhus, Denmark: BirdBuilder/Birdwash: A multipurpose programming environment for macromolecular crystallography.
McPherson, A., University of California, Irvine: Probing crystal defect with atomic force microscopy.
Navaza, J., Universite Paris Sud, Chatenay-Malabry, France: Fitting molecular structures into EM 3D reconstructions: Application to helical and icosahedral reconstruction of the rotavirus VP6 protein.
Perrakis, A., EMBL Grenoble, France: Function and structure of the MutS DNA mismatch repair protein.
Ramikrishnan, V., MRC Laboratory of Molecular Biology, Cambridge, United Kingdom: Insights from the atomic resolution structure of the 30S ribosomal subunit.
Richardson, D. and Richardson, J., Duke University Medical Center, Durham, North Carolina: The importance of packing hydrogens for improved fitting and refinement.

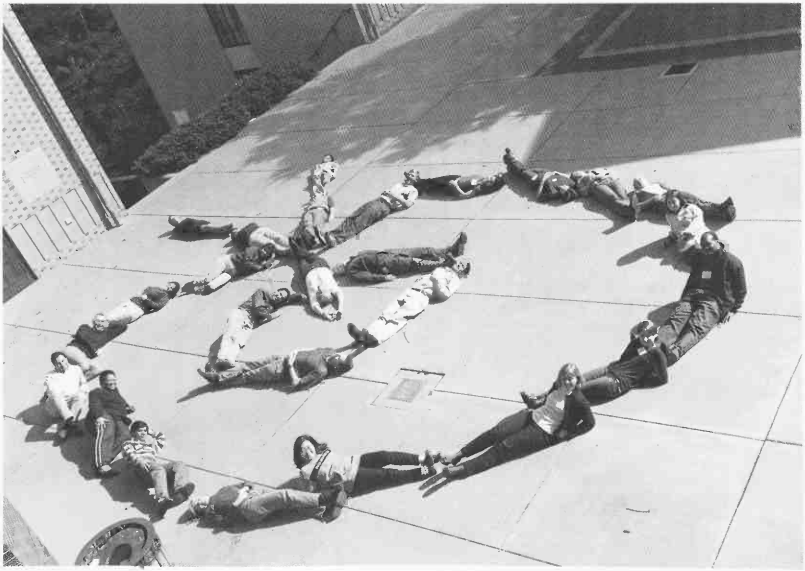
Immunocytochemistry, In Situ Hybridization, and Live Cell Imaging

October 18–31

INSTRUCTORS
J. Murray, University of Pennsylvania School of Medicine, Philadelphia
T. Ried, National Cancer Institute/NIH, Bethesda, Maryland
D. Spector, Cold Spring Harbor Laboratory
J. Swedlow, University of Dundee, United Kingdom

ASSISTANTS
A. Binnie, Oxford University, United Kingdom
M. Difilippantonio, National Cancer Institute/NIH, Bethesda, Maryland
T. Howard, Cold Spring Harbor Laboratory
M. Platani, University of Dundee, United Kingdom

This course focused on specialized techniques in microscopy related to localizing DNA, RNA, and proteins in fixed mammalian cells as well as protein dynamics in living cells. The course emphasized the use of the latest equipment and techniques in epifluorescence microscopy, confocal laser-scanning microscopy, digital image processing, and live-cell imaging. The aims of the course were designed to provide state-of-the-art technology and scientific expertise in the use of microscopic applications 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 meth-



ods presented were the preparation of tagged nucleic acid probes, fixation methods, detection of multiple DNA sequences in single nuclei or chromosome spreads, comparative genomic hybridization, cellular localization of RNA, localization of nucleic acids and proteins in the same cells, use of a variety of reporter molecules and nonantibody fluorescent tags, indirect antibody labeling, detection of multiple proteins in a single cell, and the use of GFP variants to study protein localization and dynamics. In each method, several experimental protocols were presented, allowing the students to assess the relative merits of each and to relate them to their own research. Students were encouraged to bring nucleic acid or antibody probes to the course which were used in addition to those provided by the instructors. The laboratory portion of the course was supplemented by invited lecturers who presented up-to-the-minute reports on current methods and research using the techniques presented in the course.

PARTICIPANTS

Bhuiyan, H., B.S., M.S., Stockholm University, Sweden
Bratu, D., B.A., M.A., New York University, New York, New York
Brown, A., A.B., M.D., Massachusetts General Hospital, E. Charlestown
Chin, S.-F., B.Sc., Ph.D., University of Cambridge, United Kingdom
Di Tomaso, E., B.Sc., Ph.D., Massachusetts General Hospital/Harvard Medical School, Boston
Dredge, K., B.Sc., The Rockefeller University, New York, New York
Du, J., B.S., Ph.D., Cold Spring Harbor Laboratory
Kiesslich, A., B.S., Institute for Molecular Biotechnologie, Jena, Germany
Moore, Y., M.S., Ph.D., University of Texas Southwestern Medical Center, Dallas
Morris, L., M.S., Ph.D., University of Cambridge, United Kingdom
Ott, I., B.S., M.D., Deutsches Herzzentrum der Technischen Universität, Munchen, Germany
Pfeifer, C., B.S., M.S., The Royal Veterinary and Agricultural University, Copenhagen, Denmark
Sampson, N., B.Sc., Ph.D., University of Nottingham, United Kingdom
Schelhaas, M., M.Sc., Max-Planck Institute for Neurological Research, Koln, Germany
Trevino, C., B.S., Ph.D., University of Mexico, Cuernavaca
Wood, S., B.Sc., Ph.D., Weill Medical College of Cornell University, New York, New York

SEMINARS

Dernburg, A., Stanford University School of Medicine, California: Chromosome FISH.
Eils, R., University of Heidelberg, Germany: Time-resolved analysis and visualization of dynamic processes in living cells.
Huang, S., Northwestern University Medical School, Chicago, Illinois: Fluorescence in situ hybridization to localize RNA.
Murray, J., University of Pennsylvania School of Medicine, Philadelphia: Basic introduction to light and fluorescence microscopy. Principles of confocal microscopy and deconvolution techniques.
Ried, T., National Cancer Institute/NIH, Bethesda, Maryland: Mechanisms and consequences of chromosomal aberrations in cancer cells.
Singer, R., Albert Einstein College of Medicine, Bronx, New York: Cytoplasmic organization of mRNA.
Spector, D., Cold Spring Harbor Laboratory: Immunocytochemistry.
Spring, K., National Heart, Lung, and Blood Institute/NIH, Bethesda, Maryland: Light sources and cameras.
Yasuda, R., Keio University, Yokohama, Japan: Single-molecule fluorescence imaging of F1-ATPase.
Zipfel, W., Cornell University, Ithaca, New York: GFP/multiphoton microscopy and correlation fluorescence spectroscopy.

Computational Genomics

October 26–31

INSTRUCTORS **W.R. Pearson,** University of Virginia, Charlottesville
R.F. Smith, SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania

Beyond BLAST and FASTA. This course presented a comprehensive overview of the theory and practice of computational methods for gene identification and characterization from DNA sequence data. The course focused on approaches for extracting the maximum amount of information from protein and DNA sequence similarity through sequence database searches, statistical analysis, and multiple sequence alignment. Additional topics included gene recognition (exon/intron prediction), identifying signals in unaligned sequences, and integration of genetic and sequence information in biological databases. The course combined lectures with hands-on exercises; students were encouraged to pose challenging sequence analysis problems using their own data. The course maked 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 experience with and were comfortable using the Unix operating system and a Unix text editor. The course was designed for biologists seeking advanced training in biological sequence analysis, computational biology core resource directors and staff, and for scientists in other disciplines, such as computer science, who wish to survey current research problems in biological sequence analysis.

PARTICIPANTS

Ahrens, C., B.S., Ph.D., GPC Biotech AG, Munich, Germany
Bal, H., M.S., Ph.D., Cold Spring Harbor Laboratory
Burchett, S., B.S., Ph.D., Yale University School of Medicine, New Haven, Connecticut
Chen, R., B.S., Ph.D., Baylor College of Medicine, Houston, Texas
Chinwalla, A., B.S., M.S., Washington University School of Medicine, St. Louis, Missouri
Chu, M.-L., B.S., Ph.D., Thomas Jefferson University, Philadelphia, Pennsylvania
Coleman, D., B.A., D.Phil., Princeton University, New Jersey
Cortes, D.-F., B.Sc., International Center for Tropical Agriculture CIAT, Cali, Columbia
Crignon, I., B.S., Ph.D., Novartis Pharma AG, Basel, Switzerland
Di Rienzo, A., B.S., Ph.D., University of Chicago, Illinois
Garnhart, N., B.M., University of New Hampshire, Durham
Gattu, M., B.S., Ph.D., Smithkline Beecham, King of Prussia, Pennsylvania
Goodman, M., B.S., Ph.D., Wittenberg University, Springfield, Ohio
Joubert, F., B.Sc., Ph.D., University of Pretoria, South Africa
Lin, D., B.S., Ph.D., Brookhaven National Laboratory, Upton, New York
Lopez-Jimenez, N., B.A., Ph.D., National Institutes of Health, Rockville, Maryland
Lue, N., B.S., M.D., Ph.D., Cornell University, New York, New York
Martin, S., B.S., M.S., National Resources Canada, Fredericton, Canada
McMahan, L., B.A., M.S., Montclair State University, Bloomfield, New Jersey
Schmidt, T., B.S., Ph.D., Michigan State University, East Lansing
Stajich, J., B.S., Duke University Medical Center, Durham, North Carolina
Temple, B., B.S., Ph.D., University of North Carolina, Chapel Hill
Zhang, L., B.S., University of California, Irvine
Zhang, Y., B.S., M.A., Fox Chase Cancer Center, Philadelphia, Pennsylvania

SEMINARS

Altschul, S., National Institutes of Health, Bethesda, Maryland: Statistics of sequence similarity scores. Statistics of patterns and profiles.
Cherry, M., Stanford University, California: Genome databases and genome informatics. The gene ontology project.
Gaasterland, T., The Rockefeller University, New York, New York: The whole genome perspective.
Henikoff, S., Fred Hutchinson Cancer Research Center, Seattle, Washington: Protein family databases. Blocks-based methods for finding distant relationships.
Pearson, W., University of Virginia, Charlottesville: Introduction and overview. Protein evolution–biology. Algorithms for pairwise sequence comparison. Multiple sequence comparison with hidden Markov models.
Stormo, G., Washington University Medical School, St. Louis, Missouri: Identifying sites in unaligned sequences.
Smith, R., Bioinformatics, King of Prussia, Pennsylvania: Panel discussion/review: Blocks, HMMs, and motifs–Which tool when? Introduction to multiple sequence comparison.

Phage Display of Combinatorial Antibody Libraries

November 7–20

INSTRUCTORS **C.F. Barbas,** The Scripps Research Institute, La Jolla, California
D.L. Siegel, University of Pennsylvania Medical Center, Philadelphia
G.J. Silverman, University of California, San Diego, La Jolla

ASSISTANTS **R. Fuller,** The Scripps Research Institute, La Jolla, California
C. Goodyear, University of California, San Diego, La Jolla

Recent advances in the generation and selection of antibodies from combinatorial libraries allow for the rapid production of antibodies from immune and nonimmune sources. This intensive laboratory/lecture course focused on the construction of combinatorial antibody libraries expressed on the surface of phage and selection of desired antibodies from the library. Students learned the theoretical and practical aspects of constructing combinatorial libraries from immune and nonimmune sources as well as the construction of synthetic antibody libraries. Antibodies were selected from the library by panning. Production, purification, and characterization of Fab fragments expressed in *E. coli* were also covered.

The lecture series, presented by a number of invited speakers, emphasized the 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 antibody activity, and recent results on the use of antibodies in therapy. The theory and practical implications for selection from phage displayed libraries of random peptides, cDNA products, and semisynthetic proteins were also explored.

PARTICIPANTS

Azzazy, H., Ph.D., University of Maryland School of Medicine, Baltimore
Coronella, J., Ph.D., University of Arizona, Tucson
Crawford, J., Ph.D., Paradigm Genetics, Inc., Research Triangle Park, North Carolina
Drier, E., Ph.D., Cold Spring Harbor Labortory
Enright, J., Ph.D., Dyax Corp., Cambridge, Massachusetts
Eroglu, C., B.S., M.S., European Molecular Biology Laboratory, Heidelberg, Germany
Evans, T., M.D., University of Rochester, New York
Hopf, C., Ph.D., Johns Hopkins University School of Medicine, Baltimore, Maryland
James, W., Ph.D., Intergen Corporation, Gaithersburg, Maryland
Mahler, E., B.S., Institute for Genetic Engineering and Molecular Biology Research, Capital Federal, Argentina
Maranhão, A., M.S., B.S., Universidade de Braseilia, Braseila, Brazil
Messmer, B., Ph.D., North Shore University Hospital, Manhasset, New York
Neethling, F., Ph.D., Oklahoma State University, Stillwater
Panousis, C., Ph.D., Ludwig Institute for Cancer Research, Melbourne, Victoria, Australia
Sehgal, D., Ph.D., National Institutes of Health, Bethesda, Maryland
Xiao, H., Ph.D., National Institutes of Health, Bethesda, Maryland

SEMINARS

Arap, W., University of Texas, M.D. Anderson Cancer Center, Houston: In vivo panning.
Lowan, H., Genentech, Inc., S. San Francisco, California: SAR of peptides using phage.
McHeyzer-Williams, M., Duke University Medical Center, Durham, North Carolina: Immunobiology of T-B cell responses.
Nolan, G., Stanford University School of Medicine, California: Retroviral libraries.
Noren, C., New England Biolabs, Beverly, Massachusetts: Phage peptide libraries: The PhD for peptides.
Rader, C., The Scripps Research Institute, La Jolla, California: Antibody humanization with phage display.
Webster, R., Duke Univeresity, Durham, North Carolina: The biology of filamentous phage.
Wilson, I., The Scripps Research Institute, La Jolla, California: Structural biology of the immune system.

The Laboratory would like to acknowledge the generosity of the following companies who loaned equipment and reagents to the various courses:

5 Prime-3 Prime
ALA Scientific Instruments, Inc.
Agilent Technologies, Inc.
Ambion
Amersham Pharmacia Biotech, Inc.
A.M.P.I.
Amresco
Applied Precision Inc.
Applied Scientific Instrumentation, Inc.
Astro-Med
Axon Instruments
Becton Dickinson Labware
Bioptechs
Bio-Rad Laboratories
BioVision Technologies
Boehringer Mannheim Corp.
Brinkmann Instruments
Calbiochem-Novabiochem
Carl Zeiss, Inc.
Cell & Molecular Technologies, Inc.
Chroma Technology Corp.
Codonics Inc.
Coherent Laser
Cora Styles Needles and Blocks
Corning Costar Corp.
Corning Inc.
Dagan Corp.
David Kopf Instruments
Delaware Diamond Knives
Denville Scientific Inc.
Diagnostic Instruments, Inc.
Drummond Scientific Co.
DuPont NEN
Dynal
Dynex
E.Y. Laborotories, Inc.
Eastman Kodak Co.
Epicentre Technologies
Eppendorf Scientific
Fisher Scientific
FMC Corp.
Fotodyne
General Valve
Gibco/Life Technologies
HVS Image Ltd
Hamamatsu Photonic Systems
Hampton Research
Harvard Apparatus, Inc.
Hitachi Denski America LTD.
Improvision Inc.
Improvision-Image Processing and Vision Co. Ltd.
Instrutech
Invitrogen Corp.
Jackson Immunoresearch Laboratories
Jouan, Inc.
Leica Microsystems, Inc.
Med Associates
Micro Video Instruments
Millipore
MJ Research, Inc.
Modulation Optics, Inc.
Molecular Devices Corp.
Molecular Dynamics
Molecular Probes
Nalge Nunc International
Narishige International USA, Inc.
NEN Life Science Products
New England Biolabs
Nikon Inc.
Novagen
Novex
Olympus America, Inc.
Operon Technologies Inc.
Optronics Engineering
Pel-Freez Biologicals
Perkin-Elmer Applied Biosystems
PerSeptive Biosystems
Polysciences
Promega Corp.
Qiagen Inc.
Robbins Scientific Corp.
Roper Scientific
SD Instruments
Santa Cruz Biotechnology Inc.
Savant Instruments
Scanalytics Inc.
Schleicher & Schuell
Sigma-Aldrich
Sony Medical Systems
Specialty Media
Spectra-Physics Lasers, Inc.
Statsoft
Stratagene
Sutter Instruments
Technical Manufacturing Corp.
The MathWorks Inc.
Universal Imaging Corp.
Upstate Biotechnology Inc.
Vector Laboratories
Vincent Associates
VWR Scientific Products
Warner Instruments

SEMINARS

Invited Speaker Program

Each year, Cold Spring Harbor Laboratory invites speakers from outside the institution to present their latest 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.

January

Dr. Rakesh Jain, Harvard University. A journey inside the world of solid tumors. (Host: Michael Hengartner)
Dr. Sandra Schmid, Scripps Research Institute. Dynamin: A master regulator of receptor-mediated endocytosis. (Host: Linda Van Aelst)
Dr. Nancy Hopkins, Massachusetts Institute of Technology. Large-scale insertional mutagenesis screen in zebrafish. (Host: Terri Grodzicker)
Dr. Joan Massague, Memorial Sloan-Kettering Cancer Center. The TGF-β/SMAD signaling system. (Host: Nick Tonks)
Dr. Larry Zipursky, University of California, Los Angeles School of Medicine. Axon guidance and targeting in the *Drosophila* visual system. (Host: Hollis Cline)

February

Dr. Tyler Jacks, HHMI/Massachusetts Institute of Technology, Department of Biology. Modeling cancer in the mouse. (Host: Scott Lowe)
Dr. Cori Bargmann, HHMI/University of California, San Francisco. Signaling pathways in olfaction and olfactory development. (Host: Terri Grodzicker)
Dr. Carol Prives, Columbia University. The p53 tumor suppressor protein. (Host: Terri Grodzicker)
Dr. Eric Gouaux, Columbia University. Structure and function of glutamate receptors. (Host: Leemor Joshua-Tor)

March

Dr. Andrew Belmont, University of Illinois, Urbana-Champaign. Large-scale chromatin structure and dynamics. (Host: Tatsuya Hirano)
Dr. Richard Losick, Harvard University. Asymmetry and cell fate. (Host: Terri Grodzicker)
Dr. Harold Noller, University of California, Santa Cruz. X-ray structure of functional complexes of the 70s ribosome. (Host: Winship Herr)
Dr. Stephen J. Elledge, Baylor College of Medicine. The DNA damage response in mammals. (Host: Terri Grodzicker)

April

Dr. Doug Rees, HHMI/California Institute of Technology. Structural studies of a gated mechanosensitive channel. (Host: Leemor Joshua-Tor)
Dr. Elaine Fuchs, University of Chicago. Beauty is skin deep: Mechanisms of growth and differentiation in the skin. (Host: David Spector)

October

Dr. Sandra Schmid, Scripps Research Institute. Dynamin collars: More than meets the eye. (Host: Linda Van Aelst)
Dr. Peter Mombaerts, The Rockefeller University. Targeting olfaction. (Host: Zach Mainen)

November

Dr. Jackie Lees, Center for Cancer Research, Massachusetts Institute of Technology. Role of the E2F transcription factors: Proliferation or differentiation? (Host: Bruce Stillman)
Dr. Phil Benfey, New York University. Radial patterning in *Arabidopsis:* Signaling inside out. (Host: David Jackson)
Dr. Rick Fishel, Thomas Jefferson University, Kimmel Cancer Institute. Mismatch repair and cancer: The mechanics of a molecular switch. (Host: Scott Lowe)

December

Dr. Nancy Kanwisher, Massachusetts Institute of Technology. Functional MRI investigations of human cortex: People, places, and things. (Host: Zach Mainen)
Dr. Larry Katz, Duke University Medical Center. Functional architecture of mammalian olfaction. (Host: Dmitri Chklovskii)

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. They are particularly useful for research personnel who have recently joined 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

Michael Wigler: Measuring gene copy fluctuations with microarrays.
Julius Zhu (Malinow Laboratory): Gluamate receptor trafficking and formation of functional synapses during early development.
Richard McCombie: Plant genome analysis.
Tohru Mizushima (Stillman Laboratory): Biochemical analysis of initiation of DNA replication in eukaryotic cells.

February

Nouria Hernandez: Assembly of RNA polymerase II and III transcription initiation complexes.
Dan Vaughn (Joshua-Tor Laboratory): The Apaf-1 CARD structure: A Greek key to death-fold signaling in apoptosis.
Dan Hoeppner (Hengartner Laboratory): Breaking the pathway to programmed cell death in C. *elegans*.
Alex Rai (Helfman Laboratory): Snapin is a ubiquitously expressed phosphoprotein involved in intracellular vesicle transport.

March

Karel Svoboda: Experience-dependent plasticity in the neocortex.
Scott Hammond (Hannon Laboratory): Some studies on the mechanism of RNA interference.
Patrick Reilly (Herr Laboratory): Herpes simplex virus–host cell interactions: Targeting the cell cycle.
Scott Lowe: p53 action in apoptosis and senescence.

April

Grigori Enikolopov: Stem cells, nitric oxide, and differentiation.
Junjiro Horiuchi (Yin Laboratory): Phosphorylation regulates CREB activity at multiple steps: Possible implications for memory formation.

October

Jacek Skowronski: Nef functions and AIDS.
Jannik Andersen (Tonks Laboratory): Molecular basis for recognition and dephosphorylation of the activation segment of the insulin receptor by protein tyrosine phosphatase 1B.

November

David Spector: Visualization of gene activity in living cells.
Laura Schramm (Hernandez Laboratory): Different human TFIIIB activities direct RNA polymerase III transcription from the TATA-containing and TATA-less promoters.

December

Ana Losada (Hirano Laboratory): Sister chromatid cohesion in vertebrate cells.
Luca Cartegni (Krainer Laboratory): Haystacks, needles, and genetic diseases: Designing chimeric molecules that promote exon inclusion.
Yi Zhong: A fly model of Alzheimer's disease? Insights from studies of presenilin function.

BANBURY CENTER

BANBURY CENTER DIRECTOR'S REPORT

In 2000, there were 25 meetings at Banbury Center, exceeding by two the 1999 record. Laboratory staff used Banbury Center for 11 meetings, and a notable addition to the Banbury schedule was the first of the *Topics in Biology* courses of the Watson School of Biological Sciences. There were the usual five Summer Courses, and we made the Center available to community groups on seven occasions.

More than 700 visitors came to Banbury in 2000, and the geographical distribution of the participants was much the same as in previous years; 80% of participants came from the United States, and although New York, California, and Massachusetts accounted for 44% of the participants, 39 U.S. states were represented. There were 95 participants from Europe, the majority coming from the United Kingdom.

Eugenics on the Web

This, a joint project between the DNA Learning Center and Banbury Center, completed its first stage in January, 2000. Our Advisory Board came to Banbury to review and approve the final version of the site before we went to the National Human Genome Research Institute for authority to release it to the public. The Advisory Board was, as always, constructively critical and made valuable suggestions. They approved the site, and we presented them with a certificate thanking them for their help. We have obtained a second round of funding to expand the site further, by increasing the number of images and by extending it to include European eugenics.

Neuroscience

A significant feature of the 2000 program was the large number of neuroscience meetings. This came about partly by chance, but it also reflects the increasing role that neuroscience now has in the research life of Cold Spring Harbor Laboratory. Banbury Center is well known in the worlds of molecular biology, molecular genetics, and human genetics, and I hope that neuroscience meetings like those held this year will establish the Center's reputation in the neuroscience world as well.

The *Structure, Mechanism, and Function of CaMKII* meeting was organized by Hollis Cline (Cold Spring Harbor Laboratory) and John E. Lisman (Brandeis University) and funded by the Marie H. Robertson Memorial Fund. CaMKII is an enzyme that acts as a molecular switch at synapses, and it may have a key role in synaptic plasticity and memory. Interfering with CaMKII function disrupts developmental processes and learning itself, making CaMKII a leading candidate as the molecular storage molecule for memory. Participants in this meeting study CaMKII from a variety of perspectives: its molecular structure and intramolecular reactions, its presynaptic and postsynaptic targets, and its activities at the synapse. The goal of the meeting was to examine how these properties might account for its role in memory.

Toward Animal Models of Attention and Consciousness, organized by Christof Koch (California Institute of Technology) and Anthony Zador (Cold Spring Harbor Laboratory) and funded by The Swartz Initiative for Computational Neuroscience, dealt with the fascinating topics of visual selective attention and visual consciousness. These have been treated within the realm of philosophy, but new molecular and imaging tools have led many to argue that consciousness can now be approached in an empirical and reductionist manner. This meeting brought together neuroscientists who are undertaking this task through electrophysiological and functional MRI analyses of the visual system, studies of the visual psychophysics of attention, and the development of animal model systems to study consciousness.

Meier House provides accommodations for meeting participants at Banbury Center.

Sebastian Seung (Massachusetts Institute of Technology) and David Tank (Bell Laboratories, Lucent Technologies) organized the meeting *Persistent Neural Activity,* funded by grants from the Alfred P. Sloan Foundation and The Swartz Initiative for Computational Neuroscience. "Persistent neural activity" is evoked by a short sensory stimulus, but the response persists for as long as many seconds and is believed to be involved in establishing short-term memory. This meeting was designed to examine the question: By what physiological mechanisms does a transient input cause a persistent change in neural activity? New findings, both physiological and theoretical, made this the right time to bring together researchers using different techniques, different animals, and different models to survey the field and identify emerging questions for further research.

The idea of neural networks and their application in computing began in the mid 1980s. *Neural Networks and Cognition* was organized by Anthony Zador (Cold Spring Harbor Laboratory), Zachary Mainen (Cold Spring Harbor Laboratory), and Alex Pouget (University of Rochester) to discuss the current theories of network-level computation in the brain. The organizers brought together researchers in statistical learning theory, cognitive neuroscience, and other relevant areas. The meeting was funded by Handspring Inc., and we were very pleased that Jeff Hawkins—developer of the neural network-based handwriting recognition system for the *Palm Pilot* and cofounder of Handspring Inc.—participated.

Natural Stimulus Statistics was organized by Simon Laughlin (University of Cambridge) and Pamela Reinagel (Harvard Medical School) and funded by the Cold Spring Harbor Laboratory Corporate Sponsor Program. Sensory neurons receive signals from the external world and transmit information about them to the brain. It has been suggested that a major function of the sensory neurons is to remove unnecessary information; optimal neural codes would enable peripheral cells to send vastly more information to the brain. The meeting brought together an eclectic group of scientists with backgrounds in physics, mathematics, and neurophysiology, with the goal of drawing up a list of key problems that need immediate attention.

Studies on the molecular basis of learning and memory in vivo are making use of mice with mutations in specific genes. But to do these studies properly requires careful and standardized assessments of behavior, especially if correlates are to be established with human clinical conditions. *Mouse Behavioral Phenotyping* was supported by the National Institute for Mental Health to provide an opportunity for investigators being funded under a new program to meet and discuss their findings. Organized by Michela Gallagher (Johns Hopkins University), the meeting was intended to assist existing collaborations and to promote further collaborative activities.

A large inter-institutional project is being planned for research on *Signaling Network Control–Cell Interactions*. To be funded by the National Institutes of Health, the project involves several institutes, including Cold Spring Harbor Laboratory, scattered throughout the country. A planning meeting for the project was organized by Ravi Iyengar (Mount Sinai School of Medicine), and key investigators came to Banbury for 2 days of very hard work, away from the distractions at their own institutions.

Biological Studies

Ten years ago, Irwin Fridovich (Duke University) and John G. Scandalios (North Carolina State University) organized a meeting on free oxygen radicals. In 2000, they returned and organized a meeting on the *Molecular Biology of Oxidative Stress*, funded by the Cold Spring Harbor Laboratory Corporate Sponsor Program. The main change in the intervening period has been the application of molecular approaches, with the cloning and characterization of many of the antioxidant defense genes, together with the elucidation of stress response triggers and pathways. An interdisciplinary group of experts concerned with the basic molecular biology and genetics of oxidative stress came to Banbury to discuss common mechanisms operating across evolutionary lines and to set future research goals.

We have come some way since Bishop Wilberforce asked T.H. Huxley whether it was on his grandfather's or his grandmother's side that ape ancestry was to be found. But quite what that ape ancestry is—what the genetic relationships are between the great apes, and how these are reflected in differences in phenotypes—is still uncertain. *Great Apes: Phenotypes and Genotypes* was organized by Aravinda Chakravarti (Case Western Reserve University), Svante Paabo (Max-Planck Institute for Evolutionary Anthropology), and Jan Witkowski (Cold Spring Harbor Laboratory) and funded by the Cold Spring Harbor Laboratory Corporate Sponsor Program. The meeting reviewed phenotypic differences between the great apes and what is known currently of the genetic and genomic differences between these species and discussed the feasibility of an ape genome project.

Apoptosis is a conserved program used by organisms to get rid of cells that are in excess, in the way, or potentially dangerous. Although cell killing is being studied in detail, far less is known of the subsequent steps of *Getting Rid of the Bodies*. Organized by Giovanna Chimini (INSERM-CNRS, Marseille, France) and Michael Hengartner (Cold Spring Harbor Laboratory), this meeting examined questions such as: What are the "eat-me" signals, and how are these signals generated by the apoptotic cells? What are the "eat-me" receptors, and what are the signaling pathways downstream from them? What is different between an apoptotic and a necrotic cell corpse? The meeting was funded by the Cold Spring Harbor Laboratory Corporate Sponsor Program.

Two meetings were an interesting combination of fundamental research and applied biology. *Mammalian Cloning: Biology and Practice* was held 3 years after the cloning of Dolly to review critically the current status of cloning of cows, pigs, mice, and goats. Organized by Neal First (University of Wisconsin), Peter Mombaerts (The Rockefeller University), and Jan Witkowski (Cold Spring Harbor Laboratory), the meeting was funded by the Alfred P. Sloan Foundation. Questions examined included: Why is it that there is such a high failure rate? What is known of the biology underlying mammalian cloning? What can the biology tell us about the best strategies for cloning? Participants came from academic institutions and from companies, and included pioneers of mammalian cloning, Steen Willadsen, Ian Wilmut, and Keith Campbell.

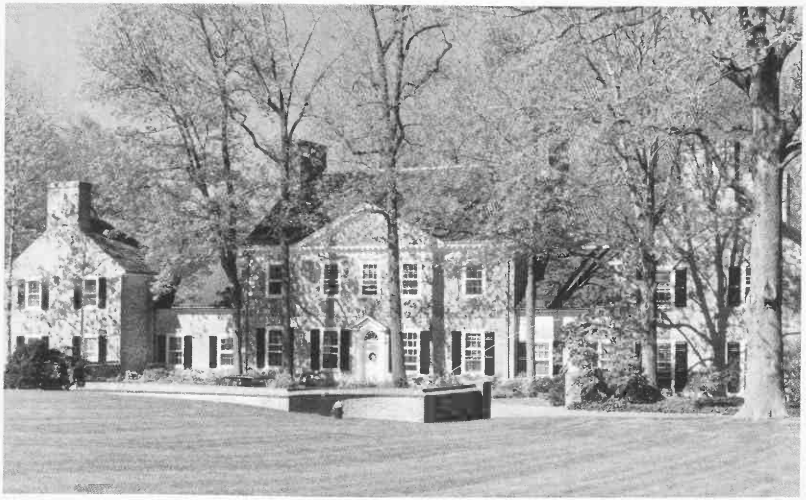
The second of these two meetings was *RNA Silencing: Functions, Mechanisms, and Applications*, organized by David Baulcombe (John Innes Centre) and Greg Hannon (Cold Spring Harbor Laboratory)

and funded by the Cold Spring Harbor Laboratory Corporate Sponsor Program. RNA silencing is a remarkable phenomenon in which overexpression of an RNA can suppress expression of a gene. Originally discovered in plants, it has become clear that the phenomenon is ubiquitous in eukaryotes. This was the first meeting that made a deliberate effort to assemble researchers working on different aspects of RNA silencing in different organisms. This was an especially timely topic, producing a most interesting and lively meeting.

Regulation and Function of Heat Shock Proteins was a meeting organized by Betty Craig (University of Wisconsin), Carol Gross (University of California, San Francisco), and Rick Morimoto (Northwestern University) for friends and colleagues of Professor Takashi Yura. Professor Yura, who made significant contributions to the field, will be retiring from the HSP Institute in 2001. It was fitting that his retirement was marked by holding a scientific meeting that covered topics such as protein folding and the regulation of the heat shock response.

Plant Biology

Plants were featured in a number of meetings, but two meetings in 2000 were largely devoted to plant science. For example, although sugar production through photosynthesis is the most important activity in plant life, sugars have vital roles in all organisms. Mark Johnston (Washington University Medical School), Jenn Sheen (Massachusetts General Hospital), and Mark Stitt (University of Heidelberg) organized the meeting *Sugar Sensing and Signaling in Plants and Other Organisms*, funded by the Cold Spring Harbor Laboratory Corporate Sponsor Program. The purpose of the meeting was to highlight recent advances in our understanding of the molecular mechanisms underlying sugar sensing and signaling. Although the meeting focused on plants, research on other organisms—bacteria, yeast, mammals—was included. As with *RNA Silencing*, this was the first meeting to include such a diverse set of researches on sugar sensing.



Robertson House also provides housing accommodations at Banbury Center.

One of the great triumphs of the genome world came in December when the complete sequence of *Arabidopsis thaliana* was published. David Luke III (former Chairman of the Laboratory's Board of Trustees) and Westvaco, Inc. have long been supporters of the *Arabidopsis* genome project, and it was a particular pleasure that Mr. Luke attended the meeting *The Application of* Arabidopsis *Genomics to Forestry and Other Complex Plant Systems*, held the week before the sequence was published. The meeting was organized by Robert Martienssen (Cold Spring Harbor Laboratory) and Ronald Sederoff (North Carolina State University) and funded by the Cold Spring Harbor Laboratory Corporate Sponsor Program. Although completion of the *Arabidopsis* genome sequence promises to revolutionize the study of all plants, its application to the biology of complex plant genomes—such as those of forest trees—remains a significant challenge. This meeting took up this challenge and concluded with a discussion of the benefits that would come from sequencing a tree genome.

Bioinformatics

Having large amounts of sequence data will not be helpful if the information cannot be integrated into the existing body of biological knowledge. The Gene Ontology (GO) Consortium has recognized that a major obstacle to such biology-wide integration of information is the absence of a unified vocabulary that is recognized across the databases devoted to particular organisms. The GO is attempting to derive such a vocabulary and nomenclature. Michael Ashburner (European Bioinformatics Institute, Cambridge) organized *Gene Ontology Annotation and the Human Genome*, a small but very intensive workshop to discuss how to extend the GO concept to the human genome.

Human Genetic Disorders

Banbury Center continued its 17-year tradition of having meetings on human genetic disorders. The first in 2000 was on *Therapeutic Approaches in Mouse Models of ALS*, organized by M. Flint Beal (Weill Medical College, Cornell University) and Don W. Cleveland (University of California, San Diego). Mouse models of human genetic disorders are important for exploring the molecular pathology of diseases and for developing therapies, and this meeting focused on current strategies for testing therapeutic approaches in mouse models of amyotrophic lateral sclerosis (ALS) and other human neurodegenerative diseases. Topics that were covered included a survey of available models, potential therapeutic agents, and methods of drug delivery. The meeting was funded by the Amyotrophic Lateral Sclerosis Association.

A most significant advance in understanding the Fragile X syndrome came with the identification of the protein, FMRP. The title of the meeting *FMRP: What Does It Do?* described exactly the goals of the meeting organized by William Greenough (University of Illinois) and David Nelson (Baylor College of Medicine) and funded by the FRAXA Research Foundation. There are fascinating reports of the localization of FMRP to the dendrites of neurons, suggesting that the absence of FMRP leads to impaired communications between neurons, which leads to mental retardation. As there is now a broad range of scientific expertise involved in research on Fragile X—biochemistry, molecular biology, neuroimaging, and behavior—the meeting provided a forum for scientists working in these different areas to meet and interact.

Pathogens

In 1991, Banbury Center held its first meeting on Lyme Disease. At that time, Lyme Disease counted as a newly emerging pathogen, the causative agent (the spirochaete *Borrelia burgdoferi*) having been identified in the early 1980s. The West Nile virus, another previously unknown pathogen, appeared in the New York area in 1999. So, the meeting *Strategies for Identification and Characterization of Unknown Pathogens*, organized by Benjamin Luft (SUNY, Stony Brook), Steven Schutzer (UMDNJ/New Jersey Medical School), and Suzanne Vernon (Centers for Disease Control) and funded by the Centers

for Disease Control, was very timely. The meeting brought together scientists from diverse backgrounds including evolutionary and molecular biology, biochemistry, immunology, epidemiology, and infectious diseases to discuss the utilization of leading-edge biotechnology for the recognition of novel pathogens.

Newly emerging pathogens afflict not only human beings, but also livestock and crops. The Agricultural Research Service of the USDA funded a discussion meeting, *Meeting the Challenge of Infectious Diseases in the 21st Century* (organized by Roger Breeze, USDA), to help define a national capacity and strategy for dealing with unresolved issues in infectious diseases of animals and plants and to prepare for meeting all new and emerging threats, of which biological terrorism is the latest. The meeting focused on science rather than policy, considering, for example, the potential impact of microbial and host genomic information.

Vaccines

One of the most effective ways of combating pathogens is through vaccination, but vaccines with a limited market will not be developed by industry. *Social Venture Capital for Neglected Vaccines: Creating Successful Alliances* explored alternative sources of funding for vaccine development. The organizer was Melinda Moree (PATH/Malaria Vaccine Initiative), with co-chairs Regina Rabinovich (PATH/Malaria Vaccine Initiative) and Philip K. Russell (The Albert B. Sabin Vaccine Institute). The meeting was funded by the Albert B. Sabin Vaccine Institute, Inc., through a grant from the Bill and Melinda Gates Foundation. It was expected that the discussions would assist public sector vaccine programs by developing strategies for managing partnerships with industry and dealing with intellectual property and other contractual commitments to assure the availability of neglected vaccines for the developing world.

The J.P. Morgan and Cold Spring Harbor Laboratory Executives' Meeting

This was the 15th in the series of meetings for senior executives in the pharmaceutical, biotechnology, and financial worlds, and for the seventh year, we are very grateful to Sandy Warner and David Deming of J.P. Morgan, Inc., for their continuing support. These meetings are always remarkable occasions, but this year's meeting was exceptional even by past standards. Entitled *Human Intelligence and Consciousness*, we drew upon an wonderful roster of speakers. Oliver Sacks began the meeting by describing extraordinary autistic savants. Other speakers were Howard Gardner, Irene Pepperberg, Charles Murray, Stephen Pinker, Vilayanur Ramachandran, and Rodney Brooks.

The President's Council Meeting

Although most activities associated with James Watson's President's Council meeting take place on the main campus, the opening talk is held at Banbury and is followed by dinner at Robertson House. The topic for this year's meeting was *The Dog and Its Genetics: Breeds, Evolution & Behavior*, an unbeatable combination of cutting-edge genetic research and humanity's closest friend. Our speaker on Friday evening was Roger Caras, known for his encyclopedic knowledge of dogs that he puts to good use in his role of Master of Ceremonies at the annual Westminster Club Show. Sitting on a stool like a story teller, Mr. Caras gave a spell-binding talk on the special relationship between human beings and animals, especially dogs.

Watson School of Biological Sciences—Topics in Biology

The Watson School of Biological Sciences has a faculty with remarkably diverse interests, but there are some areas of biology in which we are lacking deep expertise. To ensure that our students receive

instruction in these areas, we have instituted a special *Topics in Biology* course, to be held each spring at the Banbury Center. The students are taken away from the laboratory bench, enabling them to devote themselves to a concentrated program. For 2000, the selected topic was immunology, and we were very fortunate to have Hidde Ploegh from Harvard Medical School come to teach the week-long course. Hidde is an inspiring teacher, and the course was a success beyond our already high expectations. The topic for 2001 is *Evolution*.

Other Meetings

Several Laboratory science groups came to Banbury for review and planning meetings, as did the DNA Learning Center and the Cold Spring Harbor Laboratory Press. As usual, we made the Center available to a small number of local community groups, notably Lloyd Harbor Village, Cold Spring Harbor School District, and Holiday House.

Funding

The Banbury Center and the scientists who participated in meetings here are very grateful for the generosity of all those institutions that provided support for the Banbury program. Foremost among these was the Cold Spring Harbor Laboratory Corporate Sponsor Program, which provided funds for six meetings. Federal funding came from five of the National Institutes of Health (NHGRI, NICHHD, NIGMS, NIMH, NIDCD); the Centers for Disease Control; and the Agricultural Research Service (United States Department of Agriculture). Nonfederal support came from the ALS Association; the FRAXA Research Foundation; Handspring Inc.; the Marie H. Robertson Memorial Fund for Neurobiology; the Albert B. Sabin Vaccine Institute; the Alfred P. Sloan Foundation; and The Swartz Initiative for Computational Neuroscience.

Acknowledgments

Once again, Banbury could not have had such a successful year without the invaluable contributions of Bea Toliver, Ellie Sidorenko, and Katya Davey to the operation of the Center. The Meetings Office has worked with us on the increasingly difficult task of interleaving the Grace Auditorium and Banbury Center meetings, the Audiovisual Department has kept presentations running smoothly, and Housekeeping has looked after all the visitors for us. Chris McEvoy, Andy Sauer, and Joe Ellis have kept the estate looking beautiful, contributing to the special ambience of the Center. Finally, the meetings depend on the enthusiasm and work of the organizers and the contributions made by all participants.

Jan Witkowski

MEETINGS

Fourth Meeting of the Editorial Advisory Panel: Digital Image Archive on the American Eugenics Movement

January 28–30

FUNDED BY **National Human Genome Research Institute, NIH**

ARRANGED BY **D. Micklos,** DNA Learning Center, Cold Spring Harbor Laboratory
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1

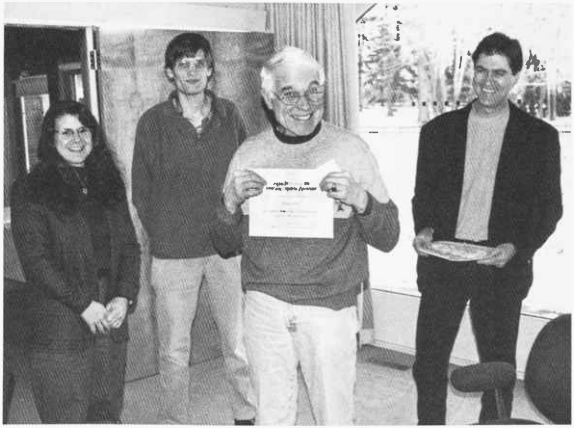
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory: Welcome, general instructions, and hospitality.
D. Micklos, DNA Learning Center, Cold Spring Harbor Laboratory: Workshop objectives and other matters.
S. Lauter and M. Christensen, DNA Learning Center, Cold Spring Harbor Laboratory: Introduction to the new interface.
Computer Work: Reactions to the site and review images in assigned topics.

SESSION 2

Review topic narratives, draft new topic narratives, and draft essay on "European connections."
Discuss NIH restriction on images.

SESSION 3

Finalize list of essential items to be completed before opening the site and those that can be completed after opening the site.
Resolutions concerning NIH restriction and opening the site.
Future developments.



S. Lauter, M. Christensen, G. Allen, D. Micklos

Therapeutic Approaches in Mouse Models of ALS

February 13–16

FUNDED BY **Amyotrophic Lateral Sclerosis Association**

ARRANGED BY **M.F. Beal,** Weill Medical College, Cornell University, New York
D.W. Cleveland, University of California, San Diego

Introduction:
R.V. Abendroth, The ALS Association, Milwaukee, Wisconsin

SESSION 1: Pathogenesis of ALS
Chairperson: M.F. Beal, Weill Medical College,Cornell University, New York

R.H. Brown, Massachusetts General Hospital, Charlestown: Overview of theories of mechanisms of disease.
D.W. Cleveland, Ludwig Institute for Cancer Research, University of California, San Diego: Mechanisms of neuronal death in ALS: Axonal strangulation, catalysis by copper, protein aggregates, and chronic caspase activation.
M. Gurney, Pharmacia & Upjohn, Kalamazoo, Michigan: Gene expression profiling in FALS transgenic mice.
J.A. Johnston, Pharmacia & Upjohn, Kalamazoo, Michigan: Aggregation of mutant SOD1 is an early event in the pathogenesis of ALS motoneuron disease.
D.R. Borchelt, Johns Hopkins University School of Medicine, Baltimore, Maryland: Studies in transgenic mice.
J. Rothstein, Johns Hopkins University School of Medicine, Baltimore, Maryland: Overview of therapeutics in mice and men.

SESSION 2: Therapeutics in ALS Mice I
Chairperson: D.W. Cleveland, Ludwig Institute for Cancer Research, University of California, San Diego

S.E. Przedborski, Columbia University, New York: Programmed cell death in ALS.
J.P. Crow, University of Alabama at Birmingham: Pharmacologic intervention at onset: Intercepting the mediators of toxicity?
R.M. Friedlander, Brigham and Women's Hospital, Boston, Massachusetts: Caspase inhibition in the treatment of ALS.
M.F. Beal, Weill Medical College, Cornell University, New York: Therapeutics with creatine.
S.H. Appel, Baylor College of Medicine, Houston, Texas: Calcium alteration in motor neuron injury: Protective effects of parvalbumin.
E.Y. Snyder, Children's Hospital, Harvard Medical School, Boston, Massachusetts: Potential role of neural stem cells in motoneuron disease.

A. McMahon, D. Cleveland

SESSION 3: Therapeutics in ALS Mice II
Chairperson: R.H. Brown, Massachusetts General Hospital, Charlestown

L.A. Shinobu, Massachusetts General Hospital, Boston: Does overexpression of heat shock protein 70 alter the natural history of the G93A mouse model of ALS?

SESSION 4: Approaches Beyond ALS I
Chairperson: R.H. Brown, Massachusetts General Hospital, Charlestown

J. Morton, University of Cambridge, United Kingdom: How good is the medicine?: Measuring clinical improvements in mice.

G.A. Cox, The Jackson Laboratory, Bar Harbor, Maine: Tissue-specific transgenic rescue in the neuromuscular degeneration (nmd) mouse.

N.L. Heintz, HHMI, The Rockefeller University, New York: A novel pathway for glutamate receptor-mediated neurore-generation.

M. Beaulieu, Montreal General Hospital Research Institute, Canada: Transgenic mice overexpressing peripherin: A new mouse model of motor neuron disease.

SESSION 5: Approaches Beyond ALS II
Chairperson: A.P. McMahon, Harvard University, Cambridge, Massachusetts

H. Mitsumoto, ALS Center, Columbia-Presbyterian Medical Center, New York, New York: An approach in a natural motor neuron disease (Wobbler mouse).

K. Duff, Nathan Kline Institute, Orangeburg, New York: MRI as a tool in the assessment of transgenic models of neu-rodegenerative disease.

S.M. Hersch, Emory University School of Medicine, Atlanta, Georgia: Neuropathology in Huntington's disease trans-genic models.

W.T. Dauer, Neurological Institute of New York, New York: Regulatable animal models of neurological disease.

P. Wong, Johns Hopkins University School of Medicine, Baltimore, Maryland: Neurodegenerative disease: Lessons from transgenic models.

T. Williamson, Trophos S.A., Marseille, France: Purified neu-ronal cultures from mouse models: High-throughput screening on the cells at risk.

SESSION 6: Oxidative Injury and Copper Metabolism
Chairperson: S.H. Appel, Baylor College of Medicine, Houston, Texas

A.C. Kato, Centre Medical Universitaire, Geneva, Switzerland: Overview of the *pmn* mouse model for studies on ALS.

M. Cudkowicz, Massachusetts General Hospital, Boston: Effects of altered levels of GSHPx in the ALS on disease phenotype in the ALS mouse model (and other therapeutic studies).

Z. Xu, University of Massachusetts Medical School, Worcester: Therapeutic effects of creatine and SOD1 cata-lase mimetics in a mouse model of ALS.

R.J. Ferrante, Bedford VA Medical Center, Massachusetts: Mechanisms of neurodegeneration and therapeutic inter-vention in ALS.

Molecular Biology of Oxidative Stress

March 5–8

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **J.G. Scandalios,** North Carolina State University, Raleigh
I. Fridovich, Duke University Medical Center, Durham, North Carolina

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
J.G. Scandalios, North Carolina State University, Raleigh

SESSION 1: Antioxidant Gene Structure, Regulation, and Expression (Prokaryotes)
Chairperson: D. Touati, Institut Jacques Monod, Paris, France

S.S. Wallace, University of Vermont, Burlington: Repair of oxidative DNA base lesions.
D. Touati, Institut Jacques Monod, Paris, France: Bacterial superoxide dismutases.
P.C. Loewen, University of Manitoba, Canada: Structure and function of bacterial catalases.
I. Fridovich, Duke University Medical Center, Durham, North Carolina: Sugars as target for O_2^-.
J. Imlay, University of Illinois, Urbana: Genetics and physiology of iron-sulfur-cluster damage and repair in *E. coli*.
I. Fita, CID, Council of Higher Scientific Investigation, Barcelona, Spain: Structure-function relationships of heme catalases.

SESSION 2: Antioxidant Gene Structure, Regulation, and Expression (Eukaryotes)
Chairperson: I. Fridovich, Duke University Medical Center, Durham, North Carolina

E.B. Gralla, University of California, Los Angeles: Iron and oxidative stress in yeast.
J.L. Pinkham, University of Massachusetts, Amherst: A single site in the promoter of the yeast *SOD2* gene mediates stress and heme-regulated transcription.
J.G. Scandalios, North Carolina State University, Raleigh: Antioxidant genes: Structure, regulation, and expression in response to oxidative stress.

W. Orr, D. Touati, N. Cole, J. Scandalios

H. Ruis, Institute of Biochemistry and Molecular Cell Biology, Vienna, Austria: Nuclear protein transport in stress signaling from the cellular environment to the nucleus.

L.M. Guan, North Carolina State University, Raleigh: Regulation of maize antioxidant catalase genes in response to environmental stress.

A.J. Slusarenko, Institut fuer Biologie III, Aachen, Germany: Role of reactive oxygen intermediates in the resistance of *Arabidopsis* to infection.

SESSION 3: Signal Perception and Transcriptional Regulation
Chairperson: J.G. Scandalios, North Carolina State University, Raleigh

G. Storz, NICHD, NIH, Bethesda, Maryland: The redox-sensitive *OxyR* transcription factor.

S.W. Ryter, University of Southern Illinois School of Medicine, Springfield: Heme metabolism and oxidative stress.

R. Kahl, University of Dusseldorf, Germany: Regulation of antioxidant enzymes.

F.C. Fang, University of Colorado Health Sciences Center, Denver: Subversion of the NADPH phagocyte oxidase by *Salmonella*.

C. Richter, ETH Zentrum, Zurich, Switzerland: Nitric oxide and peroxynitrite in mitochondria.

N. Smirnoff, University of Exeter, United Kingdom: The metabolism and function of ascorbic acid in plants.

A. Puga, University of Cincinnati Medical Center, Ohio: The Aryl hydrocarbon receptor: An environmental sensor that signals oxidative stress and cell cycle arrest.

SESSION 4: Oxidative Stress and the Aging Process
Chairperson: N.J. Holbrook, National Institute on Aging, NIH, Baltimore, Maryland

N.J. Holbrook, National Institute on Aging, NIH, Baltimore, Maryland: Signaling pathways activated by oxidative stress: Links to cell survival and aging.

W.C. Orr, Southern Methodist University, Dallas, Texas: Regulation of antioxidant gene expression in *Drosophila*.

G. Pastori, IACR-Rothamsted, Hertfordshire, United Kingdom: ROS and plant senescence.

R. Levine, National Heart, Lung, and Blood Institute, NIH, Bethesda, Maryland: Methionine oxidation.

SESSION 5: Biomedical Aspects of Oxidative Stress
Chairperson: S.S. Wallace, University of Vermont, Burlington

M.D. Jacobson, Millennium Pharmaceuticals, Inc., Cambridge, Massachusetts: Role of ROS in cell death.

M.A. Beck, University of North Carolina, Chapel Hill: Influence of oxidative stress on viral pathogenicity: Changes in the viral genome.

D. St. Clair, University of Kentucky, Louisville: Transcriptional regulation of the human *MnSOD* gene.

S.J. Chanock, National Cancer Institute, NIH, Gaithersburg, Maryland: Genetic polymorphisms and functional/biological implications of oxidative stress.

Mammalian Cloning: Biology and Practice

March 12–15

FUNDED BY **Alfred P. Sloan Foundation**

ARRANGED BY **N.L. First,** University of Wisconsin, Madison
P. Mombaerts, The Rockefeller University, New York
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

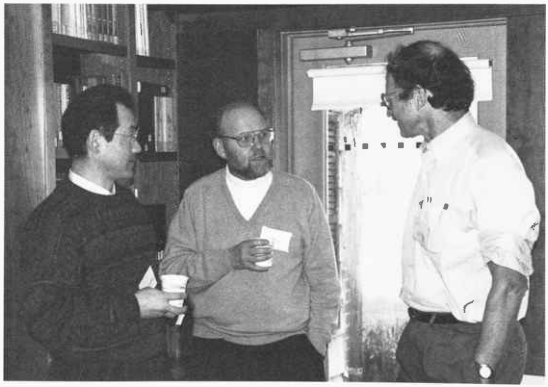
Introduction and Overview:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
I. Wilmut, Geron-BioMed, Midlothian, United Kingdom

SESSION 1: Biology of Early Development
Chairperson: R. Lovell-Badge, National Institute for Medical Research, London, United Kingdom

B. Knowles, The Jackson Laboratory, Bar Harbor, Maine: Nuclear reprogramming: Molecular mechanisms controlling the oocyte to embryo transition.
M.A. Surani, University of Cambridge, United Kingdom: Germ line, stem cells, and genomic imprinting.
A.P. Wolffe, National Institute of Child Health & Human Development, Bethesda, Maryland: The biochemical basis of nuclear reprogramming.
K.E. Latham, Temple University School of Medicine, Philadelphia, Pennsylvania: Transcriptional responses of nuclei to 1-cell cytoplasm.
R.M. Schultz, University of Pennsylvania, Philadelphia: Differential effects of culture on imprinted gene expression in the preimplantation mouse embryo: Implications for cloning of mammals.

SESSION 2: Cloning of Rodents I
Chairperson: P. Mombaerts, The Rockefeller University, New York

T. Wakayama, The Rockefeller University, New York: Cloning mice.
R. Jaenisch, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Methylation, imprinting, and nuclear cloning.
P.M. Iannaccone, Northwestern University Medical School, Chicago, Illinois: Rat blastocyst formation following nuclear transfer to adult or fetal nuclei.



H. Nagashima, I. Wilmut, H. Varmus

SESSION 3: Societal Issues
Chairperson: P. Mombaerts, The Rockefeller University, New York

L.M. Silver, Princeton University, New Jersey: Cloning: Implications for human reproduction.

SESSION 4: Cloning of Rodents II
Chairperson: N.L. First, University of Wisconsin, Madison

E. Overstrom, Tufts University School of Veterinary Medicine, North Grafton, Massachusetts: Modified oocyte activation and enucleation paradigms enhance mammalian cloning efficiency.

SESSION 5: Cloning of Cattle
Chairperson: N.L. First, University of Wisconsin, Madison

J.M. Robl, University of Massachusetts, Amherst: Factors influencing variability in cloning success.
M.D. Bishop, Infigen, Inc., DeForest, Wisconsin: Cloning, reprogramming, and rearing.
M.E. Westhusin, Texas A&M University, College Station: Characterization of developmental abnormalities in embryos and fetuses produced by nuclear transfer/cloning.
Y. Tsunoda, Kinki University, Nara, Japan: In vitro and in vivo development of nuclear transferred bovine oocytes receiving somatic cells from various tissues of adults, newborns, and fetuses.
X. Yang, University of Connecticut, Storrs: Cloning adult cows and bulls: Biological factors.

SESSION 6: Cloning of Sheep and Pigs
Chairperson: N. Zinder, The Rockefeller University, New York

A. Colman, PPI Therapeutics plc, Edinburgh, United Kingdom: Successful gene targeting in livestock.
K.H. Campbell, University of Nottingham, Leicestershire, United Kingdom: Donor cell cycle stages: Successes and problems.
H. Nagashima, Meiji University, Tokyo, Japan: Pig cloning by the Honolulu method.
R.S. Prather, University of Missouri, Columbia: Nuclear transfer in pigs.

SESSION 7: Philosophical Issues
Chairperson: P. Mombaerts, The Rockefeller University, New York

J. Burley, Queen's College, Oxford, United Kingdom: Is human cloning morally permissible?

SESSION 8: Manipulation of Primate Embryos
Chairperson: H.E. Varmus, National Institutes of Health, Bethesda, Maryland

D.P. Wolf, Oregon Regional Primate Research Center, Beaverton: Cloning by nuclear transfer and blastomere separation in rhesus macaques.
G.E. Schatten, Oregon Regional Primate Research Center, Beaverton: Cloned, transgenic, and stem-cell-derived nonhuman primates as human disease models.
J. Cohen, Saint Barnabas Medical Center, Livingston, New Jersey: Role of mitochondria in human embryos and after cytoplasmic transplantation.
J.A. Thomson, University of Wisconsin, Madison: Human ES cells: The problem of graft rejection.
J.B. Cibelli, Advanced Cell Technology, Worcester, Massachusetts: Lifespan extension of primary cells by somatic cell cloning in cattle.
P. Mombaerts, The Rockefeller University, New York, and N.L. First, University of Wisconsin, Madison: Concluding discussion and summary.
S.M. Willadsen, Saint Barnabas Medical Center, Livingston, New Jersey: Concluding remarks.

Great Apes: Phenotypes and Genotypes

March 19–22

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **A. Chakravarti,** Case Western Reserve University, Cleveland, Ohio
S. Paabo, Max-Planck Institute for Evolutionary Anthropology, Leipzig, Germany
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
S. Paabo, Max-Planck Institute for Evolutionary Anthropology, Leipzig, Germany

SESSION 1: Neural Phenotypes
Chairperson: S. Paabo, Max-Planck Institute for Evolutionary Anthropology, Leipzig, Germany

P. Rakic, Yale University School of Medicine, New Haven, Connecticut: Evolution of neocortical expansion in primates.

J.K. Rilling, Emory University, Atlanta, Georgia: What's unique about the human brain? Comparative neuroimaging studies of anthropoid primates.

K. Semendeferi, University of California, San Diego, La Jolla: Hominoid neural specializations.

S. Rice, Yale University, New Haven, Connecticut: Distinguishing novelty from heterochrony in primate brain evolution.

B.T. Shea, Northwestern University, Chicago, Illinois: Heterochrony and phenotypic variation among the large-bodied hominoids.

SESSION 2: Chromosomal Phenotypes
Chairperson: A. Varki, University of California, San Diego

E.E. Eichler, Case Western Reserve University, Cleveland, Ohio: The paralogous nature of the hominoid genome: Novel patterns of primate gene evolution.

M. Rocchi, Istituto di Genetica, Bari, Italy: Primate genome plasticity: A cytogenetic point of view.

D.L. Nelson, Baylor College of Medicine, Houston, Texas: Defining chromosome rearrangements amongst the great apes: Sequence at a chromosome 12 pericentric inversion.

M. Pagel, University of Reading, United Kingdom: LINE-1 elements and genome organization: Ancient genome-wide infections.

A. Motulsky, M.-C. King, M. Goodman

SESSION 3: Molecular Evolution
Chairperson: D.L. Nelson, Baylor College of Medicine, Houston, Texas

M. Goodman, Wayne State University School of Medicine, Detroit, Michigan: Comparative primate genomics: A search for positively selected mutations in humankind's evolutionary history.

S. Paabo, Max-Planck Institute for Evolutionary Anthropology, Leipzig, Germany: Molecular approaches to comparing humans and apes.

A. Varki, University of California, San Diego: Studies of humans as great ape "knockouts" for CMP-sialic acid hydroxylase.

P.A. Morin, Max-Planck Institute for Evolutionary Anthropology, Leipzig, Germany: Chimp SNPs from CATS: Beginning a chimpanzee SNP map.

J. Lenz, Albert Einstein College of Medicine, Bronx, New York: HERV-K, the replicating, germ line retrovirus of hominoids.

SESSION 4: Population Genetics
Chairperson: M.-C. King, University of Washington, Seattle

E.B. Hey, Rutgers University, Piscataway, New Jersey: Comparative population genetics of humans and great apes.

P. Oefner, Stanford University, Palo Alto, California: Comparative analysis of autosomal and Y chromosome genes in human and great apes.

A. Smit, University of Washington, Seattle: Repeat elements predating ape evolution.

A. Eyre-Walker, University of Sussex, Brighton, United Kingdom: Deleterious mutation rates in humans, great apes, and other animals.

SESSION 5: General Issues
Chairperson: A.G. Motulsky, University of Washington School of Medicine, Seattle

W.-H. Li, University of Chicago, Illinois: How different are human, chimp, and gorilla genomes?

N. Saitou, National Institute of Genetics, Mishima, Japan: Silver: Ape genome sequencing project.

S. Rozen, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Sequence of the human Y chromosome: Genes, gene families, and phenotypes.

M.-C. King, University of Washington, Seattle: The consequences of being a hastily made-over ape.

General Discussion:
Co-Chairpersons: S. Brenner, The Molecular Sciences Institute, Inc. Berkeley, California, and **A.G. Motulsky,** University of Washington School of Medicine, Seattle

Strategies for Identification and Characterization of Unknown Pathogens

April 2–5

FUNDED BY **National Center for Infectious Diseases, Centers for Disease Control Prevention, with additional support from Aventes Pharma AG**

ARRANGED BY **B.J. Luft**, State University of New York, Stony Brook
S.E. Schutzer, UMDNJ/New Jersey Medical School, Newark
S. Vernon, Centers for Disease Control & Prevention, Atlanta, Georgia

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
S. Vernon, Centers for Disease Control & Prevention, Atlanta, Georgia

SESSION 1: Success Stories/Lessons Learned
Co-Chairpersons: B.J. Luft, State University of New York, Stony Brook, and **R. Breeze,** U.S. Department of Agriculture, Athens, Georgia

D.H. Persing, Corixa Corporation, Seattle, Washington: Detection of novel pathogen-specific nucleic acids in human cancers and chronic inflammatory diseases.
G.H. Cassell, Eli Lilly and Company, Indianapolis, Indiana: Role of infection in chronic lung disease and arthritis.
S.B. Fisher-Hoch, Laboratoire Jean Merieux, Lyon, France: Ecology and epidemiology of emerging diseases.
M. Koopmans, Laboratory of Infectious Diseases, Bilthoven, The Netherlands: Animal pathogens infecting humans.
R.L. Berkelman, Emory University, Atlanta, Georgia: Emerging infectious diseases: A historical perspective.
J. Ellner, Case Western Reserve University, Cleveland, Ohio: How the epidemic of HIV infection changed the face of tuberculosis.
E.R. Unger, Centers for Disease Control & Prevention, Atlanta, Georgia: The sample as a window on disease: Problems in the absence of lesion.

M. Koopmans, S. Vernon

SESSION 2: Nervous System as a Target and Sampling Site
Co-Chairpersons: S. Cook, UMDNJ/New Jersey Medical School, Newark, and **J.L. Benach**, State University of New York, Stony Brook

P.K. Coyle, State University of New York, Stony Brook: Infectious versus autoimmune causes.
J. Wages, Etiogen Pharmaceuticals, Inc., Mountain View, California: Applying viral sequence tags to hunting for viruses.
W.I. Lipkin, University of California, Irvine: Lessons from Borna, Kawasaki, and West Nile. A retrospective analysis and algorithm for future work.
R.S. Fujinami, University of Utah School of Medicine, Salt Lake City: Virus can silently prime for autoimmune disease which can be triggered by infection.
M. Sha, Ciphergen Biosystems, Inc., Palo Alto, California: Detection and identification of protein biomarkers in pathogenic and other diseases.
S.E. Schutzer, UMDNJ/New Jersey Medical School, Newark: Identification of infections by protein microchip analysis.

SESSION 3: Factors Implicating Infectious Etiology
Co-Chairpersons: A. Steinberg, Mitretek Systems, McLean, Virginia, and **R.J. Dattwyler**, State University of New York, Stony Brook

W.C. Reeves, Centers for Disease Control & Prevention, Atlanta, Georgia: Epidemiology as a tool to identify infectious agents.
J.W. Casey, Cornell University, Ithaca, New York: Identification of new retroviruses and herpesviruses associated with neoplasias in aquatic animals.
D.N. Frank, University of Colorado, Boulder: rRNA-based community analysis of human inflammatory diseases of uncertain etiology.
G.P. Smith, University of Missouri, Columbia: Mimics of pathogen epitopes obtained without knowledge of the pathogen.
D.L. Rock, USDA Agricultural Research Service, Greenport, New York: Functional genomics of microbial threat agents.

SESSION 4: Successful Molecular Methods for Identification of Unknown Pathogens
Co-Chairpersons: B.J. Luft, State University of New York, Stony Brook, and **W.C. Reeves,** Centers for Disease Control & Prevention, Atlanta, Georgia

A.B. Pardee, Dana-Farber Cancer Institute, Boston, Massachusetts: Identification of pathogens by RT-PCR methods.
J.J. Dunn, Brookhaven National Laboratory, Upton, New York: SAGE-type protocol for identifying pathogens.
P.A. Demirev, University of Maryland, College Park: Identification of microorganisms by mass spectrometry and proteome.
P.J. Jackson, Los Alamos National Laboratory, New Mexico: Phylogenetic methods of detecting and characterizing new and previously identified infectious agents.
W.A. Bryden, Johns Hopkins University, Laurel, Maryland: MALDI-TOF mass spectrometry for biodetection and pathogen identification.
E.S. Raveche, UMDNJ/New Jersey Medical School, Newark: Antisense strategies in disease.
M.A. Hollis, Massachusetts Institute of Technology, Lexington: B-cell-based identification sensor.

SESSION 5: Novel Molecular Approaches to Identify Pathogens
Co-Chairpersons: S. Vernon, Centers for Disease Control & Prevention, Atlanta, Georgia, and **S.E. Schutzer**, UMDNJ/New Jersey Medical School, Newark

B. Kreider, Phylos, Inc., Lexington, Massachusetts: Utilization of PROfusion™ technology for the creation of highly sensitive detectors.
D.H. Farkas, Clinical Micro Sensors, Inc., Pasadena, California: Bioelectronic detection of nucleic acids.
A. Alizadeh, HHMI, Stanford University Medical School, California: Genome-wide expression profiling reveals molecular fingerprints of normal and malignant immune cells.
J. Boldrick, HHMI, Stanford University Medical School, California: Exploring host responses to infection using cDNA microarrays.
S. Salzberg, The Institute for Genomic Research, Rockville, Maryland: Computational methods for detecting unusual DNA sequences.

RNA Silencing: Functions, Mechanisms, and Applications

April 9–12

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **D.C. Baulcombe,** John Innes Centre, Norwich, United Kingdom
G. Hannon, Cold Spring Harbor Laboratory

Introduction and Overview:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
D.C. Baulcombe, John Innes Centre, Norwich, United Kingdom

SESSION 1: RNA Silencing in Diverse Systems
Chairperson: D.C. Baulcombe, John Innes Centre, Norwich, United Kingdom

E. Ullu, Yale University School of Medicine, New Haven, Connecticut: Insights into the mechanism of RNAi in trypanosomes.
L. Timmons, Carnegie Institution of Washington, Baltimore, Maryland: RNAi in *C. elegans*.
R. Carthew, University of Pittsburgh, Pennsylvania: RNAi in *Drosophila melanogaster.*
M. Zernicka-Goetz, Wellcome/CRC Institute, Cambridge, United Kingdom: Specific interference with gene function by dsRNA in early mouse development.
P.M. Waterhouse, CSIRO Plant Industry, Canberra, Australia: PTGS in plants is very efficiently induced by hairpin RNA and independent of methylation.
A.J. Maule, John Innes Centre, Norwich, United Kingdom: How low can you get? Size restraints on virus-induced gene silencing.
R. Jorgensen, University of Arizona, Tucson: Genetic determinants of patterns of cosuppression of pigment genes in petunia flowers.

SESSION 2: Spread and Maintenance of RNA Silencing
Chairperson: J.A. Birchler, University of Missouri, Columbia

A. Grishok, University of Massachusetts Medical Center, Worcester: Inheritance of RNA-induced gene silencing in C. *elegans*.
W.J. Lucas, University of California, Davis: Phloem long-distance translocation of RNA: Mechanisms and functions.
M. Kiebler, Max-Planck Institut fur Entwicklungsbiologie, Tubingen, Germany: Insights into mRNA transport and local translation in the mammalian nervous system.

L. Timmons, A. Grishok

SESSION 3: dsRNA and Transcriptional Silencing
Chairperson: P.M. Waterhouse, CSIRO Plant Industry, Canberra, Australia

J.A. Birchler, University of Missouri, Columbia: Transcriptional and posttranscriptional silencing of dispersed transgenes in *Drosophila*.
M. Matzke, Institute of Molecular Biology, Salzburg, Austria: RNA-directed methylation of plant promoter sequences.
M. Wassenegger, Fraunhofer Institut, Martinsried, Germany: RNA-directed RNA methylation.

SESSION 4: Biochemical and Molecular Approaches to Mechanism
Chairperson: E. Ullu, Yale University School of Medicine, New Haven, Connecticut

V.B. Vance, University of South Carolina, Columbia: A calmodulin-related protein suppresses posttranscriptional gene silencing in plants.
J.M. Kooter, Institute for Molecular Biological Sciences, Amsterdam, The Netherlands: Gene silencing in petunia by inverted repeats involves double-stranded RNAs and is associated with the production of small sense and antisense RNAs.
B.L. Bass, HHMI, University of Utah, Salt Lake City: Adenosine deaminases that act on RNA and their double-stranded RNA substrates.
S.M. Freier, ISIS Pharmaceuticals, Inc., Carlsbad, California: Mechanisms for regulation of mRNA expression in mammalian cells using synthetic oligonucleotides.
G. Hannon, Cold Spring Harbor Laboratory: Mechanistic studies of posttranscriptional gene silencing.
P. Zamore, University of Massachusetts, Worcester: RNAi: dsRNA directs the ATP-dependent cleavage of mRNA at 21–23-nucleotide intervals.
P. Green, Michigan State University, East Lansing: Determinants of mRNA stability in *Arabidopsis*.

SESSION 5: Genetical Approaches to Mechanism
Chairperson: R. Martienssen, Cold Spring Harbor Laboratory

M.B. Mathews, UMDNJ/New Jersey Medical School, Newark: Human dsRNa-binding proteins.
H. Cerutti, University of Nebraska, Lincoln: Posttranscriptional silencing of transgenes and transposons in a green alga: Role of an RNA helicase.
J.-B. Morel, Laboratorire de Biologie Cellulaire, Versailles, France: Characterization of *Arabidopsis* SGS genes involved in posttranscriptional (trans) gene silencing and virus resistance.
R.H. Plasterk, The Netherlands Cancer Institute, Amsterdam: Genetic links between mechanisms for transposon silencing, RNAi, and cosuppression in *C. elegans*.
D.C. Baulcombe, John Innes Centre, Norwich, United Kingdom: Genetic and biochemical dissection of RNA silencing in *Arabidopsis*.

SESSION 6: Biological Roles of RNA Silencing
Chairperson: G. Hannon, Cold Spring Harbor Laboratory

J. Carrington, Washington State University, Pullman: Viruses and RNA silencing: Stealth and sabotage.
S.-W. Ding, Institute of Molecular Agrobiology, Singapore: Silencing suppression by a nuclear protein.
S. Jensen, Institute Gustave Roussy, Villejuif, France: Cosuppression of transposable elements in *Drosophila*.
H. Lin, Duke University Medical Center, Durham, North Carolina: Role of *piwi* family genes in stem cell division and germ line development.
E.M. Maine, Syracuse University, New York: A possible function for RNA silencing in development of the *C. elegans* germ line.
R. Martienssen, Cold Spring Harbor Laboratory: Argonaute genetics in *Arabidopsis* and yeast.

SESSION 7: Overview
Chairpersons: G. Hannon, Cold Spring Harbor Laboratory; and **D.M. Glover,** University of Cambridge, United Kingdom

FMRP: What Does It Do?

April 16–19

FUNDED BY **FRAXA Research Foundation, with additional support from the National Institute of Child Health and Human Development, NIH, and National Institute of Mental Heath, NIH**

ARRANGED BY **W.T. Greenough,** University of Illinois, Urbana
D.L. Nelson, Baylor College of Medicine, Houston

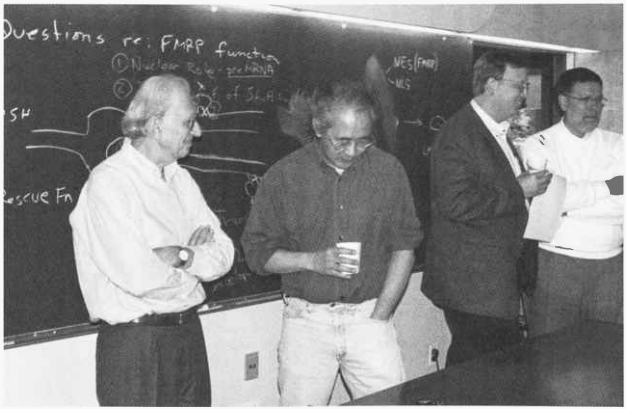
Introduction and Overview:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory: Introduction.
W.T. Greenough, University of Illinois, Urbana: Fragile X syndrome: Neural and behavioral characteristics.
D.L. Nelson, Baylor College of Medicine, Houston, Texas: Fragile X syndrome: Molecular and genetic characteristics.

SESSION 1: Fragile X Mental Retardation Protein: Insights into Neuronal Plasticity
Chairperson: D.L. Nelson, Baylor College of Medicine, Houston, Texas

J. Eberwine, University of Pennsylvania Medical School, Philadelphia: Single-cell molecular biology insights into dendritic functioning and human disease.
W.T. Greenough, University of Illinois, Urbana: Synaptic protein synthesis and FMRP.
C. Bagni, Universita' di Roma, Tor Vergata, Roma, Italy: Study of FMR1 mRNA localization and translation at the synapses.

SESSION 2: Characterization of FMRP: Genetics and Expression
Chairperson: S.T. Warren, HHMI, Emory University School of Medicine, Atlanta, Georgia

D.L. Nelson, Baylor College of Medicine, Houston, Texas: Consequences of altered FMRP expression in mice and flies.
D. Reines, Emory University School of Medicine, Atlanta, Georgia: Chromatin structure and the silent FMR1 locus in patients.
S. Adinolfi, National Institute of Medical Research, London, United Kingdom: Dissecting Fragile X mental retardation



E. Kandel, J. Yin, D. Nelson, S. Warren

protein (FMRP) in its structural and functional domains.
R.P. Bauchwitz, Columbia University, New York: Analysis of human FMR1 transgenic and fmr1(tm1Cgr) knockout mice.
E.W. Khandjian, Laval University, Quebec, Canada: Complex expression of the Fragile-X-related 1 gene in mammals.
A.T. Hoogeveen, Erasmus University Medical School, Rotterdam, The Netherlands: FMRP and the Fragile-X-related proteins (FXR1P and FXR2P).

SESSION 3: Transport and Translational Regulation
Chairperson: R.B. Darnell, The Rockefeller University, New York

A. Tartakoff, Case Western Reserve University, Cleveland, Ohio: Nucleocytoplasmic transport of RNA-binding proteins.
G.J. Bassell, Albert Einstein University, Bronx, New York: Mechanisms of mRNA targeting to neuronal dendrites and spines.
J. Esteban, Cold Spring Harbor Laboratory: Driving AMPA receptors into synapses.
B. Bardoni, CNRS/INSERM, Illkirch, France: Novel FMRP interacting proteins.
A.J. Scheetz, Yale University, New Haven, Connecticut: Cytoskeletal dynamics and translational control at developing synapses.

SESSION 4: Functional Aspects of FMRP
Chairperson: W.T. Greenough, University of Illinois, Urbana

M. Segal, The Weizmann Institute, Rehovot, Israel: FMRP involvement in formation of synapses among cultured hippocampal neurons.
E. Nimchinsky, Cold Spring Harbor Laboratory: Dendritic development in FMR1 knockout mice.
R.B. Darnell, Rockefeller University, New York: Identification of high-affinity RNA ligands for the KH-type neuronal RNA-binding proteins Nova and FMRP.
J.R. Fallon, Brown University, Providence, Rhode Island: CPEB-mediated cytoplasmic polyadenylation: A mechanism for experience-dependent local mRNA translation at synapses.
E.R. Kandel, HHMI, Columbia University, New York: Role of local protein synthesis in synapse-specific facilitation.
S.T. Warren, HHMI, Emory University School of Medicine, Atlanta, Georgia: Biochemical and neurobiological aspects of FMRP function.

SESSION 5: The Fragile X Syndrome and Mouse Models
Chairperson: I.J. Weiler, University of Illinois, Urbana-Champaign

L. Crnic, University of Colorado Health Sciences Center, Denver: Startle responses in Fragile X mice.
R. Denman, New York State Institute for Basic Research, Staten Island: The ligands of FMRP: Toward an understanding of Fragile X syndrome.
F. Tassone, University of Colorado School of Medicine, Denver: FMR1 mRNA and protein expression.
W.T. Brown, New York State Institute for Basic Research, Staten Island: Modifier genes and the Fragile X syndrome.
R.E. Paylor, Baylor College of Medicine, Houston, Texas: Behavioral characterization of *fmr1* and *fmr2* mutant mice.
M. Toth, Cornell University Medical College, New York: Abnormal processing of auditory stimuli and audiogenic seizures of FMRP knockout mice.

Regulation and Function of Heat Shock Proteins

May 2–3

ARRANGED BY **E. Craig,** University of Wisconsin, Madison
C. Gross, University of California, San Francisco
R.I. Morimoto, Northwestern University, Evanston, Illinois

SESSION 1
Chairperson: C.A. Gross, University of California, San Francisco

R.I. Morimoto, Northwestern University, Evanston, Illinois: The stress of misfolded proteins.
E.A. Craig, University of Wisconsin, Madison: Multiple Hsp70s: Clues regarding specialization.
K. Mori, Kyoto University, Japan: Analysis of the unfolded protein response.

SESSION 2
Chairperson: L.E. Hightower, University of Connecticut, Storrs

K. Nagata, Kyoto University, Japan: Substrate recognition by HSP47 and its possible functions in the collagen biosynthesis.
H. Kubota, Kyoto, Japan: Cytosolic chaperone in CCT and its eight different subunits in mammalian cells.
B. Bukau, University of Freiburg, Germany: The *E. coli* heat shock response.
T. Yura, Kyoto University, Japan: Slides

SESSION 3
Chairperson: K. Nagata, Kyoto University, Japan

S. Wickner, National Cancer Institute, NIH, Bethesda, Maryland: Protein recognition and unfolding by ClpA and degradation by ClpAP.
K. Ito, Kyoto University, Japan: Machinery for the forward and reverse movement of proteins across the *E. coli* membrane.
A.D. Grossman, Massachusetts Institute of Technology, Cambridge: DNA replication, cell cycle, and development.

SESSION 4
Chairperson: K. Mori, Kyoto University, Japan

C.A. Gross, University of California, San Francisco: Role of σ^{32} in the heat shock response.
T. Yura, Kyoto University, Japan: Heat shock response in bacteria: Recollection.
L.E. Hightower, University of Connecticut, Storrs: Bacterium meets mammal: A greeting from the heat shock response.

Toward Animal Models of Attention and Consciousness

May 14–17

FUNDED BY **The Swartz Initiative for Computational Neuroscience**

ARRANGED BY **C. Koch,** California Institute of Technology, Pasadena
A.M. Zador, Cold Spring Harbor Laboratory

SESSION 1

J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory: Introductory remarks.

C. Koch, California Institute of Technology, Pasadena: A framework for discovering the neuronal basis of consciousness.

D.J. Heeger, Stanford University, California: Attentional modulation and stimulus-evoked activity in human primary visual cortex.

G.E. Rees, California Institute of Technology, Pasadena: Linking visual attention and awareness with functional MRI.

D.A. Leopold, Max-Planck Institute for Biological Cybernetics, Tubingen, Germany: Neural correlates of multistable visual perception.

S.L. Macknik, Harvard Medical School, Boston, Massachusetts: The visibility and invisibility of spatiotemporal edges in the primate visual system.

SESSION 2

G. Tononi, The Neurosciences Institute, San Diego, California: Consciousness and complexity.

C. Brody, New York University, New York: The "unified percept" hypothesis and its quantitative neurophysiological consequences.

E.K. Miller, Massachusetts Institute of Technology, Cambridge: Executive function: The neural basis of cognitive control.

V.A.F. Lamme, Academical Medical Center, Amsterdam, The Netherlands: Neural correlates of visual awareness in Vi.

J.D. Schall, Vanderbilt University, Nashville, Tennessee: Antecedents and correlates of visual attention and awareness in prefrontal cortex.

D. Heeger, J. Reynolds, M. Sherman

SESSION 3

S.M. Sherman, State University of New York, Stony Brook: Don't forget the thalamus.
C.D. Gilbert, The Rockefeller University, New York: Attention and learning in the primary visual cortex.
P.R. Adams, State University of New York, Stony Brook: Neocortical plasticity control, thalamic bursting, and awareness.
A.K. Engel, Max-Planck-Institute for Brain Research, Frankfurt, Germany: The possible role of temporal binding for consciousness.
A.M. Zador, Cold Spring Harbor Laboratory: Is the simunculus just watching TV?

SESSION 4

Y. Miyashita, University of Tokyo School of Medicine, Japan: Top-down activation of higher-order visual representations.
N.G. Kanwisher, Massachusetts Institute of Technology, Cambridge: Mechanisms of attention in human visual cortex.
I. Fried, University of California Los Angeles School of Medicine: Single-unit recordings in the human temporal lobe during encoding and retrieval of visual stimuli.
L.R. Squire, University of California, San Diego: Conscious and nonconscious memory systems.
A.C. Nobre, University of Oxford, United Kingdom: Brain-imaging/ERP studies of attention or flexible modulation of sensor/motor processing by selective expectancies.
J.H. Reynolds, National Institute of Mental Health, NIH, Bethesda, Maryland: Visual salience, competition, and selective attention.
J. Braun, California Institute of Technology, Pasadena: Neural basis of "early" selection.
A. Pouget, University of Rochester, New York: Statistical constraints on theories of attention.
M.A. Goodale, University of Western Ontario, Canada: Dissociations between conscious visual perception and the visual control of action in neurological patients and normal observers.
C. Koch, California Institute of Technology, Pasadena: Wrap up.

Discussion: Toward Animal Models of Consciousness

Front view of Banbury Conference Center.

Mouse Behavioral Phenotyping

August 27–30

FUNDED BY **National Institute of Mental Health, NIH**

ARRANGED BY **M. Gallagher,** Johns Hopkins University, Baltimore, Maryland

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
M. Gallagher, Johns Hopkins University, Baltimore, Maryland

SESSION 1
Chairperson: J.L. Noebels, Baylor College of Medicine, Houston, Texas

Y. Benjamini, Tel Aviv University, Israel: Controlling the false discovery rate in behavior research.

W.A. Falls, University of Vermont, Burlington: Screening for sensory, motor, learning, and anxiety phenotypes in mice using startle reflex methodology.

W.N. Frankel, The Jackson Laboratory, Bar Harbor, Maine: Assessment of electroconvulsive seizure thresholds of mouse strains.

M. Gallagher, Johns Hopkins University, Baltimore, Maryland: Learning and memory assessments in mice.

SESSION 2
Chairperson: W.A. Falls, University of Vermont, Burlington

J.I. Glendinning, Columbia University, New York: High-throughput screens for assessing taste sensitivity in mice.

I. Golani, Tel Aviv University, Israel: Computerized visualization, algorithmic definition, and measurement of mouse exploratory behavior.

J. Noebels, Y. Benjamini

SESSION 3
Chairperson: B.M. Slotnick, American University, Washington, D.C.

T.A. Jones, University of Missouri, Columbia: Balance behaviors and vestibular function in four mutant strains (B6 inbred background).
D. Goldowitz, University of Tennessee, Memphis: Structure and function: Histological phenotyping of the nervous system.
G.K. Martin, University of Miami, Florida: Distortion-product otoacoustic emissions in mouse models of susceptibility to aging and noise.

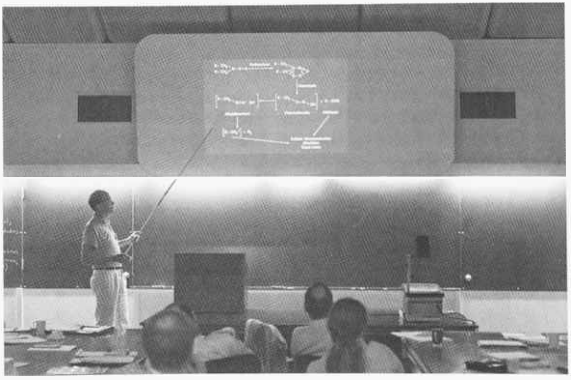
SESSION 4
Chairperson: P.C. Holland, Duke University, Durham, North Carolina

J.L. Noebels, Baylor College of Medicine, Houston, Texas: Mutational analysis of brain rhythms and cortical excitability.
B.F. O'Hara, Stanford University, California: A high throughput piezoelectric system for monitoring sleep and wake.
R.E. Paylor, Baylor College of Medicine, Houston, Texas: Organismic and experiential factors influencing the behavioral characterization of inbred mice.
E. Pugh, University of Pennsylvania, Philadelphia: Phenotyping the mouse visual system.

SESSION 5
Chairperson: R.E. Paylor, Baylor College of Medicine, Houston, Texas

B.M. Slotnick, American University, Washington, D.C.: Mouse olfaction assessed using conditioned avoidance and by olfactometry.
M.G. Tordoff, Monell Chemical Senses Center, Philadelphia, Pennsylvania: Use of the two-bottle choice test to assess mouse taste preferences.
R.W. Williams, University of Tennessee, Memphis: Efficient mapping of neuroanatomical and behavioral QTLs with subcentimorgan prevision using 5000 isogenic RIX lines.

Summary and Future Plans



Overhead slide projection presentation.

Neural Networks and Cognition

September 10–13

FUNDED BY **Handspring Inc.**

ARRANGED BY **A.M. Zador,** Cold Spring Harbor Laboratory
Z.F. Mainen, Cold Spring Harbor Laboratory
A. Pouget, University of Rochester, New York

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1
Chairperson: A. Pouget, University of Rochester, New York

A.M. Zador, Cold Spring Harbor Laboratory: Dynamic computation in the cortex: The cocktail party problem.
M.J. Tarr, Brown University, Providence, Rhode Island: Cortical brain networks automatized by expertise.
R.S. Zemel, University of Arizona, Tucson: Bayesian inference using population codes.
J. Tenenbaum, Stanford University, California: Bayesian inference in human cognition.
M. Mozer, University of Colorado, Boulder: Temporal dynamics of information transmission in a Bayesian cognitive architecture.
Y. Weiss, University of California, Berkeley: Bayesian inference in distributed networks: From psychophysics to error-correcting codes.

SESSION 2
Chairperson: Z.F. Mainen, Cold Spring Harbor Laboratory

J.C. Hawkins, Handspring Inc., Mountain View, California: Neural networks and cognition.
P. Kanerva, Swedish Institute of Computer Science, Kista, Sweden: Stochastic pattern computing as a model of computing by the brain.
J. Kristoferson, Swedish Institute of Computer Science, Kista, Sweden: On different latent semantic techniques to construct high-dimensional distributed representations of words in large corpora.
G. Sjodin, Swedish Institute of Computer Science, Kista, Sweden: Raising the abstraction level and finding semantic/syntactic concepts in large corpora by using latent semantic techniques.
B.A. Pearlmutter, University of New Mexico, Albuquerque: Independent components analysis.
S. Roweis, University College London, United Kingdom: Unsupervised learning of nonlinear manifolds.

P. Latham, A. Koulakov, Z. Mainen

SESSION 3
Chairperson: D. Chklovskii, Cold Spring Harbor Laboratory

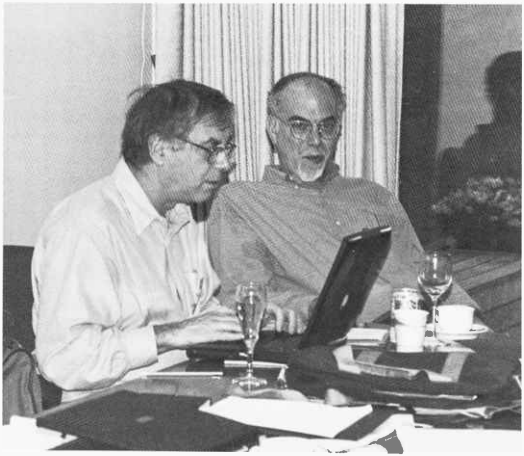
J.J. Hopfield, Princeton University, New Jersey: Large N representations, algorithms, and dynamics.
P. Latham, University of California, Los Angeles: Attractors in realistic neuronal networks: Can they exist?
A. Koulakov, Salk Institute, La Jolla, California: A sandpile model of neural integrator.
P. Dayan, University College, London, United Kingdom: Statistical model of attention and conditioning.
Z. Li, University College, London, United Kingdom: Vision in V1, a network model for the theory of pre-attentive vision.
T. Hely, The Santa Fe Institute, New Mexico: Role of feedback in information processing in the brain.

SESSION 4
Chairperson: B.W. Mel, University of Southern California, Los Angeles

B.W. Mel, University of Southern California, Los Angeles: Dendrites may be why you're so smart.
D. Chklovskii, Cold Spring Harbor Laboratory: Wire length minimization is a powerful tool for uncovering brain circuits.
B. Olshausen, University of California, Davis: Sparse coding of images in space and time.
N. Tishby, The Hebrew University, Jerusalem, Israel: Analysis of neural codes via the information bottleneck method.
A.V.M. Herz, Homboldt University, Berlin, Germany: Interplay of firing rates, firing times, and adaptation.
A. Pouget, University of Rochester, New York: Efficient computation and cue integration with population codes.

SESSION 5
Chairperson: A.M. Zador, Cold Spring Harbor Laboratory

D. Willshaw, University of Edinburgh, United Kingdom: Role of the subthalamic nucleus in the basal ganglia.
E. Todorov, University College London, United Kingdom: Constraints on neural processing in the motor system imposed by properties of the motor periphery.
D.D. Lee, Bell Laboratories, Murray Hill, New Jersey: Making a robotic dog see and hear.



E. Ziff, J. Lisman

Structure, Mechanism, and Function of CaMKII

September 17–20

FUNDED BY **Marie H. Robertson Memorial Fund for Neurobiology**

ARRANGED BY **H. Cline,** Cold Spring Harbor Laboratory
J.E. Lisman, Brandeis University, Waltham, Massachusetts

SESSION 1
Chairperson: A. Nairn, The Rockefeller University, New York

A. Nairn, The Rockefeller University, New York: CaM kinase: Structure and mechanism.
N. Waxham, University of Texas Health Science Center, Houston: The three-dimensional structure of CaMKII reveals its unique subunit organization.

SESSION 2
Chairperson: S. Halpain, The Scripps Research Institute, La Jolla, California

S. Halpain, The Scripps Research Institute, La Jolla, California: Multifunctional MAP2 as a target of multifunctional CaMKII.
T. Meyer, Duke University Medical Center, Durham, North Carolina: Molecular memory by translocation priming and postsynaptic trapping of CaMKII.
T.S. Reese, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Maryland: Distribution of CaMKII at the postsynaptic density (at rest and during activity).
A. Dosemeci, Marine Biological Laboratory, Woods Hole, Massachusetts: CaMKII clustering: Mechanism and function.

SESSION 3
Chairperson: M.H. Sheng, Massachusetts General Hospital, Boston

M.H. Sheng, Massachusetts General Hospital, Boston: Phosphorylation of PSD proteins by CaMKII.
M.B. Kennedy, California Institute of Technology, Pasadena: Postsynaptic targets for phosphorylation by CaMKII.
R.J. Colbran, Vanderbilt University School of Medicine, Nashville, Tennessee: Mechanisms of CaMKII association with postsynaptic densities.
J.W. Hell, University of Wisconsin, Madison: Regulation and physiological significance of the interaction between CaMKII and the NMDA receptor.

A. Dosemeci, S. Halpain

SESSION 4
Chairperson: H. Schulman, Stanford University School of Medicine, California

H. Schulman, Stanford University School of Medicine, California: Activity-dependent targeting and autophosphorylation both switch the functional state of CaMKII.

J.E. Lisman, Brandeis University, Waltham, Massachusetts: New mechanisms for PSD CaMKII as a memory element.

SESSION 5
Chairperson: R.A. Nicoll, University of California, San Francisco

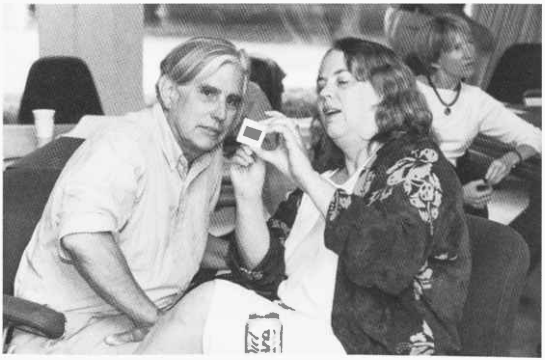
T.R. Soderling, Oregon Health Sciences University, Portland, Oregon: Regulation of AMPA-Rs by CaMKII in LTP.
R. Malinow, Cold Spring Harbor Laboratory: CaMKII controls synaptic delivery of GluR1.
R.L. Huganir, HHMI, Johns Hopkins University, Baltimore, Maryland: Regulation of AMPA receptors and synaptic plasticity.
R. Nicoll, University of California, San Francisco: Role of CaMKII in Ca channel and non-NMDA receptor-dependent synaptic plasticity.
K. Fukunaga, Kumamoto University School of Medicine, Japan: Molecular targets of CaMKII involved in the hippocampal LTP.

SESSION 6
Chairperson: J.E. Lisman, Brandeis University, Waltham, Massachusetts

E.M. Landau, Mt. Sinai School of Medicine, New York: Regulation of CaMKII activity in transmitter-dependent LTP.
R.H. Kramer, University of Miami School of Medicine, Florida: Role of CaMKII in long-term plasticity of cyclic nucleotide metabolism revealed with the "patch-cramming" technique.
N. Otmakhov, Brandeis University, Waltham, Massachusetts: Is persistent activity of calcium/calmodulin-dependent kinase required for the maintenance of LTP?
L.C. Griffith, Brandeis University, Waltham, Massachusetts: Genetic manipulation of CaMKII and its targets.
A.J. Silva, University of California, Los Angeles: CaMKII's role in short-term plasticity, LTP, and memory.
M.R. Mayford, University of California, La Jolla: CaMKII function in dendrites and during development.

SESSION 7
Chairperson: H. Cline, Cold Spring Harbor Laboratory

K. Fox, Cardiff University, Wales: Role of CaMKII in plasticity in vivo.
J.R. Fallon, Brown University, Providence, Rhode Island: Regulation of CaMKII translation at synapses.
M. Constantine-Paton, Massachusetts Institute of Technology, Cambridge: NMDAR control of CaMKII during nervous system development.
H. Cline, Cold Spring Harbor Laboratory: CaMKII mediates maturation of the visual system in vivo.



T. Reese, M. Kennedy, S. Halpain

Persistent Neural Activity

October 1–4

FUNDED BY **Alfred P. Sloan Foundation and The Swartz Initiative for Computational Neuroscience**

ARRANGED BY **H.S. Seung,** Massachusetts Institute of Technology, Cambridge
D.W. Tank, Bell Laboratories, Lucent Technologies, Murray Hill, New Jersey

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1
Chairperson: C. Brody, New York University, New York

P.S. Goldman-Rakic, Yale University School of Medicine, New Haven, Connecticut: Prefrontal microcircuits and the temporal dynamics of working memory.
M. D'Esposito, University of California, Berkeley: Toward understanding the role of prefrontal cortex in working memory: Evidence from functional MRI.
X.-J. Wang, Brandeis University, Waltham, Massachusetts: Synaptic and cellular mechanisms of working memory: Stable persistent activity and NMDA receptors.
M. Bodner, University of California, Los Angeles: Cortical attractors in working memory.
D. Durstewitz, Salk Institute for Biological Studies, La Jolla, California: Modulatory control of and transitions in working memory.

SESSION 2
Chairperson: P.S. Goldman-Rakic, Yale University School of Medicine, New Haven, Connecticut

R.A. Andersen, California Institute of Technology, Pasadena: The nature of delay activity in the posterior parietal cortex.
P.W. Mitra, Lucent Technologies, Murray Hill, New Jersey: Tuned temporal structure in neural activity during working memory.
E. Zohary, Hebrew University, Jerusalem, Israel: Strategies of visual memory: Behavioral, neuronal, and computational perspectives.
M. Griniasty, Intel Cellular Communication Division, Givat Shmuel, Israel: Correlations between patterns of persistent neural activity and the Hopfield model.
J.E. Lisman, Brandeis University, Waltham, Massachusetts: Mechanisms of multi-item working memory.

Posters:

A. Koulakov, Salk Institute, La Jolla, California: A sandpile model of neural integrator.

A. Renart, Brandeis University, Waltham, Massachusetts: Low rate and highly variable persistent activity in a micro-columnar LIF network model.

B. Pesaran, California Institute of Technology, Pasadena: Spectral measures of temporal structure in neuronal activity.

SESSION 3

Chairperson: J.E. Lisman, Brandeis University, Waltham, Massachusetts

C. Brody, New York University, New York: Single and multi-electrode recordings in primate prefrontal cortex during parametric working memory tasks.

H.I. Sompolinsky, The Hebrew University, Jerusalem, Israel: Balanced states and multiple attractors in cortical networks.

G.B. Ermentrout, University of Pittsburgh, Pennsylvania: Mechanisms underlying maintained activity.

S. Wise, National Institutes of Health, Bethesda, Maryland: Empirical dissociation of confounded spatial variables in instructed-delay tasks: Attention vs. memory vs. gaze vs. intention vs. cue vs. target.

W. Schultz, University Fribourg, Switzerland: Predictive coding of behavioral outcomes in primate basal ganglia and frontal cortex.

SESSION 4

Chairperson: M.N. Shadlen, University of Washington, Seattle

C.R. Gallistel, Rutgers University, Piscataway, New Jersey: Various behaviors that appear to require integration with respect to time.

C. Kaneko, University of Washington, Seattle: Neural integration in the oculomotor system of the alert monkey.

H.S. Seung, Massachusetts Institute of Technology, Cambridge: Recurrent network models of the oculomotor integrator.

D.W. Tank, Lucent Technologies, Murray Hill, New Jersey: Persistent activity in a goldfish oculomotor neural integrator.

R. Baker, New York University School of Medicine, New York: Neural basis and function of eye velocity storage.

E.E. Fetz, University Washington School of Medicine, Seattle: Neural mechanisms mediating persistent activity in primate motorcortical and spinal circuits.

J.J. Hopfield, Princeton University, New Jersey: What is a moment? "Cortical" sensory integration over a brief interval.

SESSION 5

Chairperson: R.A. Andersen, California Institute of Technology, Pasadena

J.S. Taube, Dartmouth College, Hanover, New Hampshire: Persistent neural activity in the head-direction cell network.

K. Zhang, The Salk Institute, La Jolla, California: Attractor theories of the head-direction system: Necessary features and difficulties.

B.L. McNaughton, University of Arizona, Tucson: Continuous and discontinuous attractor dynamics in the hippocampus.

D.S. Touretzky, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Attractor maps in the rodent hippocampus.

D.A. McCormick, Yale University School of Medicine, New Haven, Connecticut: Cellular basis for recurrent and rhythmic spontaneous activity in the cerebral cortex.

SESSION 6

Chairperson: S. Wise, National Institutes of Health, Bethesda, Maryland

M.N. Shadlen, University of Washington, Seattle: Neural integration in the parietal cortex: Accumulating the evidence.

C. Chow, University of Pittsburgh, Pennsylvania: A spiking neuron model of binocular rivalry.

Social Venture Capital for Neglected Vaccines: Creating Successful Alliances

October 10–12

FUNDED BY **Albert B. Sabin Vaccine Institute, Inc., through a grant from the Bill and Melinda Gates Foundation**

ARRANGED BY **M. Moree,** PATH/Malaria Vaccine Initiative, Seattle, Washington
R. Rabinovich, PATH/Malaria Vaccine Initiative, Rockville, Maryland
P.K. Russell, Albert B. Sabin Vaccine Institute, Inc., New Canaan, Connecticut

Introduction and Charge to the Conference:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
H.R. Shepherd, Albert B. Sabin Vaccine Institute, New Canaan, Connecticut
P.K. Russell, Albert B. Sabin Vaccine Institute, Inc., New Canaan, Connecticut
R. Rabinovich, PATH/Malaria Vaccine Initiative, Rockville, Maryland

SESSION 1: Perspectives on the Development of Neglected Vaccines

M. Moree, PATH/Malaria Vaccine Initiative, Seattle, Washington: Public sector.
L.K. Gordon, OraVax Inc., Cambridge, Massachusetts: Industry.
J.V. Scott, New York University, New York: Academia.
S.M. Ferguson, Office of Technology Transfer, National Institutes of Health, Rockville, Maryland: Government.

SESSION 2: Mechanisms for Public-Private Partnerships

H. Kettler, Office of Health Economics, London, United Kingdom: Narrowing the gap between provision and need for vaccines in developing countries.
C. Elias, PATH/Malaria Vaccine Initiative, Seattle, Washington: Public-private partnerships for contraceptives and microbicides.

SESSION 3: Alliance Management

M. McDade, Corixa Corporation, Seattle, Washington: Industry.
L. Nelsen, Massachusetts Institute of Technology, Cambridge, Massachusetts: Academia.

SESSION 4: Commitments to Ensure Availability and Accessibility of Vaccines for Developing Country Markets

P. Young, AlphaVax, Durham, North Carolina: Biotech.
L. Barreto, Aventis Pasteur, Lyon, France: Pharma.
R.P. Eddy, U.S. Mission to the United Nations, New York: News from the White House.
J.-F. Martin, Parteurop S.A., Lyon, France: The Global Children's Vaccine Fund.

SESSION 5: What should be done to move the development of neglected vaccines forward? How do we form better partnerships?
Chairperson: R. Rabinovich, PATH/Malaria Vaccine Initiative, Rockville, Maryland

PANEL:
R.J. Saldarini, Mahwah, New Jersey
C. McFadden, Dewey Ballantine, Washington, D.C.
C. Nacy, Sequella, Inc., Rockville, Maryland
T. Lakavage, SmithKline Beecham Biologicals, Rixensart, Belgium

A. Batson, W. Koff, T. Elliott

Meeting the Challenge of Infectious Diseases in the 21st Century

October 15–18

FUNDED BY **Agricultural Research Service, U.S. Department of Agriculture**

ARRANGED BY **R. Breeze,** U.S. Department of Agriculture, Athens, Georgia

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
R. Breeze, U.S. Department of Agriculture, Athens, Georgia

SESSION 1: Microbial Genomics and Pathogenesis

D.L. Rock, USDA Agricultural Research Service, Greenport, New York: Functional genomics of viruses.
L.W.J. Baillie, Defence Evaluation Research Agency, Salisbury, United Kingdom: Functional genomics of bacteria.
R. Dean, North Carolina State University, Raleigh: Functional genomics of fungi.
B. Sobral, Virginia Polytechnic Institute & State University, Blacksburg: Role of bioinformatics in biosafety.

SESSION 2: Pathogenesis, Prevention, and Control of Infectious Diseases: The Next 10 Years: Plants

S. Leath, North Carolina State University, Raleigh: Improving disease resistance of crops through breeding and genomics.
E. Buckler, North Carolina State University, Raleigh: Structural and functional genomics of complex traits in field crops.
H.C. Kistler, University of Minnesota, St. Paul: Fungal plant pathogenicity: Future directions.
S.A. Lommel, North Carolina State University, Raleigh: Plant virus pathogenicity: State of the art and future directions.
R.P. Niedz, U.S. Horticultural Research Laboratory, Ft. Pierce, Florida: Woody perennials: Biological considerations and genomic applications.

SESSION 3: Pathogenesis, Prevention, and Control of Infectious Diseases: The Next 10 Years: Animals

W. Laegreid, USDA Agricultural Research Service, Clay Center, Nebraska: Functional genomics and disease control in livestock.
G. Letchworth, USDA Agricultural Research Service, ABADRL, Laramie, Wyoming: Functional genomics of pathogen infection, replication, survival, and transmittal in vectors: Ideas for disease intervention.
M. Jutila, Montana State University, Bozeman: Analysis of bovine γ/δ T cells: Gene expression and function.
T.J. Leighton, University of California, Berkeley: Anthrax: Solving pathobiology problems with genomic information.
D. Knowles, ARS-USDA, Washington State University, Pullman: *Anaplasma marginale:* Efficient use of a small genome to generate antigenic diversity.
B.J. Luft, State University of New York, Stony Brook: Rational vaccine design.
J.J. Dunn and S. Swaminathan, Brookhaven National Laboratory, Upton, New York: OspC of *Borrelia burgdorferi:* Clues to biochemical function from genomics and structural analysis.
J.N. MacLachlan, University of California, Davis: New and emerging virus diseases: What horses can tell us.
D.E. Swayne, USDA Agricultural Research Service, Athens, Georgia: Viral ecology and pathogenesis in future diagnosis and control of avian influenza.

SESSION 4: Detection, Identification, Forensics, and Diagnosis I

W.A. Bryden, Johns Hopkins University, Laurel, Maryland and J. Jackman, Johns Hopkins University, Laurel, Maryland: MALDI-TOF mass spectrometry for biodetection and pathogen identification.

P. Keim, Northern Arizona University, Flagstaff: High-resolution pathogen typing: Epidemiological implications.

Interim Discussion:

Chairperson: R. Breeze, U.S. Department of Agriculture, Athens, Georgia

SESSION 5: Detection, Identification, Forensics, and Diagnosis II

T.J. Leighton, University of California, Berkeley: Ab initio construction of definitive anthrax group diagnostic reagents.

W.I. Lipkin, University of California, Irvine: Pathogen discovery: From Borna to West Nile and beyond.

K. Lohman, AFIERA, San Antonio, Texas: Rapid identification of pathogens from clinical specimens using real-time fluorescent PCR.

J. Mullet, Texas A&M University, College Station: Use of remote sensing and satellite imaging in conjunction with DNA diagnostics for early detection of plant pathogens.

S.E. Schutzer, UMDNJ/New Jersey Medical School, Newark: Detection of the unknown pathogen: Methods and approach.

Summing Up:

Chairperson: R. Breeze, U.S. Department of Agriculture, Athens, Georgia

Slide presentation during a meeting.

J.P. Morgan & Co., Incorporated/Cold Spring Harbor Laboratory Executive Conference on Human Intelligence and Consciousness

October 19–21

ARRANGED BY **J.D. Watson,** Cold Spring Harbor Laboratory
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1

J.D. Watson, Cold Spring Harbor Laboratory: Welcoming remarks.
O. Sacks, Department of Neurology, Albert Einstein College of Medicine, Bronx, New York: Prodigies.

SESSION 2

H. Gardner, Harvard University Graduate School of Education, Cambridge, Massachusetts: How much of intelligence is in the brain?
I. Pepperberg, University of Arizona, Tucson: Cognitive and communicative abilities of Alex the Grey Parrot.

SESSION 3

C. Murray, American Enterprise Institute for Public Policy Research, Washington, D.C.: The intersection of intelligence and policy: Two approaching problems.

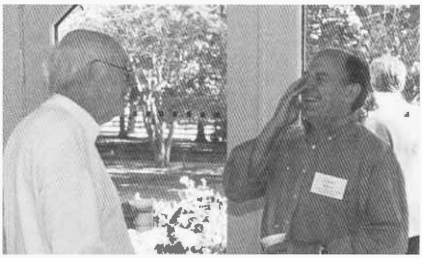
SESSION 4

Z.F. Mainen, Cold Spring Harbor Laboratory: Olfaction: A complex sensory function.
A.M. Zador, Cold Spring Harbor Laboratory: Selective hearing: The "cocktail party problem."

SESSION 5

S. Pinker, Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge: Words and rules: The ingredients of language.
V.S. Ramachandran, University of California, San Diego: What neurology can tell us about human nature, synethesia, and the meaning of art.
R.A. Brooks, Artificial Intelligence Laboratory, Massachusetts Institute of Technology, Cambridge: Building intelligent sociable machines: Understanding human intelligence.

Discussion and Closing Remarks:
J.D. Watson, Cold Spring Harbor Laboratory



J.D. Watson, C. Murray

A. Berry, J. Hawkins

R. Brooks, H. Gardner, D. Pakianathan

Natural Stimulus Statistics

October 22–25

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **S. Laughlin,** University of Cambridge, United Kingdom
P. Reinagel, Harvard Medical School, Boston, Massachusetts

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
S. Laughlin, University of Cambridge, United Kingdom,
P. Reinagel, Harvard Medical School, Boston, Massachusetts

SESSION 1
Chairperson: D.L. Donoho, Stanford University, California

W. Bialek, NEC Research Institute, Inc., Princeton, New Jersey: Finding relevant features in natural signals.
H. Barlow, University of Cambridge, United Kingdom: Help and hindrance from redundancy.
D. Mumford, Brown University, Providence, Rhode Island: Searching for an explicit stochastic model for natural scene image patches.
B. Olshausen, University of California, Davis: Sparse coding of natural images: Space, time, and color.
D. Tolhurst, University of Cambridge, United Kingdom: Measuring sparse coding: Definitions and confusions.

SESSION 2
Chairperson: S. Laughlin, University of Cambridge, United Kingdom

Y. Gousseau, CNRS-ENS Cachan, France: Morphological statistics of natural images.
E. Simoncelli, New York University, New York: Image statistics, Gaussian scale mixture models, and divisive normalization.
D.W. Dong, Florida Atlantic University, Boca Raton: Eye movements and spatiotemporal input statistics during free-viewing natural time-varying images.
K. Kording, Institute for Neuroinformatik, Zurich, Switzerland: What a cat sees and what algorithms can learn from this.
R. Kern, Universitat Bielefeld, Germany: Representation of behaviorally generated optic flow in a fly visual interneuron.
R. de Ruyter, NEC Research Institute, Inc., Princeton, New Jersey: Motion detection in the wild: Natural stimuli and information transmission in a blowfly motion-sensitive neuron.

B. Olshausen, H. Barlow, W. Geisler

SESSION 3
Chairperson: M. Meister, Harvard University, Cambridge, Massachusetts

A. Fairhall, NEC Research Institute, Inc., Princeton, New Jersey: Olfaction from the point of view of physics.
F. Grasso, Boston University Marine Program, Woods Hole, Massachusetts: Olfaction, turbulence, and odor plumes: Structure from concentration dynamics.
G. Laurent, California Institute of Technology, Pasadena: Reformatting and optimization of odor representations in the zebrafish olfactory bulb.
M.S. Lewicki, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Learning efficient codes for natural scenes and sounds: A principle for sensory coding.
P. Penev, The Rockefeller University, New York: Factorial transmission of time-varying natural stimuli with sparse, interacting unitary events: Spiking for speech and movies.

SESSION 4
Chairperson: D.J. Field, Cornell University, Ithaca, New York

F. Theunissen, University of California, Berkeley: Analyzing auditory neurons with natural and synthetic sounds.
K. Sen, University of California, San Francisco: Hierarchical processing of natural sounds in the songbird auditory forebrain.
E. Nelken, Hebrew University-Hadassah Medical School, Jerusalem, Israel: Coding of foregrounds and backgrounds in auditory scenes.
P. Reinagel, Harvard Medical School, Boston, Massachusetts: Coding of temporal visual information by LGN neurons.
Y. Dan, University of California, Berkeley: Analysis of visual coding in the LGN and V1.
J.L. Gallant, University of California, Berkeley: Using natural scenes to reveal coding properties in visual cortex.

SESSION 5
Chairperson: D. Osorio, University of Sussex, Brighton, United Kingdom

W.S. Geisler, University of Texas, Austin: Perceptual grouping and the Bayesian co-occurrence statistics of features in natural images.
J. Malik, University of California, Berkeley: Ecological statistics of Gestalt grouping factors.
M. Vorobyev, University of Maryland, Baltimore: Color coding of signals and backgrounds.
E.H. Adelson, Massachusetts Institute of Technology, Cambridge: Statistical aspects of lightness estimation.

D. Field, E. Simoncelli

Sugar Sensing and Signaling in Plants and Other Organisms

October 29–November 1

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **M. Johnston,** Washington University Medical School, St. Louis, Missouri
J. Sheen, Massachusetts General Hospital, Boston
M. Stitt, University of Heidelberg, Germany

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Transporter Proteins, GPCR, and Protein *Kinases* in Glucose Signaling
Chairperson: M. Johnston, Washington University Medical School, St. Louis, Missouri

M. Johnston, Washington University Medical School, St. Louis, Missouri: Metabolic and receptor-mediated glucose signals regulate gene expression in yeast by different mechanisms.
S. Ozcan, University of Kentucky College of Medicine, Lexington: Regulation of gene expression by glucose in yeast and in mammalian cells.
M. Carlson, Columbia University, New York: Snf1 protein kinase and glucose signaling in yeast.
G. Hardie, University of Dundee, United Kingdom: SNF1/AMP-activated protein kinases: Highly conserved kinase cascades involved in sugar sensing in plants, animals, and yeast.
J. Thevelein, Catholic University of Louvain, Belgium: Both a G-protein-coupled receptor system and glucose phosphorylation are required for glucose activation of the cAMP-PKA pathway in yeast.

SESSION 2: Glucose, Mannose, and Sucrose Sensing and Signaling in Plants I
Chairperson: K.E. Koch, University of Florida, Gainesville

C. MacKintosh, University of Dundee, United Kingdom: 14-3-3s regulate the global cleavage of their diverse binding partners in sugar-starved *Arabidopsis* cells.
U. Wobus, Institute Pflanzenetik und Kulturpflanzenforschung, Gatersleben, Germany: Sugar gradients, sugar metabolism, and sugar sensing in developing plants seeds.
K.E. Koch, University of Florida, Gainesville: A central role for sucrose metabolism in sugar signaling: A whole plant context.
I. Graham, University of York, Sand Hutton, United Kingdom: Why is trehalose metabolism essential for *Arabidopsis* seed development?
T. Roitsch, University of Regensburg, Germany: Differential regulation of source and sink metabolism by sugars.
D.R. Bush, University of Illinois, Urbana: Sucrose signaling and phloem loading.
J.-C. Jang, Ohio State University, Columbus: Signal transduction of glucose-regulated cell expansion.
M. Bevan, John Innes Centre, Norwich, United Kingdom: Genetic analysis of sucrose-mediated transcription of genes encoding enzymes of starch biosynthesis.

M. Bevan

SESSION 3: Glucose, Mannose, and Sucrose Sensing and Signaling in Plants II
Chairperson: W.-B. Frommer, University of Tubingen, Germany

S.C. Huber, North Carolina State University, Raleigh: Sucrose synthase and SNF1-related protein kinases: New components of sugar sensing in plants?
W.-B. Frommer, University of Tubingen, Germany: Sucrose transporters and sugar sensing.
P.S. Chourey, University of Florida, Gainesville: Mannose induces global gene repression in maize through a signaling pathway that is independent from sugar depletion.

SESSION 4: Glucose and *Galactose* Sensing and Signaling in Yeast/Mammals
Chairperson: M. Carlson, Columbia University, New York

C. Michels, Queens College of CUNY, Flushing, New York: Maltose sensing and signaling in *Saccharomyces* and its glucose regulation.
J.E. Hopper, Pennsylvania State University, Hershey: The Gal3p-Gal80p-Gal4p transcription switch of the yeast, *Saccharomyces cerevisiae:* A galactose-sensing switch.
C. Hoffman, Boston College, Chestnut Hill, Massachusetts: Glucose detection and adenylate cyclase activation in fission yeast.
W. Heideman, University of Wisconsin, Madison: Trying to connect the effects of glucose on growth with effects on the cell cycle in yeast.

SESSION 5: Glucokinases and Hexokinases
Chairperson: J. Sheen, Massachusetts General Hospital, Boston

M.A. Magnuson, Vanderbilt University School of Medicine, Nashville, Tennessee: Glucokinase: Role as glucose signal mediator in mammals.
D. Granot, Agricultural Research Organization, Bet Dagan, Israel: Comparative roles of hexokinase and fructokinase in tomato sugar sensing.
J. Sheen, Massachusetts General Hospital, Boston: Plant hexokinases: Novel features and functions.

SESSION 6: Sugar and Hormones and Nitrogens
Chairperson: G. Coruzzi, New York University, New York

S.C. Smeekens, University of Utrecht, The Netherlands: Interactions between sugar and hormonal signaling pathways in plants.
P. Leon, Instituto de Biotechnologia, UNAM, Morelos, Mexico: Sugar sensing and signaling in plants and other organisms.
D.R. Bush, University of Illinois, Urbana: Sucrose signaling in phloem loading.
A. Krapp, University of Heidelberg, Germany: Metabolic signaling in C/N interaction.
G. Coruzzi, New York University, New York: Sugar signaling and C:N regulation of amino acid biosynthesis in *Arabidopsis*.

M. Magnuson, K. Koch

Getting Rid of the Bodies

November 12–15

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **G. Chimini,** INSERM-CNRS, Marseille, France
M. Hengartner, Cold Spring Harbor Laboratory

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Membrane Events
Chairperson: J. Savill, Royal Infirmary, Edinburgh, United Kingdom

J. Borst, The Netherlands Cancer Institute, Amsterdam: Mechanism and relevance of sphingomyelin hydrolysis during apoptosis.
P. Williamson, Amherst College, Massachusetts: Phospholipid scramblase activation in apoptotic lymphocytes.
C.J. Fielding, University of California, San Francisco: Modification of cell surface phospholipids.
G. Chimini, INSERM-CNRS de Marseille Luminy, France: Membrane lipid architecture and competence to engulf.
R.A. Schlegel, Pennsylvania State University, University Park: Necessity and sufficiency of loss of phospholipid asymmetry by target cells and macrophages for phagocytosis.

SESSION 2: Apoptotic Receptors and Downstream Signaling
Chairperson: M. Hengartner, Cold Spring Harbor Laboratory

V.A. Fadok, National Jewish Medical and Research Center, Denver, Colorado: Engagement of a new receptor for phosphatidylserine is required for engulfment of apoptotic cells by phagocytes.
G. Matsushima, University of North Carolina, Chapel Hill: Role of Mer in macrophages and autoimmunity.
C.D. Gregory, University of Nottingham Medical School, United Kingdom: Role of CD14 in apoptotic-cell clearance.
S.C. Finnemann, Weill Medical College of Cornell University, New York: Focal adhesion kinase dynamics during $\alpha 1\beta 5$ integrin-dependent photoreceptor phagocytosis by the retinal pigment epithelium.
K. Ravichandran, University of Virginia, Charlottesville: Role for CrkII and lipid raft domains in engulfment of apoptotic cells.
M.L. Albert, The Rockefeller University, New York: Vivir La Muerte: The $\alpha v B5$ integrin recruits the CrkII/DOCK 180 molecular switch for phagocytosis of apoptotic cells.

N. Franc, P. Williamson, G. Chimini

SESSION 3: Modulation of Phagocytosis
Chairperson: C.D. Gregory, University of Nottingham Medical School, United Kingdom

S. Gallucci, National Institutes of Allergy and Infectious Diseases, NIH, Bethesda, Maryland: Induction of apoptosis in dendritic cells by an exogenous danger signal.
G. Randolph, Mt. Sinai School of Medicine, New York: Differentiation of monocytes into migratory dendritic cells after delivery of a phagocytic stimulus.
I. Dransfield, University of Edinburgh Medical School, United Kingdom: Regulation of macrophage capacity for phagocytosis of apoptotic cells.
D. Mevorach, Tel Aviv Medical Center, Israel: Getting rid of the bodies: The milieu determines patterns of uptake.

SESSION 4: Invertebrate Systems
Chairperson: S. Nagata, Osaka University Medical School, Japan

M. Hengartner, Cold Spring Harbor Laboratory: Engulfment genes cooperate with *ced-3* to promote apoptosis in C. *elegans*.
Z. Zhou, Massachusetts Institute of Technology, Cambridge: *C. elegans* CED-1 is a transmembrane receptor that recognizes cell corpses and initiates their engulfment.
M.A. Driscoll, Rutgers, State University of New Jersey, Piscataway: A monopoly of undertakers: A common set of genes mediate the removal of both apoptotic and necrotic cellcorpses in *C. elegans*.
N.C. Franc, Massachusetts General Hospital, East Charlestown: Phagocytosis of apoptotic cells by *Drosophila* (getting rid of the bodies and fly away).

SESSION 5: In Vivo Models
Chairperson: G. Chimini, INSERM-CNRS, Marseille, France

J. Savill, Royal Infirmary, Edinburgh, United Kingdom: Comparison of clearance in vitro and in vivo.
M. Botto, Imperial College School of Medicine, London, United Kingdom: Complement, apoptosis, and autoimmunity.
P.B. Martin, University College London, United Kingdom: Clearance of apoptotic debris in macrophageless PPU.1 KO mice.
S. Nagata, Osaka University Medical School, Japan: DNA fragmentation by phagocytes and its abnormality.
G. Bokoch, The Scripps Research Institute, La Jolla, California: Macropinocytosis.

Points for Discussion
Chairperson: M. Hengartner, Cold Spring Harbor Laboratory

Universality of the Apoptotic Process
Diseases and Apoptosis
Other Points

Signaling Network Control-Cell Interactions: Phase II Meeting

November 27–29

FUNDED BY **National Institute of General Medical Sciences, NIH**

ARRANGED BY **R. Iyengar,** Mount Sinai School of Medicine, New York

Overview:
R. Iyengar, Mount Sinai School of Medicine, New York

Opening:
Comments by Bridging Grant Clusters Leaders
Open Discussion (Data Exchange)

Break Out Sessions for the five Bridging Grant Clusters I
Report back of the Break Out Sessions (Integration I)
Break Out Sessions for the five Bridging Grant Clusters II
(Members participating in two Bridging Grant Clusters can switch groups in the two sessions)
Report back of the Break Out Sessions (Integration II)

Talks:
B. Ray, Science, Washington, D.C.
J.D. Scott, Oregon Health Sciences University, Portland, Oregon
R.W. Tsien, Stanford University School of Medicine, California

Role of the Facilities
Discussion Leaders:
H.E. Hamm, Northwestern University Medical School, Chicago, Illinois
H. Weinstein, Mount Sinai School of Medicine, New York

RALs and Their Relationships to Bridging Grant Clusters
Discussion Leaders:
M.P. Sheetz, Columbia University, New York
T.C. Sudhof, University of Texas Southwestern Medical Center, Dallas

Open Discussion (Integration III and Data Exchange)

Talks:
G. Mandel, HHMI, State University of New York, Stony Brook
K. Svoboda, Cold Spring Harbor Laboratory

Project Organization and Administration
Discussion Leaders:
R. Goodman, Oregon Health Sciences University, Portland
M.P. Sheetz, Columbia University, New York

Topics:
Administrative and Project Management Plans
Administrative Facility
Education and Data Dissemination

Phase II Grant Organization
Writing and Internal Review Assignments

Back view of Banbury Conference Center.

The Application of *Arabidopsis* Genomics to Forestry and Other Complex Plant Systems

December 4–7

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **R. Martienssen,** Cold Spring Harbor Laboratory
R.R. Sederoff, North Carolina State University, Raleigh

SESSION 1: Comparative Genomics I

R. Martienssen, Cold Spring Harbor Laboratory: The *Arabidopsis* genome.
D.B. Neale, University of California, Davis: Comparative genetic mapping in pine.
S.H. Strauss, Oregon State University, Corvallis: Poplar as a tool for functional genomics of trees.
G. Sandberg, Swedish University of Agricultural Sciences, Umea, Sweden: *Arabidopsis:* A necessity for the success of the Swedish poplar functional genomics program.
R.J. Kodrzycki, Westvaco Corporation, Summerville, South Carolina: Functional genomics in forest trees: An industry perspective.

SESSION 2: Comparative Xylogenesis I

M. McCann, John Innes Centre, Norwich, United Kingdom: Analysis of gene expression patterns as mesophyll cells of *Zinnia elegans trans*-differentiate to tracheary elements.
A. Jones, University of North Carolina, Chapel Hill: Signal transduction pathways in plant cell elongation, division, and differentiation.
A. Groover, University of California, Davis: Dissecting vascular development in herbaceous and woody plants using gene traps.
D. Horvath, USDA/ARS/BRL, Fargo, North Dakota: Use of *Arabidopsis* microarrays for study of heterologous systems.
S. Lev-Yadun, University of Haifa at Oranim, Tivon, Israel: Wood and fiber formation in *Arabidopsis*.

D. Luke, R. Sederoff

SESSION 3: Comparative Genomics II

V. Irish, Yale University, New Haven, Connecticut: Evolution of floral developmental mechanisms.
S. Tingey, DuPont Company, Newark, Delaware: Comparative genomics between *E. grandis* and *A. thaliana, O. sativa*, and *Z. mays*.
T. Mitchell-Olds, Max-Planck Institute for Chemical Ecology, Jena, Germany: Quantitative genetics and comparative genomics of *Arabidopsis*.
C. Loopstra, Texas A&M University, College Station: Arabinogalactan proteins and wood development: The *Arabidopsis* genome.

SESSION 4: Discussion of Policy Issues
Chairperson: R.R. Sederoff, North Carolina State University, Raleigh

SESSION 5: Comparative Xylogenesis II

E. Beers, Virginia Polytechnic Institute & State University, Blacksburg: *Arabidopsis* as a model for the study of protease function in vascular tissues.
S. Wyatt, Ohio University, Athens: Reaction wood and *Arabidopsis:* A developing model.
B.R. Franke, Purdue University, West Lafayette, Indiana: Lignin engineering with P450s.
M. Campbell, University of Oxford, United Kingdom: The developmental control of lignification.

SESSION 6: Woody Plant Systems

W. Boerjan, University of Gent, Belgium: Mapping and genetic engineering in *Populus*.
R. Alscher, Virginia Polytechnic Institute & State University, Blacksburg: Toward understanding stress response mechanism in loblolly pine: A systems approach.
R.R. Sederoff, North Carolina State University, Raleigh: The pine genome project: Comparative genomics.
J.E. Carlson, Pennsylvania State University, University Park: Environmental genomics and hardwood forests.

Robertson House

Gene Ontology Annotation and the Human Genome

December 10–12

ARRANGED BY **M. Ashburner,** University of Cambridge, United Kingdom

SESSION 1: Presentations from the Gene Ontology Consortium

M. Ashburner, University of Cambridge, United Kingdom
J. Blake, The Jackson Laboratory, Bar Harbor, Maine
S. Lewis, University of California, Berkeley

SESSION 2: Presentations from Other Public Domain Groups

R. Apweiler, European Bioinformatics Institute, Cambridge, United Kingdom
M. Clamp, Sanger Centre, Cambridge, United Kingdom
D. Maglott, National Center for Biotechnology Information, Bethesda, Maryland
S. Povey, University College London, United Kingdom

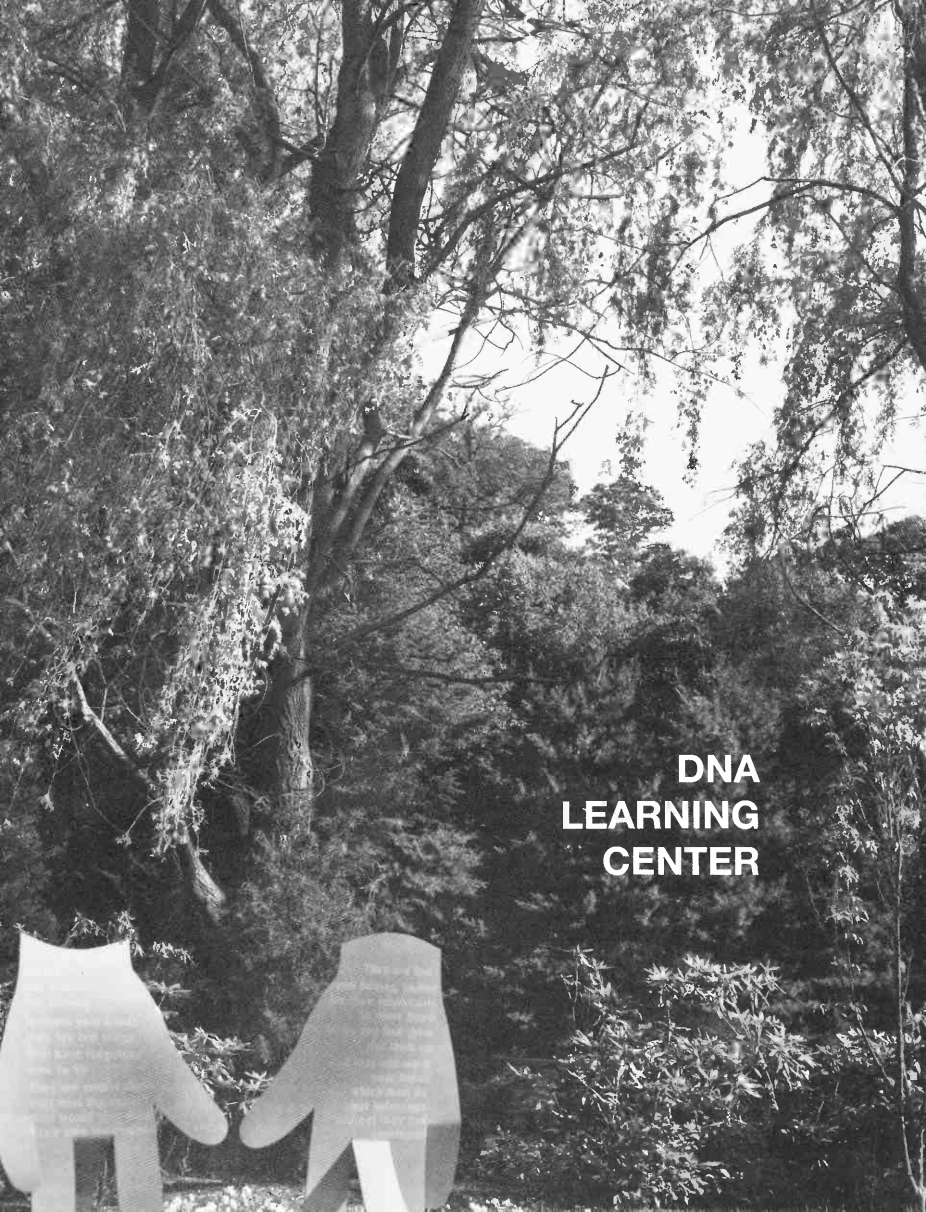
SESSION 3: Presentations from Companies

K. Roberg-Perez, Proteome, Inc., Beverly, Massachusetts
R. Mural, Celera Genomic Research, Rockville, Maryland
D. Gietzen, Incyte Pharmaceuticals, Inc., Palo Alto, California
A. Kasarskis, DoubleTwist Inc., Oakland, California
K. Fasman, AstraZeneca Inc., Waltham, Massachusetts

SESSION 4: General Discussion

L. Brooks, National Human Genome Research Institute, Bethesda, Maryland
J. Cherry, Stanford University School, Palo Alto, California
J. Eppig, The Jackson Laboratory, Bar Harbor, Maine
D. Hill, The Jackson Laboratory, Bar Harbor, Maine
M. Ringwald, The Jackson Laboratory, Bar Harbor, Maine
L. Stein, Cold Spring Harbor Laboratory, New York
P. Thomas, Celera Genomic Research, Rockville, Maryland
J. Wortman, Celera Genomic Research, Rockville, Maryland

S. Lewis, M. Ashburner


DNA
LEARNING
CENTER

DNA LEARNING CENTER

ADMINISTRATION

David Micklos
Judy Cumella-Korabik
Nancy Daidola
Vin Torti

INSTRUCTION

Scott Bronson
Amanda McBrien
Danielle Sixsmith
Veronique Bourdeau
Elna Carrasco
Maureen Cowan

MULTIMEDIA

Susan Lauter
Shirley Chan
Chun-hua Yang
Susan Conova
Uwe Hilgert

We stand at the threshold of a new century with the whole human genome stretched out before us. Messages from science and the popular media suggest a world of seemingly limitless opportunities to improve human health and productivity. Yet, at the turn of the last century, science and society faced a similar rush to exploit human genetics. The story of eugenics—humankind's first venture into a "gene age"—holds a cautionary lesson for our current preoccupation with genes.

Eugenics was the effort to apply principles of genetics to improve the human race. Most people equate eugenics with the atrocities committed for the sake of racial purity in Nazi Germany. Most are unaware of the "positive" eugenics movement, exemplified in England, which advocated voluntary efforts by families to improve their own heredity. Fewer still realize that a coercive, "negative" eugenics movement flourished in the United States, that it involved numerous prominent scientists and civic leaders, and that it made its intellectual home at the forerunner of the now prestigious Cold Spring Harbor Laboratory.

During the first decade of the 20th century, eugenics was organized as a scientific field by the confluence of Mendelian genetics and experimental breeding. This synthesis was embodied by Charles Benedict Davenport, who is considered the father of the American eugenics movement. When Charles Davenport arrived at Cold Spring Harbor in 1898, he assumed the directorship of The Biological Laboratory, a progressive, if somewhat sleepy, "summer camp" for the study of evolution. Founded in 1890, The Biological Laboratory at Cold Spring Harbor followed in the footsteps of the Marine Biological Laboratory at Woods Hole, Massachusetts (1888). Both had been established in the tradition of seaside biological stations founded along the European coastlines following the publication of Darwin's *Origin of Species*. The intersection of land and water was thought to be the ideal place to study how organisms had evolved and adapted to fill a multitude of aquatic, semi-aquatic, and terrestrial niches.

Although he had been trained in the classic observation methods of zoology and comparative morphology, Davenport became interested in the new movement that sought to directly recreate evolution in the laboratory. He and a number of other biologists, including Thomas Hunt Morgan, believed that controlled breeding experiments with plants and animals would yield new insight into evolutionary processes. Davenport gained the ear of the board of the Carnegie Institution of Washington, one of several philanthropies formed under Andrew Carnegie's will to disburse the proceeds of the divestiture of his steel holdings. From them, Davenport secured funding to establish a Station for Experimental Evolution at Cold Spring Harbor.

The dedication of the Station's first building, in 1904, offered a prediction about the future of experimental evolution—the keynote speech was delivered by Hugo de Vries, one of three researchers who had recently rediscovered Mendel's laws of genetics. Davenport populated the Station with like-minded researchers, who made agricultural plants and animals their research subjects. The Station took on the aspect of a farm with chicken coops, goat sheds, Manx cats and canaries, and fields of corn and jimsonweed. Many of the researchers at the Station for Experimental Evolution embraced Mendel's laws, and the shorthand popularized by Reginald Punnet, as a means to follow traits through their experimental crosses. For many, the intricacies of genetics eventually subsumed the experimental

The Station for Experimental Evolution, circa 1908. This view from Blackford lawn looking toward Route 25A includes the Carnegie Library beyond the chicken coops. Image source: Harry H. Laughlin Archives, Truman State University.

study of evolution, and in 1920, the Station for Experimental Evolution was quietly renamed the Carnegie Department of Genetics. Thus, the experimental evolutionists had become the first generation of geneticists.

Several years later, Davenport was introduced to Mrs. E.H. Harriman, by way of her daughter, who had taken a summer course at The Biological Laboratory. Mrs. Harriman was the widow of the railroad magnate who had founded the New York Central Railroad and its Grand Central Station in Manhattan. Davenport convinced Mrs. Harriman to contribute $10,000 to establish a Eugenics Record Office (ERO) on property adjacent to the Station for Experimental Evolution, and she later provided a $300,000 endowment.

Davenport recruited Harry Laughlin, a person with whom he shared an interest in chicken breeding, as superintendent of the ERO. Davenport's little book, *Eugenics: The Science of Human Improvement by Better Breeding*, illustrates how eugenicists sought to transfer lessons from agriculture to human beings. With almost religious zeal, Davenport and Laughlin set out to popularize eugenics. A series of ERO bulletins, including Davenport's *Trait Book* and *How to Make a Eugenical Family Study*, helped to standardize methods and nomenclature for pedigree studies. *Eugenical News*, published by the ERO from 1920 through 1938, was the dominant mouthpiece for the racist and anti-immigration agenda of eugenics research. The ERO also published a number of forms that facilitated data collection. Many families proudly submitted their pedigrees of intellectual/artistic achievement, while others sought advice on the eugenic fitness of proposed marriages. Budding eugenics researchers convened at Cold Spring Harbor each summer to learn how to conduct field work—interviewing subjects, taking medical histories, and constructing pedigrees. During its years of operation, 1910–1939, the ERO amassed hundreds of thousands of family records, pedigrees, and articles on eugenics.

Eugenics arose during the progressive era that followed in the wake of the industrial revolution, when the fruits of science and technology were improving many aspects of life. A growing middle class of professional managers believed that scientific progress offered the possibility of rational cures for social ills. At the same time, eugenicists advanced the notion that bad genes lay at the root of age-old social problems, including poverty, crime, prostitution, and insanity. They warned that bad genes—especially those purportedly entering the country with immigrants from southern and eastern

Europe—threatened to pollute the predominately Anglo "foundation stock" of America. "Data" collected by eugenicists begged an obvious question: Why build more insane asylums, poorhouses, and prisons, when the underlying problem—bad genes—could be halted by controlling human reproduction? This progressive slant appealed to many educated Americans, who also accepted the Malthusian notion that the human species required some sort of pruning to maintain its health.

American eugenics was thus transformed, by degrees, from an agricultural experiment to a popular program in social engineering. Flawed eugenic data provided a supposedly scientific basis for social legislation to separate racial and ethnic groups, restrict immigration from southern and eastern Europe, and sterilize people considered "genetically unfit." The 1927 decision of the United States Supreme Court, *Buck v. Bell*, upheld the constitutionality of eugenic sterilization and signaled the nadir of American civil liberty. By the 1930s, the Nazis applauded the scientific use of eugenic sterilization and based their own sterilization laws on a model provided by the ERO's Harry Laughlin.

Although scientific opposition to eugenics mounted in the United States during the 1930s, it did little to dampen public enthusiasm. Many sophisticated geneticists, including some whose data refuted key eugenic tenets, were ambivalent and supported some type of eugenic program at one point or another. Only growing public knowledge of the Nazi's horrific "final solution" to achieve racial purity led to a wholesale abandonment of popular eugenics, and the ERO was closed in December 1939.

The eugenicists sought an exclusively genetic explanation of human development, neglecting the important contributions of the environment. They managed to inculcate this belief into a whole generation of educated Americans. This is perhaps the greatest danger of modern genetics—that genes once again will be misconstrued as the sole determinants of human life. Genetic determinism is implicit in the Human Genome Project's objective to identify genes involved in human health. The search for disease genes, prima facie, entails value judgments about what is normal *versus* abnormal. The social and legal acceptance of such judgments may create a pressure for genetic conformity that is difficult to predict today. What will it be like when we have a precise catalog of all the good, bad, and middling genes, and the wherewithal to determine who has which? In the face of such knowledge, will society continue to acquiesce to those who prefer to let nature take its course?

The real lesson of eugenics is that it was practiced by well-intentioned persons and bigots alike, and, short of the most obvious excesses, there was often no sharp line between them. Certainly forms of behavior that pass for eugenics are with us today. The killing fields of Cambodia, Central Africa, and the Balkans differ only in method and degree from the Nazi regime of "negative eugenics." Viewed in the cold light of racial and ethnic cleansings, it may seem clear to some that strict, and amoral, adherence to scientific data will protect us from future abuses of genetics. In this sense, the cleansing of the human genome of disease genes can be viewed as merely antiseptic. However, the past tells us that assumptions about human genetics are rarely value-neutral—many of the first crop of human geneticists loaded their data and models with their own aspirations for society.

Harry Laughlin and Charles Davenport outside the Eugenics Record Office (ERO). Image source Harry H. Laughlin Archives, Truman State University.

We chuckle at eugenicists' pedigrees of musical talent, lack of moral control, and criminal tendencies. Yet today, molecular geneticists are actively searching for genes involved in perfect pitch, sexual orientation, aggression, and other constructs of human behavior. Every parent's longing for healthy, happy children, who can continue the flow of their family heritage, is an expression of the best aspirations of "positive eugenics." In the context of in vitro fertilization, what parent would not choose to implant the embryo whose gene combination favors intelligence and creativity? Therefore, rather than dismissing eugenics out of hand, it is far better that each of us attempts to discover where we, and the technologies that we are using, stand in its continuum. Then we can guard against slipping too far.

Launch of the *Image Archive on the American Eugenics Movement*

With these ideas in mind, in 1998 we embarked on a major project to collect archival materials on the American eugenics movement and publish them on the World Wide Web (WWW). On February 11, we launched the *Image Archive on the American Eugenics Movement*, as the second major content site at the DNALC's WWW Site. The project is funded by a grant from the Ethical, Legal, and Social Issues(ELSI) Program of the National Human Genome Research Institute.

With more than 1200 images and documents, the *Eugenics Archive* provides students, teachers, scholars, and the interested public with an extraordinary window into a "hidden" chapter of history. We hope that the opportunity to revisit this period will stimulate people to think critically about our current involvement in human genetics. By providing access to the eugenicists' own words and "data," we hope to challenge visitors to assume the role of historian/researcher. By focusing primarily on visual documents, we hope to engage young people and others who would not normally access a scholarly collection.

When we were conceptualizing the project, we wanted to develop a resource that could stimulate independent thinking about eugenics without espousing a "correct" interpretation of the materials. Although there was consensus that it is important to make the materials available for public scrutiny, there was also grave concern that they might be misinterpreted as "proven science" and therefore be misused to support racist views. Thus, we were challenged to assist users in understanding the historical, social, political, and ethical context in which the American eugenics movement developed, flourished, and finally collapsed. Context is built into the *Archive* on two levels. First, users are encouraged to enter the Site through a series of virtual exhibits, which introduce the key events, persons, and social conditions that contributed to the development of eugenics. Second, all images are sorted into more than 20 topic areas. Browsing by topic or searching by keyword returns a set of related images with extended captions. The topic captions are designed to help the user understand relationships among images and the relationship of the image to the eugenics movement and society. Both levels were developed in collaboration with several leading historians of eugenics. At each level, users are reminded that the vast majority of what was presented as scientific "fact" by eugenicists was fundamentally flawed and has been discredited by modern research standards.

The very act of publishing eugenic materials on the Internet set precedents for other projects dealing with the release of sensitive documents via the Internet. The documents were drawn from four major archives: the American Philosophical Society Library, Rockefeller University Archive Center, Truman State University Archives, and Cold Spring Harbor Laboratory Research Archives. At the start of the project, each archive had concerns about the large-scale publication of eugenic materials, and none had policies governing the release of their materials over the Internet. Thus, we were challenged to develop guidelines for online publication, educational "fair use" of documents, and privacy protections for subjects of the *Archive*. These policies were developed by consensus during 6 days of workshop sessions by a 15-member advisory panel:

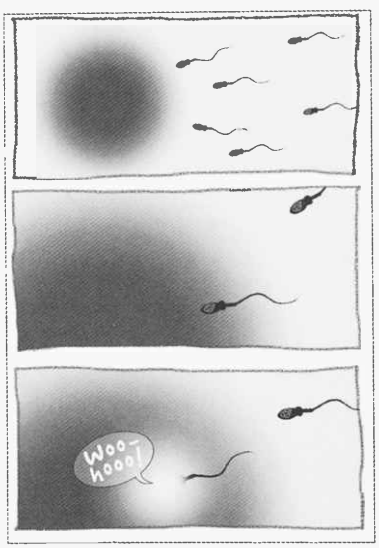
Garland Allen, Washington University, St. Louis, Missouri
Elof Carlson, SUNY, Stony Brook, New York
Pat Colbert-Cormier, Lafayette High School, Louisiana
Nancy Fisher, Regence Blue Cross, Seattle, Washington
Henry Friedlander, City University of New York, New York
Daniel Kevles, California Institute of Technology, Pasadena
Philip Kitcher, University of California, San Diego
Martin Levitt, American Philosophical Society, Philadelphia, Pennsylvania
Paul Lombardo, University of Virginia, Charlottesville
Nancy Press, Oregon Health Sciences University, Portland
Philip Reilly, Shriver Center for Mental Retardation, Waltham, Massachusetts
Pat Ryan, Carolina Biological Supply Company, Burlington, North Carolina
Marsha Saxton, World Institute on Disability, San Francisco, California
Steven Selden, University of Maryland, College Park
Terry Sharrer, National Museum of American History, Washington, D.C.

Completion of *DNA from the Beginning*

In the fall, we completed a 3-year project to publish an online "primer" on genetics, *DNA from the Beginning* (*DNAFTB*). Funded through a grant from the Josiah Macy, Jr. Foundation, *DNAFTB* is one of the most content-rich and innovative Web Sites on the WWW. Aimed at a high school audience, the Site is popular with genetics teachers, but also with regular folks who are interested in keeping up with today's news stories on cloning and genetic engineering. More than 750,000 people viewed *DNAFTB* in 2000, and it accounts for about 60% of the visits to the DNALC WWW Site. *DNAFTB*'s media-rich elements were designed to operate over fast Internet connections (such as T1, cable modem, and DSL), so in 2001, we will offer a CD version that will play quickly in situations with slow, or no, Internet connectivity.

The 41 chapters cover concepts a person needs to know to understand what is going on in biology today, from Mendelian genetics to the structure of DNA to techniques used to make "knock-out" mice. Central to each chapter is an animation in which researchers describe how they did key experiments that contributed to understanding the concept. The animation helps viewers visualize molecular events and dynamic interactions that are difficult to portray in static diagrams. Video clips and a gallery of personal photographs bring home the fact that scientists are real people with whom anyone can identify. The work of more than 80 scientists is highlighted in animations and interviews, including 25 Nobel Laureates (*) and 11 past and present CSHL scientists (+):

Garland Allen
Oswald Avery
David Baltimore*
George Beadle*
Seymour Benzer
Michael Bishop*
Theodor Boveri
Herbert Boyer
Sydney Brenner
Roy Britten
Patrick Brown
Leigh Burgoyne
Mario Capecchi
Elof Carlson
Thomas Cech*
Erwin Chargaff
Stanley Cohen
Francis Collins
Carl Correns
Francis Crick*
James Darnell
Charles Davenport+
Igor Dawid
Hugo de Vries
Walther Flemming
Stephen Fodor
Rosalind Franklin
Raymond Gesteland
Walter Gilbert*
Doug Hanahan+
Leland Hartwell
Michael Hengartner+
Alfred Hershey*+
Dean Hewish
Mahlon Hoagland
Robert Horvitz
Francois Jacob*
Arthur Kornberg*
Roger Kornberg
Phil Leder
Joshua Lederberg*
Phoebus Levene
Edward Lewis*
Scott Lowe+
Maclyn McCarty
Barbara McClintock*+
Richard McCombie+
Gregor Mendel
Matthew Meselson
Freidrich Miescher
Stanley Miller
Jacques Monod*
Thomas Hunt Morgan*
Hermann Muller*
Marshall Nirenberg*
Christiane Nusslein-Volhard*
Robert Olby
Svante Paabo
Reginald Punnett
Richard Roberts*+
Stanley Rupert
Frederick Sanger*
Thomas Sargent
Brian Sauer
Theodor Schwann
Jane Setlow
Richard Setlow
Philip Sharp*
George Shull+
Frank Stahl
Nettie Stevens
Alfred Sturtevant
Howard Temin*
Harold Varmus*
J. Craig Venter
Erich von Tschermak-Seysenegg
Ivan Wallin
James Watson*+
Eric Wieschaus*
Michael Wigler+
Maurice Wilkins*
Paul Zamecnik



Postcards were mailed in spring and winter 2000 to promote *DNA from the Beginning*. The Uncle Darwin postcard was also used to solicit votes for the Webby Awards (discussed on the following page).

Webby Nominations and Renewed Funding for the *BioMedia* Group

The success of the DNALC's Internet publishing ventures was affirmed in April when members of the *BioMedia* group traveled to San Francisco to attend the 2000 Webby Awards, which are billed as the online Oscars. *DNA from the Beginning* was a finalist in the education category, and the DNALC portal site, *Gene Almanac*, was a finalist in the science category. We also received continuation funding for our two major content projects. *Image Archive on the American Eugenics Movement* received a three-year continuation from the National Human Genome Research Institute, bringing five-year funding to $1,160,000. The Josiah Macy, Jr. Foundation approved a two-year continuation for *DNA from the Beginning*, bringing five-year contributions to $1,370,000. These renewals continue the *BioMedia* group's healthy financial situation and will allow us to be in full operation when we occupy our new *BioMedia* addition in 2001.

Macy funding will be used to develop *Your Genes, Your Health* (*YGYH*) as a companion to the existing site, *DNA from the Beginning*. *YGYH* will be a multimedia guide on genetic disorders for which causative genes have been identified and for which new diagnostics are available and rational treatments imminent. The site is specifically targeted at patients and families who are urgently looking for understandable information about a specific genetic disorder. These people are motivated to understand the meaning (the biology) behind the medical jargon they have read about or heard from a physician. The site will contain a number of resources that can help families participate more effectively in the care of a loved one:

- Animations to help visualize the unseen world of genes and molecules.
- Interviews with researchers and patients to provide insiders' perspectives on genetic disorders.
- Links to current news articles, support groups, and clinical trials to provide up-to-the-minute information on treatment options.

To make the site as responsive as possible to the needs of consumers, in December we convened a focus group of representatives from 10 genetic disorder foundations and support groups. We will continue to enlist the help of disorder-specific foundations and support groups to put us in touch with experts and families to interview for the WWW site and to review content prior to public release. By the end of the year, we were well on the way to producing the first module—on Fragile X Syndrome. By fall 2002, we expect to have published modules on 15 of the most common and most severe disorders for which genes have been identified (or are expected to be identified soon): Alzheimer, cystic fibrosis, Down syndrome, hemochromatosis, hemophilia, Huntington, Lou Gehrig, Marfan, muscular dystrophy, neurofibromatosis, Parkinson, phenylketonuria, sickle cell anemia, and Tay-Sachs.

VectorNet Portable Computer Laboratory

By year's end, we completed development of *VectorNet*, a portable computer laboratory designed as the centerpiece of a pre-college science education grant from the Howard Hughes Medical Institute. *VectorNet* was developed in response to problems we experienced conducting computer labs at institutions in the United States and abroad over several years, including lack of fast Internet service, local network unreliability, security provisions, nonuniform hardware setups, and software misconfigurations. The stand-alone system provides *all* computer hardware and software needed to deliver high-quality bioinformatics instruction at any location, regardless of existing computer infrastructure.

The *VectorNet* Laboratory consists of 12 participant notebook computers, network server-in-a-box, wireless router, wireless intranet transceivers, and video projector. The system can operate entirely from DNALC WWW Sites and Genbank subdatabases stored on the network server, or the router can provide centralized Internet access via a single T1, cable modem, or DSL connection. This equipment is packed in two wheeled containers, which can be shipped or carried as luggage to any site in the United States. Host institutions for *VectorNet* need only provide a classroom or seminar room with tables, power points, and (optionally) a fast Internet line.

We also completed development of the key Internet resources that will be used in the program's student and teacher components: three *Bioservers* and the *Genetics Origins* meta-site. *Allele Server* allows students to tabulate student *Alu* insertion data and compare two populations by contingency chi-square, genetic drift, and genetic distance. *Sequence Server* allows students to compare DNA sequences through multiple sequence alignments and construction of phylogenetic trees. Both servers have facilities to allow students to enter their own polymorphism data and to compare their data with world reference populations. *Simulation Server* allows students to model genetic changes over time, simulating the same conditions in 100 or more test populations concurrently. *Genetic Origins* contains all resources to support student experiments with two polymorphisms—an *Alu* insertion and mitochondrial sequence variations. This "super site" includes laboratory protocols, reagent recipes, supporting animations, guided use of *Bioservers*, and video interviews with molecular anthropologists.

VectorNet functioned without major difficulties during the initial rotation at John F. Kennedy High School (Bronx), where 128 8th- and 9th-grade students used the portable lab to access the *Image Archive on the American Eugenics Movement* and *DNA from the Beginning*. The *Allele Server* was also used to analyze student DNA types for the *Alu* insertion polymorphism PV92. One teacher reported that working at the *Allele Server* site really helped students understand the difficult concept of genetic equilibrium in populations. Additional rotations for the 2000–2001 academic year have been planned in collaboration with the Gateway to Higher Education Program of Mt. Sinai School of Medicine and include the following schools: Benjamin Cardozo High School (Queens), Brooklyn Technical High School, Lafayette High School (Brooklyn), Life Sciences Secondary School (Manhattan), Queens Gateway to Health Sciences High School, Science Skill Center (Brooklyn), Bayard Rustin High School for the Humanities (Manhattan), and Stevenson High School (Bronx).

Initially, we had the entire setup returned for maintenance and server updating at the DNALC. However, in the future we intend to do most maintenance on site. Future rotations will be made easier by the purchase of a second server, which will allow us to provide each school with an up-to-date mirror of the DNALC WWW site. The equipment set will be transferred directly between schools. In a single visit to the receiving school, a DNALC staff member will swap the old server for an updated one, run equipment checks, and train participating staff.

During the next several academic years, the *VectorNet* Laboratory will continue to be rotated among New York City Public Schools. However, beginning in summer 2001, the *VectorNet* Laboratory will take on an additional life. During the summer, *VectorNet* will provide the infrastructure for administering week-long workshops on bioinformatics for biology teachers at sites held around the United States. In the 1980s and 1990s, the *Vector DNA Science* Workshop brought the methods of bacterial molecular genetics to teachers around the country. Now, the *VectorNet* Bioinformatics Workshop aims to help faculty extend their expertise to human molecular genetics and genomic biology.

We Prepare to Occupy a New Facility

Despite wet conditions during much of the summer, construction of the *BioMedia* addition progressed with amazing speed. The completion of this 8000-square-foot structure in spring 2001 will double the size of our facility and will provide the physical infrastructure needed to substantially increase the DNALC's impact on modern biology education.

By year's end, it was easy to visualize the future life of the new structure. The exterior brick façade and Georgian details blend perfectly with the existing building. One immediately grasps the cleverness of the design, which nestles the structure into the hillside such that the second-floor offices open directly onto the woody glade. Prominent on the lower level is the large space that will be occupied by a new teaching laboratory—directly in line with two other labs in our existing building. One can sense that the octagonal computer lab, centered on the main entrance to the addition, will become the symbolic heart of the building. This striking space, with two tiers of glass-countered work spaces, will emphasize that the business of understanding the trove of data from the Human Genome Project has, indeed, trans-

Clockwise from upper left: view of the *BioMedia* Addition from the west parking lot; the *BioMedia* Computer Laboratory; the genetics laboratory; and the suite of offices and conference area on the upper level. Images taken in December 2000.

formed biology into an information science. Like the best of today's genome biologists, students will have the opportunity to move effortlessly between biochemical manipulations of DNA and computer manipulations of the information stored in that DNA.

A large central gallery will nearly double the size of adjacent galleries in the existing building. Here, working in collaboration with curators from the American Museum of Natural History and the Smithsonian Institution, we will install entirely new exhibits on the Human Genome Project. A mini-DNA sequencing laboratory, where high school interns prepare and sequence DNA samples submitted from students around the country, will greet visitors entering this exhibition. One module will use the Old Order Amish as a case study—both of using distinct population groups to track down disease genes and as a metaphor for our rush into the "gene age." Although this anachronistic group appears to be stuck in the 19th century, they accept modern genetic medicine and are even preparing to participate in gene therapy trials. Another module will highlight CSHL's effort to sequence plant genomes: the model plant *Arabidopsis* and the important staple, rice. A module on the American eugenics movement, drawn from the evocative materials at our WWW site, will highlight lessons that can be learned from the misuse of genetics in the first part of the 20th century.

The entire upper level of the addition will be the home of the *BioMedia* group, our Internet publishing venture that focuses on providing authoritative, multimedia content on genetics. The loft-like space includes editorial offices and multimedia editing suites to support five major WWW sites in the DNALC portfolio. A video studio will allow us to expand into broadband communications and to further exploit CSHL's position as a gathering place for the world of molecular genetics. Here, we will interview leading scientists for use on our WWW sites and, potentially, for cable distribution.

We Become the First Science Center to Perform On-Site DNA Sequencing

During the past 2 years, we have developed a relatively simple system to allow students to obtain and work with their own DNA sequences. Using a kit developed by the DNALC and distributed by 3 Carolina Biological Supply Company, students isolate DNA from hair roots or cheek cells, then mix their DNA with freeze-dried PCR reagents to amplify (enrich) a highly variable region of their mitochondrial genome. The amplified samples are then mailed to the DNALC, where high school interns perform the final DNA sequencing reactions.

The DNA sequences are placed in the *Sequence Server* database at our WWW Site, where they can be used to perform a number of analyses, including evolutionary comparisons with DNA from ancient humans and Neanderthals. The sequencer is currently set up on a Plexiglas-enclosed lab bench in our main exhibit hall, but will become a larger *Visible Sequencing Laboratory* as part of the major new exhibit on the Human Genome Project.

Initially, the actual sequencing was done in Dick McCombie's lab on the main campus, but in the spring we moved the sequencing to the DNALC. This was made possible by the gift of a new 377 DNA Sequencer, valued at $130,000, arranged by our good friend Frank Stephenson, head of technical training at Applied Biosystems. During the year, we processed 1140 samples submitted by 36 high schools, 15 universities/colleges, and 6 community colleges throughout the country. In addition, a switch to "big dye" chemistry substantially improved results. We are grateful for the help that we have received from the CSHL Genome Sequencing Center and Applied Biosystems, which has enabled us to prove the feasibility of providing a centralized sequencing facility for educational purposes.

In addition to our national program, we began offering DNA sequencing on the "menu" of lab field trips offered to students from local school districts. Students isolate and amplify their own mitochondrial DNA. After confirming polymerase chain reaction (PCR) success by electrophoresing samples in an agarose gel, students set up sequencing reactions using their own templates. Students and teachers are also introduced to the ABI 377 sequencer and to online sequence analysis tools available at the DNALC WWW site. After returning to school, the student samples are sequenced, and their sequence data are posted in the *Sequence Server* database.

During the year, we continued our collaboration with Rob DeSalle, head of the molecular biotechnology laboratory at the American Museum of Natural History in New York. Rob was curator of the highly successful exhibit "Outbreak" (1999) and is curator of the forthcoming exhibit, "The Genomic Revolution" (2001). With advice from the DNALC, Rob has included a teaching lab and DNA sequencer as integral parts of the exhibit. He plans to replicate our mitochondrial DNA sequencing program for field trips by New York City students visiting the museum's genome exhibit.

Instructional Programs Continue to Grow

The year proved to be a trying one for the DNALC staff. There was not a trace of unhappiness when mechanical preparations for the *BioMedia* addition necessitated moving everyone out of our dreary basement offices. We retasked exhibit space as a temporary office for the instructional staff, but there was no room at all for the *BioMedia* group, which ended up in very nice quarters in Williams House on the main CSHL campus. The retrenching of exhibit spaces and general overcrowding meant that overall visitation held steady at the 1999 level of 30,200. Miraculously, however, a record 21,750 students and teachers participated in hands-on labs and workshops.

During the spring, we wrapped up a successful workshop series sponsored by the Ethical, Legal, and Social Issues (ELSI) Program of the Department of Energy's Human Genome Initiative. *The Science & Issues of Human DNA Polymorphisms* introduced high school faculty to our hands-on experiments and computer software for the classroom analysis of human DNA. The final workshops were held at the Mailman Center for Child Development, University of Miami School of Medicine, and Austin Community College. During the summer, we concluded the National Science Foundation-sponsored program, *Genomic Biology*, with workshops held in Oklahoma, Utah, California, and New York.

Staff and Interns

Although several staff members departed during the year, there was a net growth of two positions in the DNALC staff in anticipation of expanded laboratory and Internet programs in the *BioMedia* addition.

In March, we were saddened to learn that Matt Christensen was leaving the *BioMedia* group to start a programming job in Chicago at unext.com. Matt was not just any programmer—he was a programmer with a passion for biology and bioinformatics. In addition to building our web site from scratch, he constructed a user-friendly interface for the bioinformatics tools that are found elsewhere on the Web but are tricky for high school students to navigate. Matt is currently enjoying the city life in Chicago, but his car has taken a beating in the 20-inch snow and 20-inch potholes.

Trish Maskiell (formerly Harrison) left us for New Hampshire when her husband got a new job. Trish started at the DNALC as a middle school teacher in 1997 and was promoted to Education Coordinator in 1998. Under Trish's management, enrollment in middle school programs increased and relationships with outside school districts solidified. Inside the classroom, Trish introduced the glowing genes lab and live *C. elegans* to young students. Kids loved Trish's mild-mannered teaching style and the analogies she came up with to help them understand genetics. Although she planned on becoming a stay-at-home mom in New Hampshire, rumors have it that she is back to coordinating, this time for a district-wide after-school program.

About the same time Trish left, we also said goodbye to Martha Mullally, a middle school teacher since 1998. Martha brought the middle school group her unique lab experience and successfully translated it for her students. One outside teacher said it best when she exclaimed, "I just love watching her teach! She's like a mad scientist!" Martha returned to her native Canada to start a 1-year education program at the University of Ottawa that will lead to a Canadian teaching certificate.

Veronique Bourdeau joined us in June to teach high school classes and develop new labs for the students. Vero recently received her Ph.D. in biochemistry from her hometown school, the University of Montreal, but grew up wanting to become a teacher. As luck would have it, her husband's work brought her to Cold Spring Harbor, and now she combines her research and teaching skills for us.

Two new middle school instructors also joined us this past summer, Elna Carrasco and Maureen Cowan. Elna knew she wanted an adventurous career in science after watching "Raiders of the Lost Ark." In love with the 70-foot Apatosaurus at the American Museum of Natural History since she was five, Elna searched for dinosaur fossils in Montana as part of her work for a degree in earth and space science from SUNY, Stony Brook. At the DNALC, she combines her interests in evolution and theater by performing/teaching the "Story of a Gene" to 5th through 8th graders.

Maureen Cowan had a teaching career in the back of her mind since high school, but it never really clicked until a friend complimented her presentation in a college genetics course. After graduating from Villanova in 1998 with a B.S. in biology, she came to Jerry Yin's lab at Cold Spring Harbor to study fruit fly behavior and worked at night on her master's degree in Secondary Education. Maureen brings her excitement about genetics into the classroom and is an excellent teacher.

"Jack-of-all-trades," Uwe Hilgert, joined the *BioMedia* group in November. Uwe (pronounced oov-eh) did his Ph.D. work at the Max-Planck Institute in Cologne, Germany, and post-doc work at the University of Arizona in Tucson. Engrossed with pathogenic fungi, Uwe was nevertheless drawn out of the lab one day to talk with an elementary school class, and the experience changed him forever. Since then, Uwe has helped develop science modules with teachers, taught molecular biology to senior citizens, and dabbled in the use of computers in teaching. At the DNALC, he is currently updating our bioinformatics tools and working with teachers to bring these tools to underprivileged schools in New York City.

Vin Torti joined us at the beginning of the year as the DNALC's own development officer. Vin spent 15 years teaching philosophy and raising money at Xaverian High School in Brooklyn before joining the development office at Cold Spring Harbor Laboratory. He enthusiastically combines his love of teaching with his love of raising money to make sure we can keep bringing the best genetics education to Long Island, New York City, and the world.

New employees, from left to right: Maureen Cowan, Uwe Hilgert, Elna Carrasco, and Veronique Bourdeau.

The DNALC's staff of high school interns continues to be a vital part of the daily "runnings" of the educational department. Interns prepare equipment and reagents for on-site and off-site lessons and, in the process, learn standard scientific techniques such as microbiological care and culturing, preparing and diluting solutions, restricting and ligating DNA, analyzing and sequencing DNA, and transforming cells. They experiment with many of the protocols when we reevaluate and upgrade the lessons. Currently, our veteran interns are Rebecca Shoer (Syosset High School), Caroline Lau (Syosset High School), Daniel Goldberg (Half Hollow Hills East High School), Yan Huang (Harborfields High School), and Janice Lee (Oyster Bay High School), and we welcomed newcomers Jordan Komisaro (Long Beach High School), Jared Winoker (Syosset High School), Benjamin Blond (Long Island School for the Gifted), and Marie Mizuno (Cold Spring Harbor High School).

After several years, many of our interns find their DNALC experience useful when they leave us for college or other jobs. Ken Mizuno, an intern for the past 2 years, headed for Carnegie Mellon University where he majors in computational biology and minors in computer science. Gina Conenello, an intern of 3 years, supervised the high school interns during the summer workshops and then left to attend Bucknell University, where she majors in biochemistry and cell biology. Greg Bautista left to attend Colgate University. Rebecca Yee, a Wellesley College student, returned during the summer to lead the middle school interns through the workshop season. Adam Frange (Wantagh High School) left his internship to begin an apprenticeship with Dr. Huifu Guo in CSHL's Beckman Laboratory.

In addition to their regular duties, many interns conduct independent research projects under the advisement of Scott Bronson. Caroline Lau and Daniel Goldberg received High Honors from the Long Island Science Congress for their research accomplished at the DNALC. Dan Goldberg's project focused on the effects of *NF-1* genes in *Drosophila melanogaster* after heat shock response. Caroline Lau studied the population genetics of Long Island's native brook trout. Janice Lee is currently researching techniques in plant PCR. Rebecca Shoer is developing techniques in gene transfer of Hydra and Jordan Komisaro has already begun developing the tobacco mosaic virus as a classroom tool in plant research.

After 6 years, the DNALC said goodbye to Jermel Watkins. Jermel was head intern for many years, assisting instructors and interns alike. Presently, Jermel is working at SUNY, Stony Brook where he is investigating the genes associated with microglial activation in the brain when exposed to glutamate and LPS. We will miss him.

The *BioMedia* group continues to be extremely fortunate in their choice of high school and college interns. High school interns in 2000 were Tracy Mak, Syosset High School, and Felix Hu, Northport High School. The summer college interns were Sheila Vyas, Wellesley College, and Joshua Cohen, University of Washington.

PUBLICATIONS

Chan S., Yang C., Conova S. Lauter S., Christensen M., Witkowski J., and Micklos D. 1999–2000. *DNA from the Beginning* (http://vector.cshl.org/dnaftb/). DNA Learning Center, Cold Spring Harbor, New York.

Micklos D. and Carlson E. 2000. Engineering American Society: The lesson of eugenics. *Nat Rev. Genet.* **1:** 153–158.

Mickos D., Lauter S., and Witkowski J. 2000. *Image Archive on the American Eugenics Movement* (http://vector.cshl.org/eugenics/). DNA Learning Center, Cold Spring Harbor, New York.

2000 Workshops, Meetings, and Collaborations

January 28–30	National Human Genome Research Institute ELSI Project, *Eugenics Image Archive*, Editorial Advisory Panel Meeting, Banbury Center
February 11	*DNA from the Beginning* interviews, Dr. Richard Setlow and Dr. Jane Setlow, Brookhaven National Laboratory
February 11	*DNA from the Beginning* interview, Dr. Elof Carlson, State University of New York, Stony Brook
February 17–20	American Association for the Advancement of Science Annual Meeting, Washington D.C.
February 27–March 2	Department of Energy Contractor-Grantee Meeting, Santa Fe, New Mexico
March 7	*DNA from the Beginning* interview, Dr. Richard McCombie, CSHL
March 16	Site visit by Steve Israel, Huntington Town Board Councilman, and Beverly Wayne, Institute on Holocaust and Law
	National Institute of Social Sciences Issues Discussion Group, New York, New York
March 20	Loomis Chaffee Convocation, Windsor, Connecticut
March 22	Huntington Township Chamber of Commerce Economic Summit II Meeting, Huntington, New York
	Gateway to Higher Education Planning Meeting, Mount Sinai School of Medicine, New York, New York
March 26–30	BIO2000 International Meeting & Exhibition, Boston, Massachusetts
March 29	National Human Genome Research Institute ELSI Review Panel, Bethesda, Maryland
March 31	Educational Advisory Board Meeting on the Human Genome Exhibit, American Museum of Natural History, New York, New York
April 3–5	Department of Energy ELSI Workshop, *The Science and Issues of Human DNA Polymorphisms*, The Mailman Center for Child Development, University of Miami School of Medicine, Florida
April 6	Laboratory for National Institute of Science, Beta Kappa Chi, Nashville, Tennessee
April 6–9	National Science Teachers Association Annual Meeting, Orlando, Florida
April 7–9	Department of Energy ELSI Workshop, *The Science and Issues of Human DNA Polymorphisms*, Austin Community College, Rio Grande Campus, Texas
April 11	Hutton House lecture, Long Island University, Brookville, New York
April 17	*Great Moments in DNA Science* Honors Students Seminar, CSHL
April 18	Hutton House lecture and laboratory, Long Island University, Brookville, New York
April 20	Site visit by Dr. June Osborn, Josiah Macy, Jr. Foundation, New York, New York and David Luke, Cold Spring Harbor Laboratory Board of Trustees
April 21	Seminar on Eugenics, Anne Arundel Community College, Arnold, Maryland
April 22	Laboratory for Rampart and Sierra High Schools, Colorado Springs, Colorado
April 25	Hutton House lecture, Long Island University, Brookville, New York
May 3	National Institute of Social Sciences Award Luncheon, Harvard Club, New York, New York
May 8	*Great Moments in DNA Science* Honors Students Seminar
May 11	Webby Awards, San Francisco, California
May 12	*DNA from the Beginning* interview, Dr. James Cleaver, University of California, San Francisco
May 12	*DNA from the Beginning* interview, Dr. Stanley Prusiner, University of California, San Francisco
May 22	Site visit by Carolyn Gusoff, Channel 4 News
May 22	*Great Moments in DNA Science* Honors Students Seminar
June 1	Site visit to Clinic for Special Children, Strasbourg, Pennsylvania
June 2	Site visit by Christopher Perez, Pfizer Foundation, New York, New York
June 5–10	National Science Foundation Workshop, *Genomic Biology*, Oklahoma City Community College, Oklahoma
June 16	Site visit by Frank Stephenson, Applied Biosystems, Foster City, California
June 19–24	National Science Foundation Workshop, *Genomic Biology*, Eccles Institute of Human Genetics, University of Utah, Salt Lake City
June 26-30	*Fun With DNA* Workshop, DNALC
	DNA Science Workshop, DNALC
June 26–July 7	*Genomic Biology & PCR* Minority Workshop, Central Islip High School, New York
June 28	Interview by Lea Tyrrell, Channel 12 News
June 30	Interview by Roland Pease, BBC worldwide
July 5	*DNA from the Beginning* interview, Dr. James Darnell, Rockefeller University, New York
July 10–15	National Science Foundation Workshop, *Genomic Biology*, California State University, Fullerton
July 10–15	*Fun With DNA* Minority Workshop, PS 38, Rosedale, New York
	World of Enzymes Workshop, DNALC
	DNA Science Minority Workshop, Brooklyn Technical High School, New York
	DNA Science Workshop, DNALC

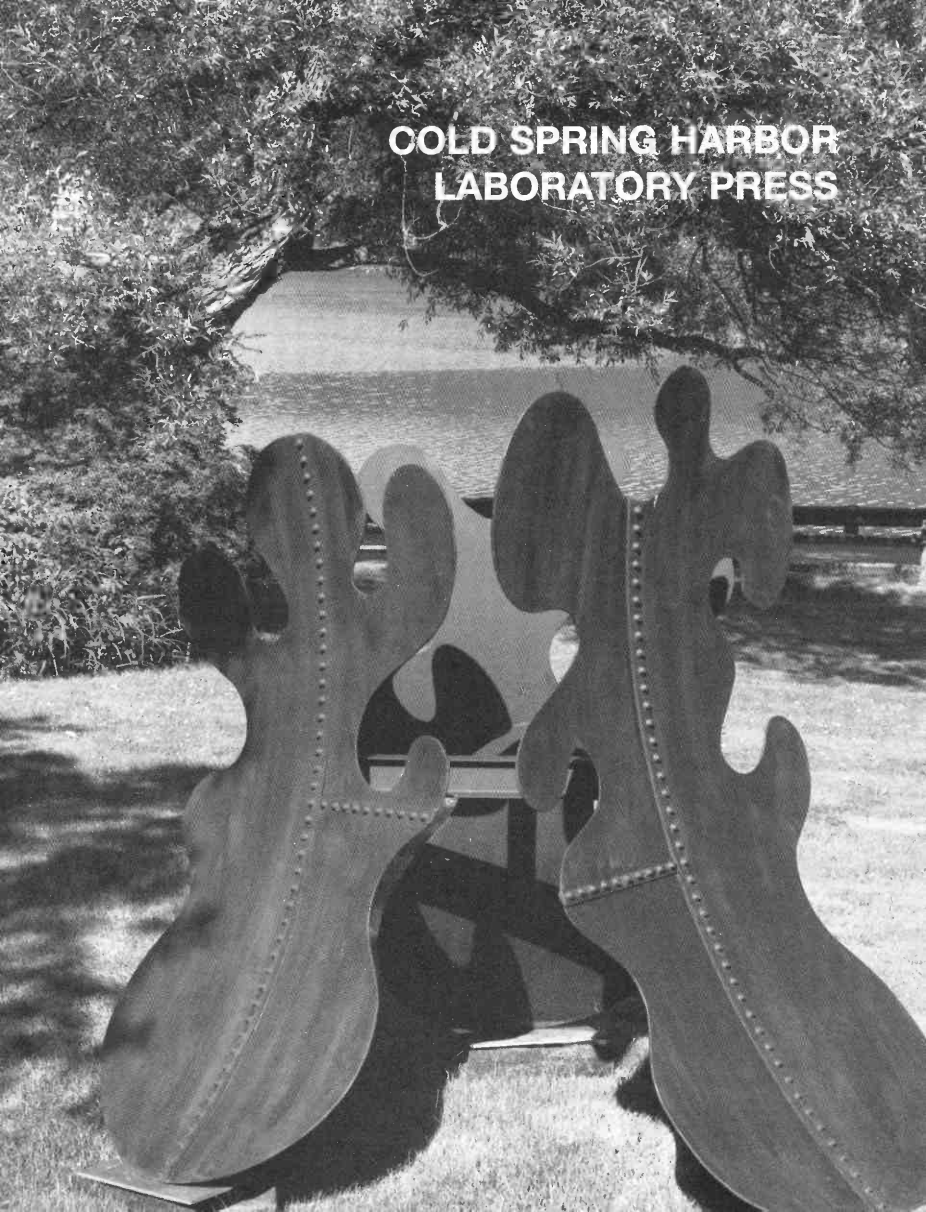
July 17–21	*Fun With DNA* Workshop, DNALC
	DNA Science Minority Workshop, John F. Kennedy High School, Bronx, New York
July 17–22	National Science Foundation Workshop, *Genomic Biology*, DNALC
July 24–28	*Fun With DNA* Workshop, DNALC
	DNA Science Minority Workshop, Brooklyn Technical High School, New York
	DNA Science Workshop, DNALC
July 31–August 4	*Fun With DNA* Workshop, DNALC
	Genomic Biology & PCR Workshop, DNALC
August 4	Site visit by Alan Alda, *Scientific American Frontiers*
August 7–10	*World of Enzymes* Workshop, DNALC
	Green Genes Workshop, DNALC
August 14–18	*Fun With DNA* Workshop, DNALC
	DNA Science Workshop, DNALC
August 15	National Human Genome Research Institute ELSI Project, *Eugenics Image Archive*, collection visit to Museum of the City of New York
	National Human Genome Research Institute ELSI Project, *Eugenics Image Archive*, collection visit to American Museum of Natural History
August 21–25	*Fun With DNA* Workshop, DNALC
	Genetic Horizons Workshop, DNALC
	Genomic Biology & PCR Workshop, DNALC
August 28	Site visit by Dr. Nicholas Chiorazzi and Dr. Bette Steinberg, North Shore-Long Island Jewish Research Institute, Manhasset, New York
August 28–31	*World of Enzymes* Workshop, DNALC
	Green Genes Workshop, DNALC
September 7	Site visit by Rob DeSalle, American Museum of Natural History, New York, New York
September 9	Macmillan Genetics Encyclopedia Editorial Board Meeting, New York, New York
September 15	*DNA from the Beginning* interview, Dr. Michael Wigler, CSHL
September 18	*DNA from the Beginning* interview, Dr. Michael Hengartner, CSHL
September 19	Site visit by Dr. Frederick Volp, Cold Spring Harbor School District, New York
September 20	*DNA from the Beginning* interview, Dr. Eric Wieschaus, Princeton University, New Jersey
September 22	*DNA from the Beginning* interview, Dr. Scott Lowe, Cold Spring Harbor Laboratory, New York
September 24–25	American Association for the Advancement of Science–American Physiological Society Meeting, Warrenton, Virginia
September 28–29	National Human Genome Research Institute ELSI Project, *Eugenics Image Archive*, collection visit to State University of New York, Albany
October 3	Site visit by the Cosmopolitan Club, New York, New York
October 10–12	Howard Hughes Medical Institute Program Directors Meeting, Chevy Chase, Maryland
October 11	Site visit to Clemson University, Clemson, South Carolina
October 14–17	Association of Science–Technology Centers Conference, Cleveland, Ohio
October 16	Site visit by Philip Palmedo, Steven Barry, and Jerry Sandler, The Long Island Museum of Science & Technology, Garden City, New York
	Site visit by Stephen Speer, CSIRO Discovery, Canberra, Australia
October 25–28	National Association of Biology Teachers Annual Meeting, Orlando, Florida
October 27–28	National Science Foundation Program Directors Meeting, Washington D.C.
November 3–5	Department of Energy Meeting, *Science Education on the Internet*, Salt Lake City, Utah
November 7	Site visit to North Shore–Long Island Jewish Research Institute, Manhasset, New York
November 9	Howard Hughes Medical Institute *City Genes* meeting, CSHL
November 10	Site visit by Dr. Harold Cheatham and Dr. Jerry Trapnell, Clemson University, Clemson, South Carolina
November 13	EAB presentation, DNALC
November 21	Site visit to American Museum of Natural History, New York, New York
November 22	Site visit by Dr. John Reiher, The Center for Occupational Research and Development, Waco, Texas
November 29	Presentation for faculty and parents, Green Vale School, Glen Head, New York
December 4	National Institute of Social Sciences Gold Medal Award Dinner, Union Club, New York, New York
December 5	National Human Genome Research Institute ELSI Project, *Eugenics Image Archive*, collection visit to Ellis Island, New York
December 13	Site visit by Anne Dhanaraj, Singapore Science Centre
December 15	National Human Genome Research Institute ELSI Project, *Your Genes, Your Health* Planning Meeting, Banbury Center
December 19	*DNA from the Beginning* interview, Dr. Walter Gilbert, Harvard University, Cambridge, Massachusetts
December 21	Site visit by Lisa Darma and Lawrence Wallace, Carolina Biological Supply Company, Burlington, North Carolina

Sites of Major Faculty Workshops 1985–2000

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**
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
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**
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**
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**
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
	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
	U.S. Military Academy, West Point	**1996**
	Stuyvesant High School, New York	1998–1999
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**
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
UTAH	University of Utah, Salt Lake City	1993
	University of Utah, Salt Lake City	**1998**
	University of Utah, Salt Lake City	**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
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
SWEDEN	Kristineberg Marine Research Station, Fiskebackskil	1995


COLD SPRING HARBOR
LABORATORY PRESS

2000 PUBLICATIONS

LABORATORY MANUALS

Molecular Cloning: A Laboratory Manual, Third Edition
Joseph Sambrook and David W. Russell

Phage Display: A Laboratory Manual
Carlos F. Barbas III, Dennis R. Burton, Jamie K. Scott, and Gregg J. Silverman

Methods in Yeast Genetics 2000
Dan Burke, Dean Dawson, and Tim Stearns

Drosophila *Protocols*
William Sullivan, Michael Ashburner, and R. Scott Hawley

CSHL MONOGRAPH SERIES

Translational Control of Gene Expression
Nahum Sonenberg, John W.B. Hershey, and Michael B. Mathews (eds.)

SYMPOSIUM

Signaling and Gene Expression in the Immune System
Cold Spring Harbor Symposia on Quantitative Biology, Volume LXIV

OTHER TITLES

A Passion for DNA: Genes, Genomes, and Society
James D. Watson

The Coiled Spring: How Life Begins
Ethan Bier

We Can Sleep Later: Alfred D. Hershey and the Origins of Molecular Biology
Franklin W. Stahl (ed.)

Abraham Lincoln's DNA and Other Adventures in Genetics
Philip R. Reilly

Dorothy Hodgkin: A Life (U.S. edition)
Georgina Ferry

Landmark Papers in Cell Biology: Selected Research Articles Celebrating Forty Years in The American Society for Cell Biology
Joseph G. Gall and J. Richard McIntosh (eds.)

JOURNALS

Genes & Development (Volume 14, 24 issues)
T. Grodzicker and D. Solter (eds.)

Genome Research (Volume 10, 12 issues)
A. Chakravarti, R. Gibbs, E. Green, R. Myers, M. Boguski, and L. Goodman (eds.)

Learning & Memory (Volume 8, 6 issues)
J.H. Byrne (ed.)

OTHER

CSHL Annual Report 1999

Banbury Center Annual Report 1999

Administration and Financial Annual Report 1999

COLD SPRING HARBOR LABORATORY PRESS

Publishing at the Laboratory made important advances in 2000. The scope of the book program was broadened and its sales support was strengthened through contracts with major booksellers in the United States and the creation of a European sales team centered in Oxford. Advance orders for the new edition of the celebrated laboratory *Molecular Cloning* were strong. There was significant growth in institutional journal subscriptions and journal advertising sales. And solid progress was made in Internet-based publishing and marketing. As a result, the financial results for the year were very satisfactory. Revenue rose by 35% and the net operating excess by 56%.

Books for Scientists

Nine new books were published for the use of scientists at the graduate student level and beyond. The most eagerly anticipated was a new edition of the laboratory manual that can be said to have changed biology. *Molecular Cloning: A Laboratory Manual* was first published in 1982. It contained technical wisdom distilled by Tom Maniatis, Ed Fritsch, and Joe Sambrook from their 2 years of teaching a course in recombinant DNA technology. Joe recalled recently that in the course's first year, none of the techniques worked, and students learned the hard way that experimental science was unpredictable. The methods then were new and known to just a few pioneers, but their power was clear and scientists were eager to learn them. In the Laboratory's Annual Report for 1982, Jim Watson gleefully reported that the demand was so strong it seemed possible that the manual would sell as many as 25,000 copies. Two editions and more than 150,000 copies later, the manual has become the definitive work of reference in its field and can be seen as an important catalyst in the transformation of many biological disciplines from the descriptive to the analytical.

For the third edition, written by Joe Sambrook and David Russell, with associate authors Nina Irwin and Kaaren Janssen, *Molecular Cloning* was handsomely redesigned in two colors, bound as a classic book, rather than a manual, and published in a new and compelling print and on-line combination. To be truly useful, scientific information now must be networked, so purchasers of the book are given access to a private Web Site from which they can print protocols for bench use, link to reference data bases, take part in discussions about methods of interest, and receive new and updated information.

Thanks to a vigorous marketing and sales campaign, advance orders for *Molecular Cloning* were strong, and the book was published to great enthusiasm in December. As in 1982, we believe that its prospects are good.

Three other laboratory manuals were published this year, more narrowly focused but written with the authority of experienced teachers and investigators. *Phage Display: A Laboratory Manual* and *Methods in Yeast Genetics 2000* both emerged from long-standing courses at the Laboratory. Drosophila *Protocols* broadened and updated the scope of Michael Ashburner's classic book of methods for work on fruit flies. Our two major book series were extended, with the addition of the valuable monograph *Translational Control of Gene Expression* and publication of the 64th Annual Symposium volume *Signaling and Gene Expression in the Immune System*.

The professional list was completed by three unusual books. In a collaboration with the American Society of Cell Biology, to mark its 40th anniversary, we published *Landmark Papers in Cell Biology*, a selection of classic research papers published since the Society's foundation that was greeted warmly by college teachers and investigators alike. And we were delighted with the appearance of *The Coiled Spring: How Life Begins*, an elegant account of embryonic development by Ethan Bier in a beautifully designed and illustrated volume of remarkable breadth. Finally, we were proud to produce a tribute to one of the giants of science at Cold Spring Harbor, Alfred Hershey. A series of essays by colleagues, collaborators, and friends, *We Can Sleep Later* portrays a man who was not easy to know but

commanded immense respect for his intelligence, insight, and diligence—a consummate scientist and a leading architect of the new discipline of molecular biology in the 1940s and 1950s.

Sales to scientists were also strongly supported by the backlist of established books, particularly *At the Bench, Using Antibodies*, and *Transcriptional Regulation in Eukaryotes*.

Books for the Public

The steady sales of our 1992 re-issue of the classic history of molecular biology—Horace Judson's *The Eighth Day of Creation*—and Max Perutz's essay collection *I Wish I'd Made You Angry Earlier* have encouraged us to believe that we can find an audience for interesting science among curious but non-specialist readers. Jim Watson is a proven master of such prose, and we were fortunate to have the opportunity to anthologize some of the essays he has written in the past 20 years for a variety of purposes and publications. With a sparkling introduction by Walter Gratzer, *A Passion for DNA* was published in March and simultaneously in Europe by Oxford University Press. Assisted by the author's willingness to make media and bookstore appearances as he traveled the country, the book sold very well and attracted excellent reviews. During the year, agreements were made for its translation into five languages.

This title was the first of three new general interest titles to be published this year, and publicity through press releases, book signings, and media coverage was arranged to promote them. *Abraham Lincoln's DNA and Other Adventures in Genetics*, by the distinguished scientist and attorney Philip Reilly, proved to be very popular and attracted favorable press comment. The United States edition of the biography *Dorothy Hodgkin: A Life* was also published, and its British author Georgina Ferry visited Cold Spring Harbor in December to give a talk and sign books. The success of these titles is encouraging us to seek opportunities to publish similar books for a general audience.

Books for Students

The development of undergraduate textbooks is a lengthy process, involving authors, editors, and artists in several years of work before anything is produced. The undergraduate textbook program initiated in 1999 moved closer to fruition this year, and the number of books being developed increased sharply. Recognizing the special expertise and deep pockets required to ensure the widespread adoption of textbooks for course teaching, our strategy is to co-publish these books with larger, commercial partners that have proved themselves in the markets concerned. In 2000, three co-publication contracts were either signed or brought to final draft. The first book to be published will be the volume on gene regulation being written by Mark Ptashne and Alex Gann, the textbook program's senior editor, that is scheduled for 2001. Other titles to appear in 2002 and 2003 will include the fifth edition of Jim Watson's classic text *Molecular Biology of the Gene*, with authors from Harvard, Berkeley, MIT, and Cold Spring Harbor.

In the development of all of these projects, the writing center at the Meier House has proved to be an extraordinary asset, and more than a dozen authors have spent productive and pleasant periods in residence during the year.

Books On-line

The Web Site for Cold Spring Harbor Laboratory Press, CSHLPress.com, was redesigned and rebuilt during the latter months of 2000, for release early the next year. The Site provides sophisticated e-commerce for books and journals, a dynamic home page that assists targeted promotional activities, and

software for communicating directly with purchasers. In 2000, on-line purchasing rose to account for 6% of book sales. The redesigned Site will enhance the development of this marketing channel.

Like the manual *Molecular Cloning*, the advanced textbook *Bioinformatics: Sequence and Genome Analysis*, to be published early in 2001, will have an associated Web Site, BioinformaticsOnline.org, that will be accessible only to book purchasers. The Site will provide live links to the software and other resources described in the book and will be continually updated. Sponsorship and advertising support for the Site are in discussion.

During the year, there was continuous appraisal of the opportunities for book publishing offered by emerging electronic technologies. Contracts were signed with NetLibrary.com for the conversion of back volumes of the series *Cancer Surveys* into e-books that can be read by library patrons. Work continued at the National Center for Biotechnology Information on the creation of on-line editions of the books *Retroviruses* and C. elegans *II*. There is not yet a consensus on the best file formats for electronic books, or the best device on which to read them. Nevertheless if the market for e-books does catch fire, it is likely to happen first in academic circles, and we want to be prepared.

Journal Publishing

All three of our journals had a successful year, gaining institutional subscriptions at a rate of 2–10%, receiving more submitted manuscripts than ever, and achieving a level of advertising sales higher even than last year's record-breaking total. These results were particularly satisfying under business conditions that resulted in an industry-wide average decline of science journal subscriptions by 5%.

The journals' impact factors continued to be satisfactory. *Genes & Development*'s impact factor rose to 19.22, ranking it among the top ten journals in all biology.

On-line full-text access to the current year's issues of Cold Spring Harbor journals remained available only with a print subscription. Issues older than 12 months were made accessible free of charge on-line. More than 70% of subscribers made use of the electronic edition in 2000. The possibility of introducing site licenses for on-line-only access next year was opened in discussion with academic institutions, library consortia, and companies worldwide.

Throughout the year, preparations were made for the transition of *Protein Science* to publication by Cold Spring Harbor in 2001. The journal, owned by The Protein Society, was elegantly redesigned, and a complementary Web Site was built in collaboration with our electronic publishing partner, The HighWire Press of Stanford University Library. After much detailed effort to engineer a smooth transition of manuscript submission and subscription handling, the first issue of the journal to be published by Cold Spring Harbor, dated January 2001, appeared on schedule in December. Editorially, the journal will begin now to expand its scope to include reviews, commentaries, meeting reports, and papers from the emerging field of proteomics.

At the suggestion of Mr. and Mrs. Takemori, owners and publishers of *Newton*, a lavishly illustrated science magazine with a mass audience in Japan, Spain, and Italy, an unusual collaboration was initiated in 2000. Twelve lengthy articles on aspects of biology were commissioned from freelance science writers and edited for publication in the magazine by our journal editors Laurie Goodman and Emily Huang. *Newton* assigned to the project a team of very talented illustrators, whose computer graphics brought to vibrant life the science described in the articles. The finished articles are visually striking, broadly educational, and their re-publication in book form is now being considered.

Sales, Marketing, and Distribution

Promotional activities in 2000 included direct mail, meeting exhibits, and advertising in print publications and on-line. We attended 11 professional meetings, displaying new and established books in such

numbers that a double-wide exhibit booth has now become a standard necessity. Valuable use was made of the conferences at the Laboratory to draw scientists' attention to our new publications.

As part of our increasingly vigorous sales strategy, visits were made to the Tokyo (April), London (March), Beijing (August), and Frankfurt (October) Book Fairs, where extensive contacts were made with potential distributors and translators of our books. The previous year's efforts to establish a sales presence in Europe culminated in the establishment of CSHL Press U.K., a warehouse and order-processing facility in Oxford where orders can be taken in pounds sterling and goods shipped to book stores and academic institutions more quickly than is possible from the United States. To support the expansion of sales efforts in Europe, a group of experienced commission sales representatives was appointed to cover several countries and has rapidly become most effective.

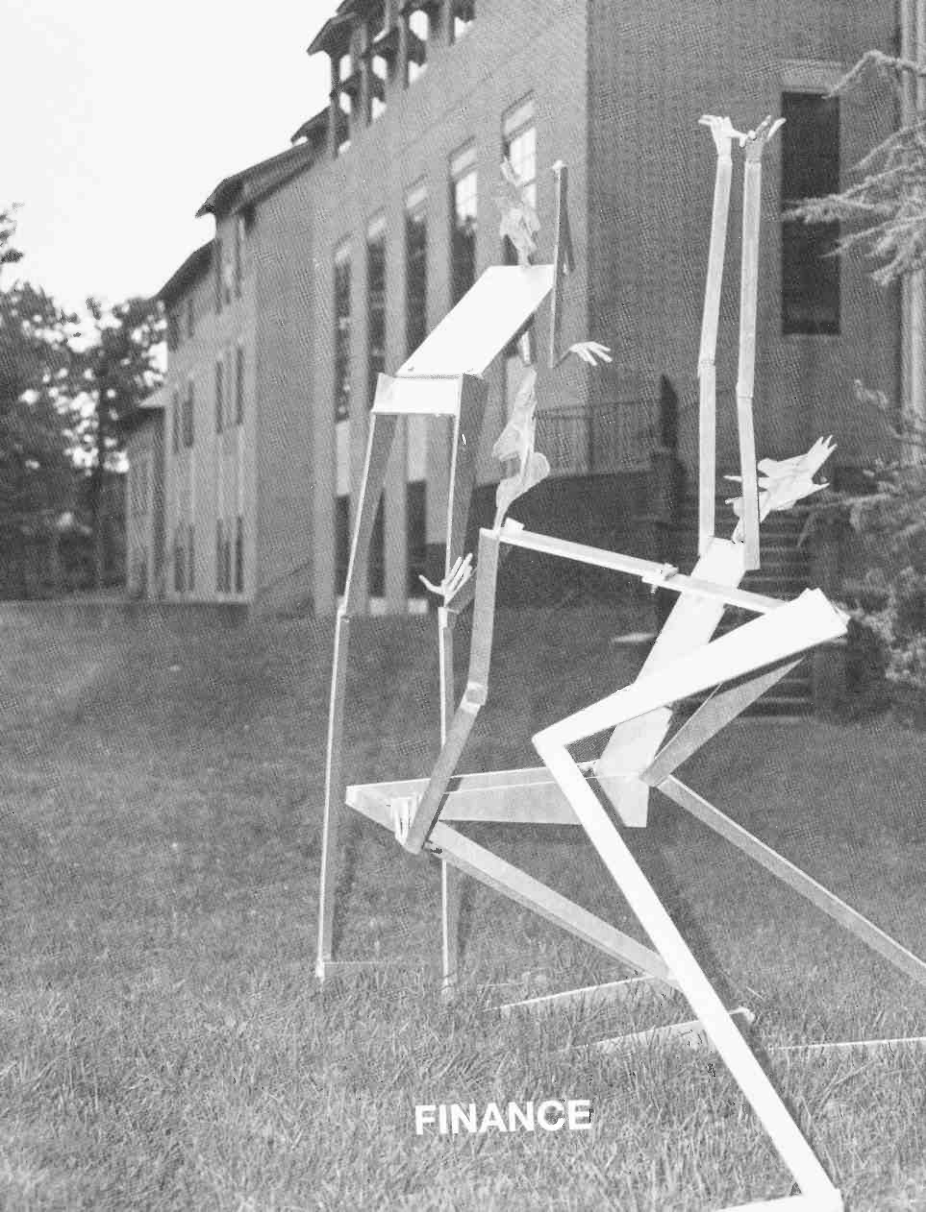
Staff

The staff members of the Press (as of December 2000) are listed at the end of this volume. It is a pleasure to acknowledge the dedication and high standards they bring to their duties.

In 2000, we welcomed several new members to the staff: Kathryn Fitzpatrick as Marketing Manager; Dawn Canfora as Journal Secretary; Heather Cosel-Pieper as Editorial Intern; Arlen Feldwick-Jones as Production Assistant; Anne Wenzel as Copy Editor; Geraldine Jaitin, Joan Pepperman, Mary Sabala, and Kathleen Watras as Fulfillment Assistants; Katya Gurbeloshvili as Sales Assistant; and Christopher Vacca as Warehouse Assistant.

With a staff roster of 50, located at three sites within the Laboratory and one nearby at Plainview, and with three staff members working from home offices in San Diego, California; Guildford, Connecticut; and Questa, New Mexico, CSHL Press has become a larger and more complex organization to manage. I am therefore particularly grateful for the intelligence, efficiency, and attention to detail of my assistant Liz Powers and the senior staff: Jan Argentine, Editorial Development Manager; Ingrid Benirschke, Marketing Manager; Kathryn Fitzpatrick, Marketing Manager; Marcie Ebenstein, Advertising Manager; Nancy Hodson, Business Manager; Guy Keyes, Sales and Fulfillment Manager; Denise Weiss, Production Manager; and the editors of our journals, Terri Grodzicker at *Genes & Development* and Laurie Goodman at *Genome Research*.

John R. Inglis



FINANCE

FINANCIAL STATEMENTS

CONSOLIDATED STATEMENTS OF FINANCIAL POSITION
December 31, 2000 and 1999

	2000	1999
Assets:		
Cash and cash equivalents	$ 27,456,990	34,830,286
Accounts receivable:		
Publications	2,187,811	837,564
Other	937,537	333,310
Grants receivable	3,652,363	2,759,349
Contributions receivable	8,130,610	4,878,895
Publications inventory	2,327,313	2,138,874
Prepaid expenses and other assets	1,691,542	1,321,313
Investments	208,166,091	203,853,845
Investment in employee residences	3,047,429	2,751,580
Land, buildings, and equipment:		
Land and land improvements	14,157,625	12,614,303
Buildings	84,495,326	80,460,206
Furniture, fixtures and equipment	7,907,387	5,427,259
Laboratory equipment	14,606,551	12,405,968
Library books and periodicals	365,630	365,630
Construction in progress	15,899,784	4,514,827
	137,432,303	115,788,193
Less accumulated depreciation and amortization	(38,108,559)	(34,481,784)
Land, buildings, and equipment (net)	99,323,744	81,306,409
Total assets	$ 356,921,430	335,011,425
Liabilities and Net Assets:		
Liabilities:		
Accounts payable and accrued expenses	$ 7,928,869	4,840,651
Notes payable	227,932	251,918
Bonds payable	45,200,000	45,200,000
Deferred revenue	2,695,061	2,878,365
Total liabilities	56,051,862	53,170,934
Net assets:		
Unrestricted		
General operating	13,903,989	13,408,682
Designated by board for:		
Research programs	2,197,000	1,900,000
Endowment	107,942,756	99,533,294
Net investment in plant	53,895,812	46,536,091
Total unrestricted	177,939,557	161,378,067
Temporarily restricted	9,796,302	8,587,520
Permanently restricted	113,133,709	111,874,904
Total net assets	300,869,568	281,840,491
Total liabilities and net assets	$ 356,921,430	335,011,425

CONSOLIDATED STATEMENT OF ACTIVITIES
Year ended December 31, 2000
With comparative totals for the year ended December 31, 1999

	Unrestricted	Temporarily Restricted	Permanently Restricted	2000 Total	1999 Total
Revenue, gains, and other support:					
Public support (contributions and nongovernment grant awards)	$ 9,489,893	9,687,797	5,625,670	24,803,360	23,878,182
Government grant awards	20,097,385	–	–	20,097,385	17,658,439
Indirect cost allowances	12,885,076	–	–	12,885,077	11,377,795
Other revenue:					
Program fees	2,551,373	–	–	2,551,373	2,385,687
Rental income	374,757	–	–	374,757	358,934
Publications sales	8,683,809	–	–	8,683,809	6,400,303
Dining services	2,616,113	–	–	2,616,113	2,445,184
Rooms and apartments	1,985,658	–	–	1,985,658	1,865,047
Royalty and licensing fees	1,093,573	–	–	1,093,573	606,691
Realized gain on sale of investments (net)	23,856,792	–	2,604,143	26,460,935	6,519,465
Investment income (interest and dividends)	8,132,622	–	–	8,132,622	6,967,857
Miscellaneous	173,074	–	–	173,074	146,841
Total other revenue	49,467,771	–	2,604,143	52,071,914	27,696,009
Net assets released from restrictions	8,479,015	(8,479,015)	–	–	–
Total revenue, gains, and other support	100,419,140	1,208,782	8,229,813	109,857,735	80,610,425
Expenses:					
Research	35,923,808	–	–	35,923,808	31,454,215
Educational programs	9,801,786	–	–	9,801,786	9,153,276
Publications	8,281,363	–	–	8,281,363	6,159,371
Banbury Center conferences	1,165,989	–	–	1,165,989	1,111,921
DNA Learning Center programs	1,105,658	–	–	1,105,658	1,059,168
General and administrative	9,252,253	–	–	9,252,253	7,767,286
Dining services	3,635,324	–	–	3,635,324	3,117,043
Total expenses	69,166,181	–	–	69,166,181	59,822,280
Increase in net assets from operations	31,252,959	1,208,782	8,229,813	40,691,554	20,788,145
Other changes in net assets					
Net unrealized (loss) gain on fair value of investments	(14,691,469)	–	(6,971,008)	(21,662,477)	39,572,760
Increase in net assets before extraordinary item	16,561,490	1,208,782	1,258,805	19,029,077	60,360,905
Extraordinary item (loss on refinancing of debt)	–	–	–	–	(598,061)
Increase in net assets	16,561,490	1,208,782	1,258,805	19,029,077	59,762,844
Net assets at beginning of year	161,378,067	8,587,520	111,874,904	281,840,491	222,077,647
Net assets at end of year	$ 177,939,557	9,796,302	113,133,709	300,869,568	281,840,491

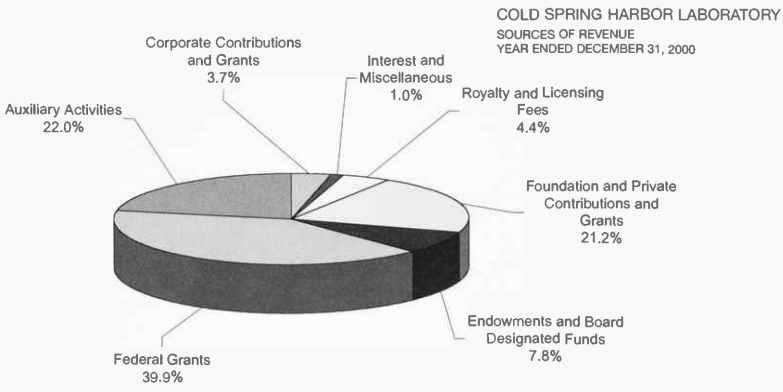
CONSOLIDATED STATEMENTS OF CASH FLOWS
Years ended December 31, 2000 and 1999

	2000	1999
Cash flows from operating activities:		
Increase in net assets	$ 19,029,077	59,762,844
Adjustments to reconcile increase in net assets to net cash provided by operating activities:		
Depreciation and amortization	3,974,311	3,526,229
Extraordinary item (loss on refinancing of debt)	–	598,061
Net appreciation in fair value of investments	(4,798,458)	(46,092,225)
Contributions restricted for long-term investment	(9,350,612)	(10,043,571)
Changes in assets and liabilities:		
Increase in accounts receivable	(1,954,474)	(215,871)
Increase in grants receivable	(893,014)	(374,642)
Increase in contributions receivable	(3,600,936)	(1,903,687)
Increase in publications inventory	(188,439)	(215,090)
(Increase)decrease in prepaid expenses and other assets	(370,229)	140,163
Increase in accounts payable and accrued expenses	3,088,218	2,030,863
Decrease in deferred revenue	(183,304)	(480,357)
Net cash provided by operating activities	4,752,140	6,732,717
Cash flows from investing activities:		
Capital expenditures	(21,991,646)	(12,545,862)
Proceeds from sales and maturities of investments	71,313,900	70,618,250
Purchases of investments	(70,827,688)	(70,270,898)
Net change in investments in employee residences	(295,849)	(287,320)
Net cash used in investing activities	(21,801,283)	(12,485,830)
Cash flows from financing activities:		
Permanently restricted contributions	5,625,670	6,291,765
Contributions restricted for investment in land, buildings, and equipment	3,724,942	3,751,806
Decrease(increase) in contributions receivable	349,221	(317,460)
Repayment of bonds payable	–	(27,000,000)
Issuance of bonds payable	–	42,200,000
Deferred financing costs incurred	–	(576,994)
Repayment of notes payable	(23,986)	(24,245)
Net cash provided by financing activities	9,675,847	24,324,872
Net (decrease)increase in cash and cash equivalents	(7,373,296)	18,571,759
Cash and cash equivalents at beginning of year	34,830,286	16,258,527
Cash and cash equivalents at end of year	$ 27,456,990	34,830,286
Supplemental disclosures:		
Interest paid	$ 2,063,031	1,494,414

COMPARATIVE OPERATING HISTORY 1996–2000
(Dollars in Thousands)

	1996	1997	1998	1999	2000
Revenue:					
Main Lab:					
Grants & contracts	$ 20,879	22,743	24,025	27,397	31,027
Indirect cost reimbursement	9,704	9,910	11,054	11,207	12,718
Other	7,859	8,472	9,441	9,426	10,618
CSHL Press	4,805	5,238	6,341	6,400	8,684
Banbury Center	1,214	1,495	1,444	1,848	1,856
DNA Learning Center	754	875	1,334	1,392	1,471
Total income	45,215	48,733	53,639	57,670	66,374
Expenses:					
Main Lab:					
Research & training	20,879	22,743	24,025	27,397	31,027
Operation & maintenance of plant	5,446	5,274	5,549	5,765	6,589
General & administrative	3,438	3,625	3,378	3,844	6,162
Other	5,367	5,759	7,328	7,863	7,075
CSHL Press	5,032	5,320	6,141	6,077	8,186
Banbury Center	1,225	1,437	1,321	1,614	1,702
DNA Learning Center	781	887	1,228	1,280	1,362
Total expenses, excluding depreciation	42,168	45,045	48,970	53,840	62,103
Excess before depreciation, amortization and release of designated funds	3,047	3,688	4,669	3,830	4,271
Depreciation and amortization	(2,988)	(3,371)	(3,443)	(3,526)	(3,974)
Designation of funds (1)	–	–	(750)	–	(297)
Net operating excess	$ 59	317	476	304	1,244

The above amounts are presented on a combined basis for all funds for which Cold Spring Harbor Laboratory prepares operating budgets.
(1) Funds designated to underwrite future direct and indirect expenses of the neuroscience, imaging, computational neuroscience and other research programs.



FINANCIAL SUPPORT OF THE LABORATORY

Cold Spring Harbor Laboratory, Banbury Center, and the 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 2000.

GRANTS January 1, 2000–December 31, 2000

COLD SPRING HARBOR LABORATORY

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*2000 Funding*	
FEDERAL GRANTS				
NATIONAL INSTITUTES OF HEALTH				
Program Projects	Dr. Herr	1/92–12/01	$ 4,206,852	
	Dr. Stillman	8/90–7/05	3,473,216	*
Research Support	Dr. Cline	12/99–11/04	438,640	
	Dr. Cline	3/98–2/01	284,918	
	Dr. Enikolopov	8/99–5/04	325,299	
	Dr. Enikolopov	9/94–11/03	392,359	
	Dr. Futcher	4/93–3/01	283,628	
	Dr. Grewal	3/00–2/05	281,847	*
	Dr. Hannon	9/00–8/05	283,333	*
	Dr. Helfman	9/99–8/04	374,872	
	Dr. Hengartner	5/00–4/04	291,355	*
	Dr. Hernandez	7/00–6/04	200,101	*
	Dr. Hirano	5/96–4/01	328,964	
	Dr. Joshua-Tor	12/01–11/04	288,523	*
	Dr. Krainer	7/89–6/02	438,566	
	Dr. Lowe	7/99–6/04	315,853	
	Dr. Malinow	5/00–4/05	504,941	*
	Dr. Malinow	4/95–3/03	429,090	
	Dr. McCombie	1/99–12/03	877,686	
	Dr. McCombie	9/99–9/02	1,499,223	
	Dr. Neuwald	10/98–8/01	344,771	
	Dr. Skowronski	4/98–3/03	473,529	
	Dr. Spector	4/90–3/03	477,968	
	Dr. Stillman	6/00–5/04	517,992	*
	Dr. Svoboda	12/98–11/03	368,943	
	Dr. Tonks	8/91–3/01	536,041	
	Dr. Tonks	5/97–4/01	299,173	
	Dr. Tully	4/94–3/02	352,460	
	Dr. Tully	10/00–9/03	567,290	*
	Dr. Van Aelst	12/97–11/01	381,829	
	Dr. Wigler	7/98–4/02	910,736	
	Dr. Wigler	9/00–7/03	750,111	*
	Dr. Xu	1/98–12/02	294,209	
	Dr. Yin	9/99–4/02	355,953	
	Dr. Zhang	9/00–8/03	416,667	*
	Dr. Zhang	8/00–7/03	300,150	*
	Dr. Zhong	6/00–5/04	291,813	*

*New Grants Awarded in 2000
*Includes Direct & Indirect Cost

Grantor	Program/Principal Investigator	Duration of Grant	2000 Funding*	
Fellowships	Dr. Sacco-Bubulya	7/00–1/02	32,416	*
	Dr. Groover	5/99–4/01	14,569	
	Dr. Haas	7/98–6/01	39,232	
	Dr. Hofmann	12/00–12/02	34,832	*
	Dr. Horiuchi	10/98–9/00	36,700	
	Dr. Nimchinsky	9/98–8/01	40,936	
	Dr. Ruthazer	3/99–2/02	39,232	
Training Support	Training in Cancer Cell Biology and Tumor Virology	7/94–12/03	305,184	
Course Support	Cancer Research Center Workshops	4/92–3/05	280,319	
	Neurobiology Short-term Training	5/82–4/01	159,099	
	Analysis of Large DNA Molecules	4/91–3/01	67,224	
	Computational Genomics	9/91–8/01	93,149	
	In Situ Hybridization and Immunocyto-chemistry	7/98–6/03	86,396	
	Automated Genome Sequencing	4/95–3/01	93,087	
	Molecular Biology and Development of *Xenopus laevis*	4/96–3/01	18,000	
	C. elegans	8/98–6/01	41,243	
	Macromolecular Crystallography	9/00–8/01	48,359	*
Meeting Support	Genome Mapping and Sequencing	4/90–3/02	35,947	
	65th CSHL Symposium: Biological Responses to DNA Damage	6/98–5/01	10,000	*
	Zebrafish Development and Genetics	4/00–3/03	18,270	*
	Axon Guidance and Neural Plasticity	6/00–5/01	20,000	*
	Cancer Genetics and Tumor Suppressor Genes	8/00–7/05	15,000	*
	Mouse Molecular Genetics	8/00–7/05	16,500	*
	Germ Cells	9/00–8/01	6,000	*
	Gene Therapy	8/00–8/01	6,000	*
	Molecular Chaperones and Heat Shock Response	3/00–2/01	8,000	*
NATIONAL SCIENCE FOUNDATION				
Cooperative Agreement	Dr. McCombie	2/99–1/02	1,068,426	
Research Support	Dr. Cline	5/99–8/03	115,000	
	Dr. Helfman	2/99–1/03	108,590	
	Dr. Jackson	2/98–1/01	100,000	
	Dr. Martienssen	11/98–10/01	559,979	
Training Support	URPs	6/00–5/03	56,920	*
Course Support	Advanced Bacterial Genetics	5/99–4/02	77,264	
	Arabidopsis Molecular Genetics	4/00–3/03	63,500	*
	Early Development of *Xenopus laevis*	9/98–8/02	20,674	
	Computational Neuroscience: Vision	8/00–7/02	18,135	*
Meeting Support	Zebrafish Development and Genetics	4/00–3/01	9,781	*
	Cell Cycle	5/00–4/01	8,000	*
	Molecular Genetics	7/00–6/01	10,000	*
	Early Development of *Xenopus laevis*	9/00–8/01	19,980	*
	Axon Guidance and Neural Plasticity	9/00–8/01	7,000	*
	Germ Cells	9/00–8/01	12,840	*
	Molecular Genetics	7/00–6/01	10,000	*

*New Grants Awarded in 2000
*Includes Direct & Indirect Cost

Grantor	Program/Principal Investigator	Duration of Grant	2000 Funding*	
DEPARTMENT OF ENERGY				
Research Support	Dr. Martienssen	8/91–2/01	102,000	
UNITED STATES DEPARTMENT OF AGRICULTURE				
Research Support	Dr. Martienssen	12/00–11/01	72,000	*
	Drs. McCombie/Martienssen	7/98–8/01	95,100	
	Dr. Jackson	9/99–8/01	105,000	
	Dr. Stein	9/00–8/04	515,097	*
	Dr. Stein	9/00–8/02	336,939	*
UNITED STATES DEPARTMENT OF THE ARMY				
Research Support	Dr. Hannon	6/00–5/03	126,000	*
	Dr. Helfman	7/99–6/02	117,600	
	Dr. Van Aelst	7/99–6/02	117,600	
	Dr. Zhong	9/99–8/02	240,793	
Fellowship Support	Dr. Hamaguchi	4/00–3/04	59,000	*
	Dr. Hannon	6/00–5/01	59,000	*
	Dr. Jin	5/99–5/02	42,000	
	Dr. Mendez	7/99–6/02	42,000	
	Dr. Saitoh	1/01-12/03	48,191	*
	Dr. Samuelson	6/98–5/01	21,500	
MISCELLANEOUS GRANTS				
Research Support				
Alzheimer's Association	Dr. Barria	8/99–7/01	40,000	
	Dr. Malinow	8/99–7/01	60,000	
American Cancer Society	Dr. Wigler, Professorship	1986–2012	50,000	
	Dr. Wigler, Supply Allowance	2000	10,000	
	Dr. Lowe	7/99–6/02	150,000	
	Dr. Joshua-Tor	7/99–6/02	125,000	
Andrew's Buddies Corporation	Dr. Krainer	1/00–12/01	40,000	*
Michael Scott Barish Human Cancer Grant sponsored by the 1 in 9: The Long Island Breast Cancer Action Coalition	Dr. Wigler	2000	205,000	*
The Louis Berkowitz Family Foundation, Inc.	Dr. Lazebnik	2000	23,000	*
Breast Cancer Research Foundation	Dr. Wigler	10/00–9/01	1,000,000	*
Llewellyn Burchell Charitable Trust	Dr. Lazebnik	12/00–11/01	20,000	*
Dr. and Mrs. Burke A. Cunha Gift	Dr. Van Aelst	2000	1,000	*
Davenport Family Foundation	Cancer Research Programs	1/00–12/00	325,000	*
Devgen N.V.	Dr. Hengartner	4/98–3/01	200,000	
W.H. Donner Foundation	Dr. Lazebnik	9/00–8/01	45,000	*
The Fraxa Foundation	Dr. Svoboda	6/99–6/01	44,000	*
Joseph Flaherty Gift	Dr. Wigler	2000	500	*
Genetica	Dr. Hannon	11/00–10/01	133,652	*
Thomas S.T. Gimbel and Lesley B. Gimbel Gift	Utrophin Medical Research Project	2000	12,500	*

*New Grants Awarded in 2000
·Includes Direct & Indirect Cost

Grantor	Program/Principal Investigator	Duration of Grant	2000 Funding*	
Huntington Breast Cancer Action Coalition	Dr. Wigler	2000	25,000	*
Human Frontier Science Program Organization (HFSP)	Dr. Hirano	7/98–6/01	55,000	
	Dr. Svoboda	9/98–8/01	67,947	
	Dr. Tully	8/99–8/02	46,670	
Illinois Neurofibromatosis	Dr. Van Aelst	2000	10,000	*
Edward and Patricia Karsch Fund	Dr. Lazebnik	2000	500	*
Long Island Foundation for the Elimination of Breast Cancer	Dr. Wigler	2000	8,000	*
Long Islanders Against Breast Cancer	Dr. Wigler	2000	5,515	*
Long Beach Breast Cancer Coalition	Dr. Wigler	2000	2,500	*
Manhasset Women's Coalition Against Breast Cancer	Dr. Wigler	2000	25,000	*
Louis Morin Charitable Trust	Dr. Dubnau	2000	45,740	*
	Dr. Joshua-Tor	2000	79,260	*
March of Dimes	Dr. Hengartner	6/99–5/00	54,402	
Elizabeth McFarland Breast Cancer Research Grant	Dr. Wigler	9/00–8/01	58,486	*
G. Harold and Leila Y. Mathers Charitable Foundation	Dr. Svoboda	3/99–2/02	290,140	
The Maxfield Foundation	Dr. Lazebnik	12/00–11/01	10,000	*
Monsanto, Inc.–Plant Consortium Program	Dr. Martienssen	1/98–8/00	202,500	
Neurofibromatosis Foundation of Arizona	Dr. Van Aelst	9/99–8/00	10,000	*
Neurofibromatosis, Inc., Massachusetts Bay Area	Dr. Van Aelst	9/99–8/00	10,000	*
Neurofibromatosis Foundation–Illinois and Massachusetts Bay Chapters	Dr. Zhong	7/00–6/01	75,000	*
NIH/Cal Tech Consortium Agreement	Dr. Stein	9/00–6/03	268,074	*
NIH/Nanoprobes, Inc. Consortium Agreement	Dr. Spector	4/99–3/01	31,263	
NIH/Sloan Kettering Consortium Agreement	Dr. Wigler	9/95–8/01	333,333	
NIH/Sloan Kettering Consortium Agreement	Drs. Tonks/Van Aelst	8/97–5/01	471,211	
NIH/University of Pennsylvania Consortium Agreement	Dr. Yin	10/97–9/01	100,000	
NIH/Washington University Consortium Agreement	Dr. Stein	3/99–2/03	36,123	
	Dr. Stein	9/99–11/01	138,141	
	Dr. Stein	9/99–9/02	10,105	
Mr. and Mrs. Edmond Nouri	Dr. Tully	4/00–3/03	41,795	*
Novartis–Plant Consortium Program	Dr. Martienssen	1/98–12/02	135,000	
NSF/Clemson Consortium Agreement	Dr. McCombie	10/99–9/02	323,000	
NSF/Rutgers University Consortium Agreement	Drs. Spector/Martienssen/McCombie	10/00–9/05	463,472	*
NSF/University of Wisconsin Consortium Agreement	Dr. Martienssen	9/00–8/05	210,653	*
Offin Charitable Trust	Dr. Lazebnik	12/00–11/01	25,000	*
Michael Rankowitz and Shelia Heffron	Dr. Zhong	2000	25,000	*
Rap Publishing	Dr. Lazebnik	2000	508	*

*New Grants Awarded in 2000
*Includes Direct & Indirect Cost

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*2000 Funding*-	
Fannie E. Rippel Foundation	Equipment: Advanced Neuroscience Imaging Center	2000	355,000	*
Robin and Enrique Senior Philanthropic Fund	Utrophin Medical Research Project	2000	5,000	*
Seraph Foundation	Dr. Enikolopov	11/00–4/01	54,000	*
	Dr. Huang	11/00–4/01	25,000	*
	Dr. Lazebnik	1/00–12/00	23,000	*
Sewanhaka Central High School	Dr. Wigler	2000	200	*
Dr. Jeffrey B. Shellan Gift	Dr. Wigler	2000	500	*
Alfred P. Sloan Foundation	Dr. Watson	4/00–12/00	45,000	*
SNP Consortium	Dr. Stein	4/99–9/01	656,877	
	Dr. Stein	11/99–9/01	131,299	
Ambassador Felix Schnyder Memorial Fund, administered by The Lauri Strauss Leukemia Foundation	Dr. Lowe	1/00–12/00	15,000	*
St. Giles Foundation	Drs. Wigler/Hatchwell	8/00–1/01	178,677	*
Sungene Plant Consortium Program	Dr. Martienssen	7/00–6/01	135,000	*
Texas Neurofibromatosis Foundation	Dr. Van Aelst	2000	10,000	*
Tularik, Inc.	Dr. Wigler	10/97–10/03	660,000	
U.S.D.A./Clemson Consortium Agreement	Dr. McCombie	10/99–9/02	323,000	
West Islip Breast Cancer Coalition for L.I., Inc.	Dr. Wigler	2000	5,000	*
Westvaco–Plant Consortium Program	Dr. Martienssen	1/99–12/02	135,000	
Zeneca Ltd.–Plant Consortium Program	Dr. Martienssen	7/99–7/03	135,000	
The Whitaker Foundation	Dr. Svoboda	6/98–7/01	46,750	
Donnamarie Zuzzolo Gift	Dr. Wigler	2000	50	*
Fellowships				
Rita Allen Foundation	Dr. Lowe	9/99–8/02	50,000	
	Dr. Hannon	10/00–9/03	50,000	*
Alzheimer's Association	Dr. Esteban	8/99–7/01	40,000	
American Cancer Society	Dr. Drier	7/99–6/02	32,000	
	Dr. Hastings	1/00–12/02	30,000	*
	Dr. Saitoh	4/00–12/00	34,000	*
Arnold and Mabel Beckman Foundation	Watson School of Biological Sciences	9/00–8/05	250,000	*
Burroughs Wellcome Fund	Dr. Mainen	9/97–8/00	115,500	
Canadian Insitutes of Health Research (CIHR)	Dr. Querido	8/99–7/02	35,000	
Cancer Research Fund–Damon Runyon Walter Winchell	Dr. Z. Zhang	6/99–5/02	42,500	
CSHL Association	Fellowship and Start up Fund support	2000	205,821	*
Danish Academy of Technical Sciences	Drs. Tonks/Andersen	2000	13,510	*
Demerec-Kaufman-Hollander Fellowship	Dr. Grewal	1/00–12/00	5,971	*
Deutsche Forschungsgemeinschaft (DFG)	Dr. Schmitz	6/99–5/01	28,000	
The Ellison Medical Foundation	Dr. Grewal	9/99–9/01	50,000	
Engelhorn Scholars Program Funded by European Foundation for the Advancement of Medicine	Watson School of Biological Sciences	2000	104,200	*
The Eppley Foundation for Research	Dr. Huang	9/00–8/01	22,000	*
Johns Hopkins/Ellison Foundation	Dr. Kass-Eisler	11/99–10/00	70,635	*

*New Grants Awarded in 2000
-Includes Direct & Indirect Cost

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*2000 Funding**	
Joseph G. Goldring Foundation	Dr. Stillman	7/99–6/00	60,000	*
Helen Hoffritz Foundation	Dr. Cline	12/97–12/00	30,000	*
Howard Hughes Medical Institute	Watson School of Biological Sciences	9/00–8/01	133,000	*
	Graduate Student Support	6/99–5/04	64,200	*
Human Frontier Science Program Organization (HFSPO)	Dr. Davenne	5/00–4/02	35,000	*
	Dr. Nakayama	4/00–3/02	42,000	*
	Dr. de Stanchina	1/00–12/02	36,000	*
Irving A. Hansen Memorial Foundation	Dr. Tonks	2000	10,000	*
Japan Society for the Promotion of Science (JSPS)	Dr. Fukada	4/00–3/02	51,000	*
Cynthia Jay Gift	Dr. Cline	11/00–12/00	3,600	*
Sidney Kimmel Foundation	Dr. Tansey	7/99–6/01	100,000	
Esther A. and Joseph Klingenstein Fund, Inc.	Dr. Svoboda	7/98–6/01	40,000	
Charles Henry Leach II Foundation	Dr. Enikolopov	1/97–12/01	25,000	
Lehrman Institute	Dr. Svoboda	4/00–3/03	53,000	*
	Dr. Watson Archives	4/00–3/03	72,000	*
The Leukemia and Lymphoma Society Special Fellow Award	Dr. Duelli	12/00–12/03	35,000	*
	Dr. Julien	7/99–6/02	33,250	
	Dr. Pendergrast	7/98–6/01	39,700	
	Dr. Schmitt	7/00–6/03	42,000	*
	Dr. Shibahara	7/00–6/01	45,000	*
	Dr. Soengas	7/99–6/02	39,700	
Life Science Research Foundation	Dr. Vollbrecht	6/99–5/02	42,000	
Ministerio de Educacion y Cultura	Dr. Cantallops	5/99–4/01	17,694	
NARSAD	Dr. Esteban	7/99–6/00	30,000	
	Dr. Zhu	7/00–6/01	30,000	*
Pew Charitable Trust	Dr. Hannon	7/97–6/01	60,000	
	Dr. Svoboda	7/98–6/02	60,000	
Rockefeller Foundation	Dr. Vanavicht	11/99–10/01	11,674	
Roche Foundation Fellowship	Dr. Duelli	1/00–12/00	20,880	*
Schwarz Foundation	Drs. Svoboda/Maravall	2000	25,000	
Schwarz Foundation	Initiative for Computational Neuroscience	1/00–12/00	48,050	*
Searle Scholars Program	Dr. Grossniklaus	7/98–6/01	60,000	
Andrew Seligson Memorial Fellowship	Fellowship Support	2000	35,000	*
Alfred P. Sloan Foundation	Dr. Zador	9/00–9/02	20,000	*
Spanish Dept. of Education and Science (Ministerio de Educacion y Ciencia)	Dr. Maravall	7/00–12/01	11,270	*
Tularik, Inc.	Fellowship Support	1/98–12/03	200,000	
The V Foundation	Dr. Myers	8/99–7/01	50,000	
	Dr. Hamaguchi	4/00–3/02	50,000	*
Helen Hay Whitney Foundation	Dr. Sabatini	4/99–3/02	37,000	
	Dr. Zito	7/00–6/03	35,000	*
Course Support				
Grass Foundation	Scholarships	2000	15,000	*
Howard Hughes Medical Institute	Advanced Neurobiology Courses	2000	330,000	*
Meeting Support				
Hampton Research	Crystallography Workshop	2000	1000	*
Hoffmann-LaRoche, Inc.	Crystallography Workshop	2000	1000	*
MSC Molecular Structure Corp.	Crystallography Workshop	2000	1000	*
Merck Research Laboratories	Crystallography Workshop	2000	1000	*
Pfizer	Crystallography Workshop	2000	750	*
Protein Solutions, Inc.	Crystallography Workshop	2000	500	*

*New Grants Awarded in 2000
·Includes Direct & Indirect Cost

BANBURY CENTER

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*1999 Funding*[a]
FEDERAL SUPPORT			
CDC–National Center for Infectious Diseases	Strategies for Identification and Characterization of Unknown Pathogens	2000	$ 44,500 *
NIH–National Human Genome Research Institute	Digital Image Archive on the American Eugenics Movement Editorial Advisory Panel Workshops	2000	9,122
NIH–National Institute of Child Health and Human Development	FMRP: What Does It Do?	2000	10,000 *
NIH–National Institute of General Medical Sciences (through a grant to Mount Sinai School of Medicine)	Signaling Network Control-Cell Interactions: Phase II Planning	2000	24,219 *
NIH–National Institute of Mental Health	FMRP: What Does It Do?	2000	10,000 *
NIH–National Institute of Mental Health	Mouse Behavioral Phenotyping	2000	25,000 *
USDA–Agricultural Research Service	Meeting the Challenge of Infectious Diseases in the 21st Century	2000	43,406 *
NONFEDERAL SUPPORT			
Meeting Support			
ALS Association	Therapeutic Approaches in Mouse Models of ALS	2000	36,167 *
FRAXA Research Foundation	FMRP: What Does It Do?	2000	20,975 *
Handspring Inc.	Neural Networks and Cognition	2000	25,000 *
Marie H. Robertson Memorial Fund for Neurobiology	Structure, Mechanism, and Function of CaMKII	2000	20,000
Albert B. Sabin Vaccine Institute, Inc. (through a grant from the Bill & Melinda Gates Foundation)	Social Venture Capital for Neglected Vaccines: Creating Successful Alliances	2000	23,551 *
Alfred P. Sloan Foundation	Mammalian Cloning: Biology and Practice	2000	40,180 *
Alfred P. Sloan Foundation (through a grant to Massachusetts Institute of Technology)	Persistent Neural Activity	2000	30,000 *
The Swartz Initiative for Computational Neuroscience	Persistent Neural Activity	2000	17,565 *
The Swartz Initiative for Computational Neuroscience	Toward Animal Models of Attention and Consciousness	2000	36,772 *

*New Grants Awarded in 2000
[a]Includes Direct & Indirect Cost

DNA LEARNING CENTER

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*2000 Funding**
FEDERAL GRANTS			
NATIONAL INSTITUTES OF HEALTH ELSI Research Program	Creation of a *Digital Image Archive on the American Eugenics Movement*	3/98–3/01	$ 201,289
NATIONAL SCIENCE FOUNDATION	*A Partnership to Develop Advanced Technology Units on Genomic Biology*	8/97–7/01	166,270
DEPARTMENT OF ENERGY	*The Science and Issues of Human DNA Polymorphisms: An ELSI Training Program for High School Biology Teachers*	1/97–9/01	62,642
NONFEDERAL GRANTS			
Howard Hughes Medical Institute	Precollege Science Education Initiative for Biomedical Research Institutions	7/94–8/01	66,732
Josiah Macy, Jr. Foundation	*DNA from the Beginning*	10/97–9/01	297,484

The following schools each awarded a grant for the *Genetics as a Model for Whole Learning Program:*

Bethpage Union Free School District	$ 1,050	Locust Valley Central School District	15,175
Commack Union Free School District	125	Mamaroneck Union Free School District	1,625
Community School District #29	33,375	Massapequa Union Free School District	1,425
East Meadow Union Free School District	4,065	Northport–East Northport Union Free School District	5,600
Farmingdale Union Free School District	1,995	Old Westbury School of the Holy Child	1,650
Garden City Public School	6,005	Oyster Bay–East Norwich Central School District	2,770
Great Neck Union Free School District	5,200	Plainedge Union Free School District	1,075
Green Vale School	2,800	Port Washington Union Free School District	6,770
Half Hollow Hills Central School District	6,375	Rockville Center Union Free School District	4,525
Harborfields Central School District	8,785	St. Dominic Elementary School	5,650
Jericho Union Free School District	5,500	Syosset Central School District	22,100
Lawrence Union Free School District	3,640		

The following schools each awarded a grant for *Curriculum Study:*

Commack Union Free School District	$1,100	Massapequa Union Free School District	2,200
East Woods School	1,100	Oceanside Union Free School District	1,100
Elwood Union Free School District	2,200	Oyster Bay–East Norwich Central School District	2,200
Friends Academy	2,200	Plainview–Old Bethpage Central School District	1,100
Garden City Union Free School District	1,100	Portledge School	1,100
Green Vale School	1,100	Port Washington Union Free School District	1,100
Half Hollow Hills Central School District	1,100	Ramaz School	2,200
Harborfields Central School District	1,100	Roslyn Union Free School District	1,100
Herricks Union Free School District	1,100	Sachem Central School District	1,100
Island Trees Union Free School District	1,100	South Huntington Union Free School District	1,100
Jericho Union Free School District	1,100	Syosset Central School District	1,100
Locust Valley Central School District	1,100	West Hempstead Union Free School District	1,100
Long Beach City School District	2,200		

We wish to express the genuine gratitude of the trustees, administration, and most especially the scientists of Cold Spring Harbor Laboratory for the generous financial support of those persons, corporations, and foundations whose names appear on the following pages of the Annual Report.

Richard L. Cosnotti, Chief Development Officer

Cold Spring Harbor Laboratory is a nonprofit research and educational institution chartered by the University of 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."

METHODS OF CONTRIBUTING TO COLD SPRING HARBOR LABORATORY

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 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: Most wills probably need to be updated. 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 Chief Development Officer, Cold Spring Harbor Laboratory, One Bungtown Road, Post Office Box 100, Cold Spring Harbor, NY 11724, 516-367-8840.

CAPITAL AND PROGRAM CONTRIBUTIONS

January 1, 2000–December 31, 2000

Contributions of $5,000 and above, exclusive of Annual Fund

In 2000, Cold Spring Harbor Laboratory received significant support in the form of capital and program contributions from individuals, foundations, and corporations.

Rita Allen Foundation, Inc.
The Michael Scott Barish Memorial Grant sponsored by 1 in 9: The Long Island Breast Cancer Action Coalition
Andrew's Buddies Corporation
Anonymous
Arrow Electronics
Louis Berkowitz Family Foundation, Inc.
The Breast Cancer Research Foundation
Llewellyn Burchell Charitable Trust
Arthur and Barbara Crocker Charitable Trust
The Ira W. DeCamp Foundation
Devgen N.V.
Oliver S. & Jennie R. Donaldson Charitable Trust
William H. Donner Foundation
Ellison Medical Foundation
The Eppley Foundation for Research
Lawrence Flinn, Jr. Charitable Trust
Fraxa Research Foundation
Samuel Freeman Charitable Trust
Bernard F. and Alva B. Gimbel Foundation
Goldring Family Foundation
Irving A. Hansen Memorial Foundation
The Helen Hoffritz Foundation
Huntington Breast Cancer Action Coalition
Illinois Neurofibromatosis, Inc.
Sidney Kimmel Foundation for Cancer Research
The Esther A. and Joseph Klingenstein Fund, Inc.
Charles Henry Leach II Foundation
The Lehrman Institute
Mrs. Joy Levy
Long Island Foundation for the Elimination of Breast Cancer
Long Islanders Against Breast Cancer (L.I.A.B.C.)
Mr. and Mrs. David L. Luke III
Marks Family Foundation
G. Harold & Leila Y. Mathers Charitable Foundation
The Maxfield Foundation
Breast Cancer Awareness Day in memory of Elizabeth McFarland
Monsanto, Inc.
Louis Morin Charitable Trust
NARSAD
Neurofibromatosis of Arizona
Neurofibromatosis, Inc. Mass Bay Area
Mr. and Mrs. Edmond J. Nouri
Novartis
Offin Charitable Trust
Pew Charitable Trust
William E. and Maude S. Pritchard Charitable Trust
Michael L. Rankowitz and Sheila Heffron
John R. Reese
Fannie E. Rippel Foundation
The Rockefeller Foundation
Mr. and Mrs. Jerry Saslow
Mr. and Mrs. Alan Seligson
Robin and Enrique Senior Philanthropic Fund
The Seraph Foundation
Alfred P. Sloan Foundation
SNP Consortium
St. Giles Foundation
Laurie Strauss Leukemia Foundation
SunGene GmbH & Co.
Swartz Initiative for Computational Neuroscience
Texas Neurofibromatosis Foundation
Tularik, Inc.
Mary G. Turner
The V Foundation
Dr. and Mrs. James D. Watson
West Islip Breast Cancer Coalition
Westvaco
The Whitaker Foundation
Helen Hay Whitney Foundation
Zeneca, Ltd.

Total	**$10,359,907**

WATSON SCHOOL OF BIOLOGICAL SCIENCES CAPITAL CAMPAIGN

January 1, 2000–December 31, 2000

Contributions of $10,000 and above, exclusive of Annual Fund

The Watson School of Biological Sciences at Cold Spring Harbor Laboratory was established for the purpose of granting the Ph.D. degree. In its second year, the Watson School matriculated 9 additional students bringing the total number to 15. In 2000, the laboratory received significant support from individuals, foundations, trusts, and corporations.

Arnold and Mabel Beckman Graduate Studentship	Arnold and Mabel Beckman Foundation	
Core Course	Arnold and Mabel Beckman Foundation Mr. Nicholas C. Forstmann Mr. and Mrs. Leslie C. Quick, Jr.	
Dean's Chair	Lita Annenberg Hazen Foundation Annenberg Foundation	
Faculty Lectureships	The Esther A. and Joseph Klingenstein Fund, Inc. Mrs. Mary D. Lindsay Quick & Reilly Group, Inc. Dr. George B. Rathmann	
Fellowships	Bristol-Myers Squibb Company The Charles A. Dana Foundation European Foundation for the Advancement of Medicine Mr. Alan Goldberg	The Florence Gould Foundation Mr. and Mrs. David H. Koch Mr. Robert D. Lindsay Mr. and Mrs. David L. Luke III The Miller Family Foundation
Knight House	Mrs. Gertrude W. Conner Mr. Edward Giles J.P. Morgan and Co. Inc.	
Pall Fund	Alan B. Slifka Foundation, Inc.	
Visiting Lectureships	The Michel David-Weill Foundation Fairchild Martindale Foundation Mr. and Mrs. Charles E. Harris Dr. Daniel E. Koshland, Jr. Dr. and Mrs. Mark A. Ptashne	
Watson Fund	Biogen, Inc. Mrs. John H. Livingston Dr. and Mrs. Walter C. Meier Dr. Michael Wigler Dr. Mark J. Zoller	
Total	**$14,794,970**	

ANNUAL CONTRIBUTIONS

Corporate Sponsor Program

The Corporate Sponsor Program continues to be an essential factor in the overall success of the Laboratory's meetings programs, for meetings held in Grace Auditorium on the main Laboratory campus and at Banbury Center. The funding provided by the Program enables us to plan ahead for the following year's meetings, and we are able to use it to select new and unusual topics. However, the wave of mergers in the pharmaceutical and biotechnology industries is severely reducing the pool of companies that we can approach, and we are therefore evermore grateful to the 38 corporate sponsors and affiliates who joined us in 2000 and are listed below.

The Corporate Affiliates and Contributors Program began in 1999 with the expectation that smaller biotechnology companies which must, necessarily, devote their resources to research might nevertheless find it useful to have some of the benefits of the Corporate Sponsor Program.

The members of the Program receive special privileges in acknowledgment of their contributions. We waive all on-site fees for eight representatives of each company at our meetings. The number of meetings has increased, so that in 2000, no fewer than 17 meetings took place in Grace Auditorium. Three scientists from member corporations may attend meetings at Banbury Center, where attendance is otherwise only by invitation of the organizers. Corporate Sponsors receive gratis copies of Cold Spring Harbor Laboratory Press publications, including the journals *Genes and Development*, *Learning and Memory*, *Genome Research*, and *Protein Science*. We acknowledge our Sponsors in all relevant publications, including the books of abstracts given to each of the 7000 participants who come to the meetings each year. The names of the sponsoring companies are listed on the poster describing the meetings; this is mailed to approximately 17,000 scientists throughout the world. In addition, the companies are listed on the Cold Spring Harbor Laboratory Web Site, on the Meetings and Banbury Center pages.

COLD SPRING HARBOR LABORATORY CORPORATE SPONSORS

Amgen Inc.
AstraZeneca
Aventis Pharma AG
BASF Bioresearch Corporation
BioVentures, Inc.
Bristol-Myers Squibb Company
Chiron Corporation
Chugai Research Institute for Molecular Medicine, Inc.
Diagnostic Products Corporation
DuPont Pharmaceuticals Company
Forest Laboratories, Inc.
Genentech, Inc.
Genetics Institute
Glaxo Wellcome Inc.
Hoffmann-La Roche Inc.
Johnson & Johnson
Kyowa Hakko Kogyo Co., Ltd.
Eli Lilly and Company
Merck Research Laboratories
New England BioLabs, Inc.
Novartis Pharma Research
OSI Pharmaceuticals, Inc.
Pall Corporation
Palmetto Partners, Ltd.
Parke-Davis Pharmaceutical Research
PE Biosystems
Pfizer Inc.
Pharmacia & Upjohn, Inc.
Research Genetics, Inc.
Schering-Plough Corporation
SmithKline Beecham Pharmaceuticals

PLANT CORPORATE ASSOCIATES

Monsanto Company
Novartis Agricultural Discovery Institute
Pioneer Hi-Bred International, Inc.
Westvaco Corporation

FOUNDATIONS

Albert B. Sabin Vaccine Institute, Inc.

CORPORATE AFFILIATES AND CONTRIBUTORS

Affymetrix, Inc.
Alexis Biochemical Corp.
Applied Imaging
Biogen, Inc.
CuraGen Corporation
Digital Gene Technologies, Inc.
Double Twist, Inc.
Dynal, Inc.
Eppendorf - 5 Prime, Inc.
Genetix Limited
Genome Systems, Inc.
Genomic Instrumentation Ser.
Genomica Corporation
Informax, Inc.
Lexicon Genetics, Inc.
PharMingen
Proteome, Inc.
Qiagen GMBH
Research & Diagnostic Systems, Inc.
Stressgen
Third Wave Technologies, Inc.
Zymo Genetics

Total	**$1,043,000**

DNALC Corporate Advisory Board Annual Fund

The Corporate Advisory Board, established in 1992, serves the DNA Learning Center as a liaison to the corporate community and assists in securing unrestricted dollars for annual support. As a means of raising awareness, the Board conducts the annual CSHL Golf Tournament as well as the Annual Fund. The proceeds benefit the DNA Learning Center and the Partners for the Future program. The Partners program teaches principles and methods of basic scientific research to eight Long Island high school seniors, who work with scientists 10 hours per week after school, from October through March.

Chairman: John J. Leahy, Citibank Private Bank

Michael Aboff, Aboff's Inc.
Andrew D. Ackerman, Chase Manhattan Bank
Brett Ashley, Cole Communications
Rocco S. Barrese, Esq., Dilworth and Barrese
Howard M. Blankman, The Blankman Group
Jane S. Block, Pall Corporation
Thomas J. Calabrese, Daniel Gale Agency
Richard A. Catalano, KPMG, LLP
Edward A. Chernoff, Motors and Armatures, Inc.
Paul Czeladnicki, Rivkin, Radler and Kremer
Robert Diller, Brinkmann Instruments
Candido E. Fuentes-Felix, M.D., F.A.C.S.
Dave Garbus, Huntington Business Products Centre
John Henry, EAB
Arthur D. Herman, Herman Development Corporation
John Kean III, Kean Development Company
Laurie J. Landeau, V.M.D.
Ralf Lange, Luitpold Pharmaceuticals, Inc.
Lilo Leeds, Institute for Student Achievement
James Mattutat, The Shattan Group
Patricia Petersen, Daniel Gale Agency
Christiaan B. Reynolds, ASI Solutions, Inc.
William D. Roche
Wendy Vander Poel Russell, CSHL Honorary Trustee
Horst K. Saalbach, Ph.D., Festo Corporation
Peter G. Schiff, Northwood Ventures LLC
Charles Schueler, Cablevision Systems Corp.
Dixie Scovel, Newsday
Jeffrey K. Tupper, U.S. Trust Company of New York
Robert Van Nostrand, OSI Pharmaceuticals, Inc.
Frederick Volp, Ph.D., CSH Central School District
William N. Wham, ISC Inc.

ANNUAL FUND CONTRIBUTORS

GENOME SUPPORTERS
Contributions of $10,000 or more

EAB
Edward A. Chernoff, Motors and Armatures, Inc.
Mr. and Mrs. Arthur M. Spiro

CHROMOSOME SUPPORTERS
Contributions of $5,000 or more

Mr. and Mrs. G. Morgan Browne
Laurie J. Landeau, V.M.D.
Newsday
Mr. Denis J. O'Kane
Pall Corporation

GENE SUPPORTERS
Contributions of $2,500 or more

Anonymous
Citibank Private Bank
Louis and Virginia Clemente Foundation, Inc.
Dilworth and Barrese
Herman Development Corporation
KPMG LLP
Meenan Oil Company, LP
Northwood Ventures, LLP
OSI Pharmaceuticals, Inc.
Pittway Corporation Charitable Foundation
Rivkin, Radler and Kremer
Tilles Family Foundation

EXON SUPPORTERS
Contributions of $1,000 or more

Aboff's Inc.
Mrs. and Mrs. Joseph Azznarra
Brinkmann Instruments
Daniel Gale Agency
DNALC Fund
Gale and Wentworth Fund
Harweb Foundation
Mr. and Mrs. Gerard Leeds
Mr. George Roche
Mr. and Mrs. William D. Roche
Mr. Harold Rosenberg and Dr. Laurie Sayet Rosenberg
The Blankman Group

INTRON SUPPORTERS
Contributions of $500 or more

Arthur Andersen, LLP
Mr. and Mrs. Robert Beuerlein
Ms. Rosemary Bourne
Dr. and Mrs. Burke A. Cunha
Dr. and Mrs. Candido E. Fuentes-Felix
Monterey Fund, Inc.
Petunia Productions
Ms. Wendy Vander Poel Russell
Topspin Partners, LP

TRIPLE CODON SUPPORTERS
Contributions of $250 or more

John V. and Carole S. Aksak
Mr. and Mrs. Michael Boylan
Emerson Specialty Motor Division
Dr. and Mrs. Steven Goldberg
North Country Medical Associates
State Bank of Long Island

NUCLEOTIDE PAIR SUPPORTERS
Contributions of $100 or more

Drs. John B. Amodio and Eileen M.K. Fontanetta
Dr. and Mrs. Thomas Arcati
Dr. and Mrs. Pierre Beaubrun
Mr. and Mrs. Steven Corwin
Dunetz, Marcus, Brody and Weinstein LLC
Mr. and Mrs. John Genovese
Ms. Louise Johnston
Mr. and Dr. Candido Llovera
Mr. and Mrs. Joseph Lo Cicero
Ms. Anita MacDougall
Mr. and Mrs. Kevin McCarthy
Mr. and Mrs. James Mattutat
Mr. and Mrs. James J. Murphy
Mr. and Mrs. Richard Nicklas, in honor of Leslie C. Quick, Jr.
Joseph P. O'Brien Agency
Dr. Christopher Perrone
Mr. and Mrs. John Pufahl
Mr. and Mrs. Thomas K. Reimels
Dr. and Mrs. Newell B. Robinson
Mr. and Mrs. Alexander J. Smith
Mr. and Mrs. Emil Stenger
Mr. David Strahl
The Torti Family
Mr. and Mrs. Thomas Tucker

NUCLEOTIDE SUPPORTER
Contributions of $50 or more

Mr. and Mrs. Thomas Amberger
The Bakshi Family
Mr. and Mrs. Richard Canner
Ms. May-Loo Dai
Damin Sales/Mr. Steven Gershman
Dr. Michael Gaffney and Ms. Patricia Lynch
Ms. Jennifer Giwa
Mr. and Mrs. Warren Herman
Ms. Christina Jackowski
Mr. and Mrs. Arthur Lagrega
Ms. Mary Lou Lysaght
Mr. and Mrs. Alan Marks
Dr. and Mrs. Lawrence B. Mogan
Mr. and Mrs. Donald Morrongiello
Mr. and Mrs. Kevin O'Donnell
Ms. Stephanie G. Ovaida, Esq.
Mr. and Mrs. Kenneth Packert
Mr. and Mrs. Luis Paniagua
Ms. Elizabeth D. Pessala
Mr. and Mrs. Philip Rao, Jr.
Mr. Marc Schafler
Mr. and Mrs. Jeffrey Stern
Ms. Norma Whitley

GIFTS IN MEMORY OF MARYBETH ZOEGAL
Mr. and Mrs. Christopher Biagini
Mr. and Mrs. Richard Blum
Mr. and Mrs. Henry Brengel
Mr. and Mrs. Michael J. Costello
Ms. Judith Ann Dazzo
Mr. and Mrs. Thomas Grosse
Mr. and Mrs. Victor E. Rerisi
Mr. Arnold A. Sabino

OTHER CONTRIBUTIONS
Mr. and Mrs. Daniel Golub
Mr. and Mrs. Thomas LoBasso, Jr.
Mr. and Mrs. Anthony Sabino
Ms. Dorothy Schumejda
Mr. and Mrs. John Vargas

Total $110,045

The Campaign for the *BioMedia* Addition

To effectively meet the needs of an ever-increasing number of students and the DNA Learning Center's expanding programs, The Campaign for the *BioMedia* Addition is under way. The expansion, to include a suite of teaching labs, a student research lab, new exhibits space, a broadcast media studio, and multimedia computer lab, will ensure that future generations of students and teachers receive the latest and most up-to-date molecular biology education that is the hallmark of the DNA Learning Center.

Through the generous support of the Dolan Family Foundation, the DNA Learning Center will be renamed *The Dolan DNA Learning Center*.

Campaign Contributors

Construction	
The Dolan Family Foundation	$ 3 million
CSHL Funding	$ 500,000
New York Stock Exchange Foundation	$ 10,000
Mr. Denis O'Kane	$ 35,000
Edwin Webster Foundation	$ 80,000
Equipment	
P.E. Biosystems	$ 130,000

DNA Learning Center Grants

Josiah Macy, Jr. Foundation

With a grant of $550,000, the DNA Learning Center will build a companion site to its existing Web Site, *DNA from the Beginning*, titled *Your Genes/Your Health. YGYH* is targeted at patients and families dealing with genetic illness interested in learning the biology behind the medical terminology. Further-more, this new site will provide resources that can assist families participating more effectively in the care of a loved one. This recent grant from the Macy Foundation brings their total investment in the project to $1.37 million.

The Pfizer Institute

With a 2-year grant of $150,000, the DNA Learning Center will offer the *Pfizer Foundation Leadership Institute in Human and Molecular Biology* during the month of July in 2001 and 2002. The *Institute* will provide high-level high school science teachers throughout the country an opportunity for hands-on laboratory experiences at the DNA Learning Center which will assist them in integrating molecular genetics into their high school programs.

SEVENTH ANNUAL GOLF TOURNAMENT SPONSORS

GOLF TOURNAMENT COMMITTEE

Tournament Chairman
Edward A. Chernoff, Motors & Armatures Inc.

Sponsorship Co-Chairmen
W. Dillaway Ayres, CSHL
G. Morgan Browne, CSHL

Photography Chairman
Howard M. Blankman, The Blankman Group

Raffle Co-Chairpersons
Carol Carson, Motors & Armatures Inc.
James Mattutat, The Shattan Group

Tournament Committee
W. Dillaway Ayres, CSHL
Art Brings
G. Morgan Browne, CSHL
Richard L. Cosnotti, CSHL
William Keen, CSHL
Charles Prizzi, CSHL
Vincent P. Torti, CSHL

Guest Celebrities
Clark Gillies
Laura Savini

Founding Tournament Sponsor
J.P. Morgan & Co., Inc.

Major Corporate Sponsors
Cablevision Systems Corp.
Fortunato Son, Inc.
Luitpold Pharmaceuticals, Inc.
Marsh Inc.
Motors & Armatures, Inc.
Quick & Reilly Group, Inc.

Corporate Sponsors
Badge Insurance Agency Inc.
KPMG, LLP
Festo Corporation

Journal Sponsor
Conde Nast Publications

Hole-In-One Sponsors
Badge Insurance Agency Inc.
Competition BMW of Smithtown

Brunch Sponsor
Centerbrook Architects

Cocktail Reception Sponsor
Motors & Armatures, Inc.

Auction Prize
Lufthansa

Registration Table Sponsor
U.S. Trust Company of New York

Refreshment Sponsor
OSI Pharmaceuticals, Inc.

Driving Range Sponsor
Chase Manhattan Bank

Putting Green Sponsor
Jack Berleth & Sons, Inc.

Gold Sponsors
ASI Solutions, Inc.
Barnes & Noble Bookstores
Brinkmann Instruments, Inc.
Chase Manhattan Bank
Ciardullo Landscapes & Designs, Inc.
Citibank Private Bank
Cortel Business Systems
Daniel Gale Agency
Dilworth & Barrese
Herman Development Corporation
ISC, Inc. (3)
Kean Development Company
Lufthansa
Luitpold Pharmaceuticals, Inc.
Northwood Ventures LLC
OSI Pharmaceuticals, Inc.
Rivkin, Radler & Kremer
Sbarro Corporation
The Seligson Foundation
Squadron, Ellenoff, Plesent & Sheinfeld LLP
Tradition N.A. Derivative Products
U.S. Trust Company of New York

Silver Sponsors
Bee, Eisman & Ready
Thomas J. Calabrese
Frank Crystal & Company, Inc.
Electronix Systems
The Hartford
Huntington Business Products Centre
State Bank of Long Island
U.S. Trust Company of New York

Bronze Sponsors
Aboff's Inc.
Advantage Title
Burt Building Materials Corp.
Callan, Regenstreich, Koster & Brady, LLP
Dr. Candido Fuentes-Felix
Margolin & Margolin, Esqs.
MCS/Canon

Commemorative Journal Sponsors
Aboff's, Inc.
A Partymaker
Arrow Linen Supply
Charmer Industries
Dimension II Lawn Sprinklers
Farmingdale Ironworks
Hamilton, Brooks, Smith & Reynolds
Janvey & Sons
KeySpan Energy
Lewisy Fuel Oil, Inc.
New York Biotechnology Assn.
Rampart Insurance
Paul Rosenvold Inc.
Wendy Vander Poel Russell
The Sheridan Press

Tee & Green Sponsors
The Blankman Group
CII Technologies
Jeff's Seafood
J & M Bakery Products
Josten's
Riverhead Building Supply
Stirling Creek Associates, Inc.
TIAA-CREF
ToJo Provisions, Inc.
UnitedHealthCare

Raffle Prize Contributors
Aboff's Inc.
Ace One-Hour Photo
A Rise Above
All-Star Video
Barmonde Studios
Broadway Cares
Clearwater Restaurant
Cold Spring Harbor Laboratory
Crossroads Restaurant
J. DiCarlo's Restaurant
Distinctive Products Company
Ecco Environmental
Boomer Esiason Foundation
Daniel Gale Agency
Clark Gillies
Huntington Business Products Centre
Lemongrass Restaurant
London Jewelers
Marsh's of Huntington
Mill River Inn
New Age Technologies & Peter Tosto
OC Tanner
Port-To-Port
Ragtime
Rivkin, Radler & Kremer
John Rosenberg
Sharp Electronics
Spinnaker Travel
Pat Demarest, Sunderland of Scotland
Texas Instruments
Tourneau
Tupelo Honey Restaurant
Tuxedo Park
Winebow Inc., Limited

Favor Bag Sponsors
Fortune Magazine
CSHL Tee Shirts by Barnes & Noble Bookstores
Golf Balls by Chase Manhattan Bank
Golf Towels by Daniel Gale Agency
Golf Balls by Festo Corporation
Golf Shirts by GE Industrial
Golf Balls by KPMG, LLP
Golf Balls by Vin Torti, CSHL
Golf Tees and Umbrellas by United Healthcare

Special Thanks
The Harold Betters Quartet
Howard Blankman
Eddie Chernoff, Golf Chairman
CSHL & DNALC Staff
Francis X. Harrington
Brian Lynch & Boston Brewery
Jim Mattutat & Pat Petersen
Don Vultaggio, Arizona Beverages

Other Contributors
Anonymous
CDW
Moser & Moser LLP
William D. Roche

Total	**$142,500**

Total DNA Learning Center Annual Fund $252,545

Cold Spring Harbor Laboratory Association (CSHLA)

Officers

James L. Spingarn, President
Trudy H. Calabrese, First Vice President
John S. Grace, Vice President
Cathy Cyphers Soref, Secretary
Antoine C. Kemper, Jr., Treasurer (February 1999–July 2000)
Joseph T. Donohue, Treasurer

Directors

Nicholas J. Bartilucci
George W. Cutting, Jr.
David H. Deming
Frances N. Elder
Linda Franciscovich
Robert A. Gay
William F. Gerry
Lynn M. Gray
Susan Hollo
Allen Dulles Jebsen
E. Coe Kerr
Alan M. Kisner, M.D.
Mary Alice Kolodner
Gilibert R. Ott, Jr.
Eileen K.S. Pulling
Lawrence Remmel
Henry Salzhauer
Ann C. Seifert
Cynthia R. Stebbins
Bruce Stillman, Ph.D.
Marjorie von Stade
James D. Watson, Ph.D.

Honorary Directors

John P. Cleary
Mary D. Lindsay
Anne R. Meier

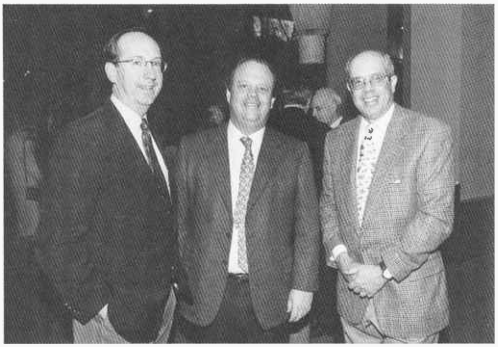
President's Report

The year 2000 kept us very busy, as usual, trying to keep up with the innovation, accomplishments, new happenings, and results at Cold Spring Harbor Laboratory. In many instances, as the "public" face of the Laboratory, it is we who design, implement, and enact programs, both of educational and entertainment value, intended to encourage support for this great institution.

The support of generous donors enables the Laboratory to staff its various research laboratories with top scientific research talent. A significant portion of the Annual Fund goes directly to support promising young postdoctoral fellows. These people are vital to the Laboratory's continuing success. This year, the Cold Spring Harbor Laboratory Association raised $763,721.95 in our Annual Fund. This is a new record, which with Annual Fund endowment gifts totaling $250,000 makes our success even more impressive, surpassing $1 million for the first time.

Leadership is key to the Association's success. In 2000, George W. Cutting, Jr., William F. Gerry, Lynn M. Gray, Susan Hollo, and Allen Dulles Jebsen were elected new Directors. We thank Mary Ann Charlston and Mary D. Lindsay, our retiring Directors, who gave talented and devoted time and effort to the Association. I am delighted to add that after many unstinting and wonderfully productive years with the Association, Mary D. Lindsay has been named as Honorary Director. Later on in the year, we bid farewell to both Linda Franciscovich and Antoine C. Kemper, Jr., who resigned as Directors due to personal commitments. Many thanks to them for their devoted service.

Our February 5 Annual Meeting was followed by a cocktail buffet dinner and featured Dr. Steven Pinker, who is Professor of Psychology, Department of Brain and Cognitive Sciences at the Massachusetts Institute of Technology. Dr. Pinker's fascinating talk related to matters we deal with every day: "Words and Rules: The Ingredients of Language." On March 11, we had a most productive 1-day "define our mission and come up with new ideas" retreat. The next Association event was our annual Jazz at the Lab Concert on April 15, followed by a cocktail buffet dinner. Again, our own Rick Cosnotti was crucial to its great success as were Directors Joe Donohue and Gil Ott. Featured were the Harold Betters Quartet, Kenny Blake, and Eric Johnson.



CSHLA Directors Joseph Donohue and David Deming and CSHLA President James Spingarn at 2000 Annual Meeting.

On May 25, the New York City Spring Event was held at the University Club. A Genetic Engineering Panel discussed "Industrial Strength Food: Commercial Implications of Genetic Engineering," a most pertinent topic today.

June 4 was the date of our annual blockbuster Dorcas Cummings Lecture when renowned and younger scientists from around the globe gather to focus upon a particularly important theme. Our guest speaker was Jan H.J. Hoeijmakers, Ph.D., from Erasmus University of Rotterdam. He eloquently spoke about "Maintaining Nature's Perfection: Cancer and Aging and the Condition of Our Genes." Dinner parties that followed were organized by Trudy Calabrese, Frances Elder, Bob Gay, Eileen Pulling, Ann Seifert, and Cathy Soref. We thank them and the generous hosts of these delightful get togethers:

Ms. Mary Lenore Blair
Mr. and Mrs. Thomas J. Calabrese
Mr. and Mrs. John P. Cleary
Mr. and Mrs. Guy A. Fritts
Mr. and Mrs. John S. Grace
Mr. and Mrs. William F. Gerry
Mr. and Mrs. Richard M. Jackson
Mr. and Mrs. E. Coe Kerr
Mr. and Mrs. Michael L. Kolodner
Mrs. Mary D. Lindsay
Mr. and Mrs. David L. Luke III
Mr. and Mrs. Robert O'Connor
Mr. and Mrs. James J. Pirtle, Jr.
Mr. and Mrs. Henry E. Salzhauer
Mr. and Mrs. William E. Sheeline
Mr. and Mrs. Douglas S. Soref
Mr. and Mrs. James L. Spingarn
Mr. and Mrs. Thomas R. Vandeventer
Mr. and Mrs. Robert P. Walton

Two other past events reappeared this year to their great success and our great delight. They were the CSHLA Partnership Picnic on September 24, which promotes relationships between Laboratory scientists and members of the Association. It was hosted, most beautifully again, by David and Betsy Clark. On October 3, members of the Cosmopolitan Club in New York City came for a tour of the Laboratory and DNA Learning Center. It was a smash hit and was cohosted by Elizabeth Watson and Mary Lindsay.

October 18 saw our Second Annual Biotechnology Luncheon, which was again generously sponsored by The U.S. Trust Company. The main speakers were Thomas O'Karma, M.D., Ph.D., President and Chief Executive Officer of Geron Corporation, a biopharmaceutical company, and Alan Colman, Ph.D., Research Director of PPL Therapeutics, a biotechnology firm. Their topic of discussion, which was introduced by our Director and Chief Executive Officer, Dr. Bruce Stillman, was "Intimations of Immortality." Many thanks to Eileen Pulling and Larry Remmel for all of their hard work in making this event such a success. The most gracious Mrs. John (Robin) Hadley hosted our November 11 Major Donor Cocktail Dinner, at which time we expressed our special appreciation to our exceptionally generous donors. It was elegant, and the food was delicious. Thank you Robin!

We thank each of you for your wonderfully generous interest, time, and support.

January 2001 **James L. Spingarn**

Members of the Cold Spring Harbor Association

ANGELS
Contributions of $10,000 or more

The Ada Howe Kent Foundation
Mr. and Mrs. G. Morgan Browne
Clarkson Family Foundation
Mr. and Mrs. John P. Cleary
The Dorothy Schiff Foundation
Robert B. Gardner, Jr.
Mrs. Oliver R. Grace
Mr. and Mrs. Austen T. Gray, Jr.
J. Aron Charitable Foundation, Inc.
Mrs. Mary D. Lindsay
Mr. and Mrs. David L. Luke III
Mr. and Mrs. William L. Matheson
Dr. and Mrs. Walter C. Meier
Mr. Robert G. Merrill
The Miller Family Foundation
Morris Charity Fund/Mr. and Mrs. Douglas P. Morris
Mr. and Mrs. Denis J. O'Kane
Mr. and Mrs. Edward L. Palmer
Edward M. Scolnick, M.D.
Mr. and Mrs. James L. Spingarn
Marilyn and Jim Simons
Dr. and Mrs. James D. Watson

BENEFACTORS
Contributions of $5,000 or more

The Alex J. Weinstein Foundation, Inc.
Anonymous
Roger and Lori Bahnik
Mr. William Braden
Mrs. Helen M. Brooks
The Craigmyle Foundation
Norris Darrell, Jr.
Mr. and Mrs. David H. Deming
Robin T. Hadley
The Howard Phipps Foundation
Mrs. John H. Livingston
Robert and Joyce Marcus
The Mary G. Turner Charitable Lead Unitrust
Merlin Biomed Investment Advisors
Mr. and Mrs. Charles E. Murcott
Norwood Foundation, Inc./The Bancroft Families
Joyce and John Phelan
Mr. and Mrs. Philip R. Von Stade
The William H. Donner Foundation, Inc.

PATRONS
Contributions of $2,500 or more

Abby & George O'Neill Trust
Mr. and Mrs. W. Dillaway Ayres, Jr.
Mr. and Mrs. Timothy Broadbent
Mr. and Mrs. George W. Cutting, Jr.
Mr. and Mrs. Edward E. Fessenden
Mr. and Mrs. Robert A. Gay
Michael J. Griffin and Joyce A. Bertoldo
Mr. and Mrs. William H. Grover
Mr. and Mrs. Allen D. Jebsen
Mr. Robert M. Jenney
Joyce Green Family Foundation
Mr. and Mrs. Townsend J. Knight
Laurie J. Landeau V.M.D.
Mrs. Henry Lewis III
Mr. Henry Luce III
Mrs. Ellen Hallowell Pratt
Mr. and Mrs. Thomas L. Pulling
Mr. and Mrs. Preston V. Pumphrey
Quality King/The Nussdorf Family
The Richard A. and Amelia Bernstein Foundation, Inc.
John and Hope Reese
The Schiff Foundation
Wellington Foundation, Inc.
The William Ewing Foundation
Mr. and Mrs. Douglas A. Warner III

ASSOCIATES
Contributions of $1,000 or more

Mr. Warren S. Adams II
Robert and Betty Albertson
Anonymous
The Anthony & Claire Pace Foundation, Inc.
Robert H. Arnow
Mr. and Mrs. Joseph Aznara
Mr. and Mrs. Horace C. Bailey
Banbury Fund
David and Pamela Banker
Mr. and Mrs. Nicholas J. Bartilucci
Mr. Edmund Bartlett
Mr. and Mrs. Cornelius N. Bliss III
Mr. and Mrs. Allen L. Boorstein
Dr. David Botstein and Dr. Renee Fitts
Mary Griggs Burke
Mr. Leonard Butterman
Mr. and Mrs. Thomas J. Calabrese, Jr.
Ceptyr, Inc.
Mr. and Mrs. Thomas H. Choate
Mr. and Mrs. David C. Clark
Dr. and Mrs. Bayard Delafield Clarkson
Mrs. Robert L. Clarkson, Jr.
Mr. James W. Cleary
Mr. and Mrs. John H. Coleman
Mr. and Mrs. Peter O. Crisp
Mr. and Mrs. Arthur M. Crocker
Mr. and Mrs. Curtis Cushman
The Davidson Family Foundation
Mr. and Mrs. Donald L. Deming
William Theodore Denslow Foundation
Jerrold Doros
Nelson Doubleday
Dr. Lester Dubnick
Mr. and Mrs. William T. Dunn
Duplex Electrical Supply Corporation
Jan and Jim Eisenman
Mr. and Mrs. Johnston L. Evans
Mr. and Mrs. William Everdell
Dr. Robert and Marjorie Feeney
Jeffrey Keller and Dolores Fredrich
E.M. Geddes, Jr.
The General William Mayer Foundation
Mr. and Mrs. E.T. Gerry, Jr.
Helen and Monte Getler
Mr. and Mrs. Edward M. Giles
Mr. and Mrs. Oliver R. Grace, Jr.
Mr. and Mrs. Edward Greenberg
Mr. and Mrs. Gordon S. Hargraves
Henry U. Harris, Jr.
Mr. Lindsay C. Herkness III
Mr. and Mrs. Mark G. Hollo
Mr. and Mrs. Waldo Hutchins III
Mr. Frederic B. Ingraham
Walter B. James Fund #2
Mr. and Mrs. Barclay Jones
Mr. and Mrs. E. Coe Kerr
Julia B. Key
Andrea B. and Peter D. Klein
Mr. and Mrs. Michael L. Kolodner
Drs. Egon and Marcia Kramer-Mayer
Mr. and Mrs. Gordon E. Lamb
Mr. Edward M. Lamont
Mrs. Lois A. Lenahan
Leo Model Foundation, Inc.
Robert V. Lewis, M.D.
Mr. and Mrs. Mario Lipari
Mrs. Vladimir S. Littauer
Dr. David M. Livingston
Mr. and Mrs. Luke D. Lynch, Jr.
Thomas and Ellen McPartland
Mrs. Cynthia R. Mead
Clarence F. Michalis
Mr. and Mrs. Keith M. Moffat
Francis W. Murray III
Mr. and Mrs. William J. Nammack
Mr. and Mrs. Michael S. O'Brien
Thomas W. and MaryEllen Ostrander
Mr. and Mrs. Philip F. Palmedo
Mr. and Mrs. Nicholas B. Paumgarten
Mr. and Mrs. Howard Phipps, Jr.
Mr. and Mrs. Whitney Pidot
Mr. and Mrs. Robert A. Pilkington
Mr. and Mrs. Joseph D. Posillico, Jr.
Mr. and Mrs. Lawrence Remmel
Chuck Riggs and Sheila Mahony
Mr. and Mrs. Luis E. Rinaldini
Mr. John J. Roche
Mr. and Mrs. Arthur M. Rogers, Jr.
Mr. and Mrs. J. Wright Rumbough, Jr.
Mrs. William F. Russell
Salzhauer Family Fund
Mr. and Mrs. Thomas L. Seifert
Mr. and Mrs. Enrique F. Senior
Mr. and Mrs. Owen T. Smith
Mr. and Mrs. Peter Solbert
Mr. and Mrs. Douglas S. Soref
Dr. Axel Stawski
Mr. and Mrs. James F. Stebbins
Grace and Bruce Stillman
Mr. and Ms. Henry Stockman
Mrs. Richard Storrs
Mrs. James Van Alen
Mrs. Martin Victor
Mrs. Taggart Whipple
Mr. Gerald I. White
Mr. and Mrs. Samuel H. Wolcott III
Mr. Mayer Wolf

FRIENDS
Contributions of $300 or more

Mr. and Mrs. William Achenbaum
Albrecht, Viggiano, Zureck & Company, P.C.
Norman E. Alexander
Ms. Jean E. Andersen
Mr. and Mrs. David F. Andersen
Dr. and Mrs. Glenn Anderson
Anonymous
Mr. and Mrs. Edward H. Auchincloss
Mr. and Mrs. Richard Aurelio
Henry and Erica Babcock
Mrs. George de B. Bell
Mr. John Berejka and Dr. Diane Moriarty
Berger, Torquato & Davis, LLP
William L. Bernhard
Pam and Gene Bernstein
Bancker Construction Corporation
Mr. John C. Bierwirth
Bladykas Engineering, P.C.
Ms. Rosemary Bourne
Mrs. Walter E. Brunauer
Eleanor and John Campbell
Eleanor Chernick, Ed.D.
James C. Childress
The Christopher D. Smithers Foundation
Cord Meyer Development Company
Donna and Barry Cosel-Pieper
Mr. and Mrs. Richard T. Cunniff
Claus & Ariane Dannasch
Mr. and Mrs. Daniel C. De Roulet
Mrs. W. T. Dean
James and Sophie Donaldson
Mr. and Mrs. Kostas Douzinas
Mr. and Mrs. Joseph P. Downer
Mayor E. Richard Droesch
Eileen and Charles Dubroff
Mr. and Mrs. Don Durgin
Mrs. Julie C. Eichenberger
Jerry and Gail Elstein
Claire and Joel Fairman
Mr. and Mrs. Harold L. Fates
Anne Marie Fields
Mr. and Mrs. Eric O. Fornell
Dr. and Mrs. Candido E. Fuentes-Felix
Mr. and Mrs. Richard Gachot
Mr. and Mrs. William C. Gay, Jr.
William and Maxine Glenn
Stephen M. and Judith F. Goodman
Rosalyn C. Gordon
Dr. and Mrs. Maurice Green
Mr. and Mrs. Harold W. Hawkey
Melvin Heller
Mrs. Albert L. Hoffman
Sealy and Mark Hopkinson
Mr. and Mrs. Francis C. Huntington
Imowitz Koenig & Co., LLP
Michael and Joanne Ingham
Laura J. Ingraham
Thomas Iovino/Judlau Contracting
Jaspan Schlesinger Hoffman, LLP
Mr. and Mrs. Jeffrey Kelter
Kiwanis Club of Floral Park
Mrs. James Knott
Barbara B. Knowles and Davor Solter
Mort and Deborah Kunstler
Maureen and Tony Leness
Mr. and Mrs. Robert V. Lindsay
George N. Lindsay, Jr. and Nancy Metz
Candido and Irene Llovera
Cynthia J. MacKay, M.D.
William H. Mathers
Mr. and Mrs. William B. Matteson
Linda H. McLaughlin
Kim Anh and Jeffrey H. Miller
Mr. and Mrs. John Robinson Miller IV
Mr. and Mrs. George M. Milne
Elihu and Dorothy Modlin
Mr. and Mrs. James D. Mooney, Jr.
Mr. and Mrs. Charles F. Morgan

Mr. and Mrs. John P. Morgan II
Jo Anne and Howard Olian
Mr. and Mrs. Peter O'Neill
Mrs. Martha M. Osterhus
Gilbert R. Ott, Jr.
Mrs. Anita H. Page
Mrs. John H. Page
Mr. and Mrs. Charles G. Papalcure
Sterling Pile, Jr.
Mr. and Mrs. James Pirtle
Scott and Diane Powers
The Richard Foundation
The Richard M. Rossbach Memorial Fund
Susan L. Rose
Mr. and Mrs. Michael Evan Saffran
Steven M. Schlussel, Esq.
Gloria and Marvin Sharfin
Mr. Edward E. Shorin
Dr. and Mrs. Walter Shropshire, Jr.
Mary and Robert Sibert
Mr. and Mrs. Howard Simpson
Herbert L. Smith III
Georgette A. Spingarn
Mr. and Mrs. Erwin P. Staller
Drs. Joan and Thomas Steitz
Mr. Byam K. Stevens, Jr.
Judith and Irwin Tantleff
Mrs. David S. Taylor
Think Tank Consultants, Inc.
Mr. and Mrs. George H. Tilghman
James and Mary Gay Townsend
Stanley S. Trotman
Carl Vernick, P.E./Soil Mechanics Drilling Corp.
Mrs. Sherwood Waldron
Henry and Rosalind Walter
Walter K. Pulver Landscape Architect
Charles W.B. Wardell, Jr.
Winifred I. Warner
Mr. and Mrs. George M. Wheatley III
Mr. and Mrs. Douglas Williams
Don M. Wilson III
Frederick C. Witsell, Jr.
Dr. Charles Yanofsky
YEC, Inc.

BASIC MEMBERSHIP
Contributions of $75 or more

Mr. and Mrs. Thomas H. Ackerman
Mr. and Mrs. Charles O. Ames
Carl and Mary Anderson
Mr. and Mrs. J. Bryan Anthony
Mrs. S. Reed Anthony
Marion and Jens Bakke
Daniel P. Barbiero
Dr. Henry H. Bard, M.D.
Mr. and Mrs. David Barrett
Mr. Robert S. Barron and Ms. Susan Holgate
Phyllis Bartlett
Dr. and Mrs. Neil Barton
Mr. Richard Battenfeld
Mr. and Mrs. Frederick Baum
Philip C. Beers, Ph.D.
Mr. and Mrs. Robert E. Benson
Peter J. Bergen
Mr. and Mrs. Loren C. Berry
Marjorie M. Bhavnani
Mrs. Georgina M. Bissell
Mr. and Mrs. Peter H. Blohm
Lilias K. Bostwick
Dr. and Mrs. Gerald Brogan
Shelley and John Broven
Mr. and Mrs. Willard and Jeanette Bunn, III
Charles Buntschuh and Joan Caly
Mrs. Jean Cattier
Mr. and Mrs. Robert Citrino
Dr. and Mrs. Howard and Ruth Cohen
Drs. John E. Coraor and Hanna Nekvasil
Dr. Gerald J. Cordani
Cosmopolitan Club
Mr. and Mrs. Richard L. Cosnotti
Mr. and Mrs. P. Coster
Mr. and Mrs. Miner D. Crary, Jr.
Drs. Michael and Carol Dannenberg
Dr. E. William Davis
Mr. and Mrs. Daniel P. Davison
George Davison and Judith Rivkin
Edward F. DelFoe III
Mr. and Mrs. John Deming
John R. Drexel IV
Dr. and Mrs. Gerard L. Eastman
Mr. and Mrs. John E. Eckelberry
Mr. and Mrs. Duncan Elder
Mr. Stephen Ely
Kristina J. Eng
Mrs. James M. Estabrook
Mr. and Mrs. William M. Evarts, Jr.
Bob and Diane Fagiola
Ms. Emily F. Fairchild
Dr. Francine L. Falco
Mr. and Mrs. John Falk
Mrs. John Fennebresque
Rosemary Ann Foehl
Fort Hill Animal Hospital
Alan Foxman and Linda Swanson
Phyllis R. Fritts
Mrs. David Fuchs
Clarence E. Galston
Sam Gandy
Annette Gangitano
Mrs. Robert C. Gardner
Howard J. Gary
Martin Gellert
Mr. and Mrs. Roger M. Gerber
Hugh Jay Gershon
Dr. and Mrs. Stephen A. Gettinger
Mr. and Mrs. Stephen E. Gilhuley
Dr. Morton D. Glantz
Dr. and Mrs. Lewis A. Goldfinger
Mr. and Mrs. James M. Goldrick
George Gorelick
Mr. and Mrs. John S. Grace
Mrs. Austen T. Gray
Dr. and Mrs. Raymond B. Griffiths
Mr. and Mrs. Frank Gundersen
Susan Gunduz, M.D., F.A.A.P.
Mrs. Hamilton Hadden
Louisa Thomas Hargrave
Bunny Hart
Dr. and Mrs. Chester Hartenstein
Oliver C. Hazard
Huyler C. Held
Mrs. W. Peter Henderson
Anthony S. Hendrick
Arno and Elsie Herzog
Charles A. Hickmann
Mr. and Mrs. Lawrence P. Hilbert
Mr. and Mrs. Charles R. Holcomb
Mary Ann Holthausen/The Pall Family Fund
Irving and Edna Holtzman
Rodney and Deborah Hornbake
George H. Howard
Charles and Elizabeth Hubbard
Hubbinette-Cowell Associates, Inc.
Philip and Gretchen Hull
Rudolf C. Hunermund
I. Janvey & Sons, Inc.
Jitka and Pavel Illner
Beatrice W. Ingham
Mr. and Mrs. Herbert S. Ingham
Mr. and Mrs. Richard M. Jackson
Mr. and Mrs. Howard S. Jacobs
Mrs. Robert D. Jay
Ms. Charlotte Escande Julhiard
Howard and Sandra Kane
Ms. Bonnie J. Kansler
Mr. and Mrs. Thomas J. Kelly
Mr. and Mrs. A. Claude Kemper
Mr. Antoine C. Kemper, Jr.
Arnold E. Keppler
Doris Ketcham
Brian and Kristi King
Dr. Stanley Kitzis
Mr. and Mrs. Jerome Klein
Angela Koenig
Ms. Phyllis L. Kossoff
Mr. and Mrs. John W. Lapsley
James L. Larocca
Mrs. Thomas LeBoutillier
Harvey Levine
Sanford H. Levy
Dr. and Mrs. Lawrence J. Lippert
Mr. and Mrs. Alan J. Long
Mr. and Mrs. John B. LoRusso
Ms. Mary Anne Lubanko
Mr. Wilfred G. Mackey
Mrs. Henry R. Macy
Mr. Ralph Martins
Prof. Ernst Mayr
Malcolm McClintock
McCormack Smith Engineers, PLLC
Alfred McDerrow, M.D.
Angus and Barbara McIntyre
Dr. Katherine McIntyre
Mr. and Mrs. Randall P. McIntyre
Mr. Robert M. McLane
Elizabeth Meyer
Mr. and Mrs. George S. Meyer
Richard W. Meyer
Mr. and Mrs. Garfield L. Miller III
William H. Miller
Jonathan Mogen
George G. Montgomery, Jr.
Jonathan and David Mosenson
Dr. Gisela Mosig
Mr. and Mrs. Robert Mountain
Mr. and Mrs. Brent M. Mowery, Jr.
Tess Mullarkey
Elizabeth B. Munier
Rem and Marion Myers
Bob and Anne Petrocelli
Andrea and Barry Newburger
Benny K.C. Ng, M.D.
Junichi Nikawa
North Country Garden Club
North Fork Bank
Mr. and Mrs. David C. Noyes
Mr. and Mrs. Hugh O'Kane, Jr.
Innis O' Rourke, Jr.
Kathleen Pagliughi
Michael and Nancy Paloian
Mr. and Mrs. Peter J. Pell
Mr. and Mrs. Sterling Pile III
Graham L. Platt
Byrd S. and Thomas C. Platt
Mr. and Mrs. Edward Everett Post
Demetri T Poulis, M.D., F.A.C.S.
Michael Powers
Dr. and Mrs. Richard M. Powers
Mr. and Mrs. Daren A. Rathkopf
Mrs. Ewing W. Reilley
Mr. and Mrs. Charles J. Reina
Mrs. Anne Remsen
Bernard J. Reverdin
Mr. and Mrs. Bernard Richards
Mr. and Mrs. Joseph Rizza, Jr.
Francis and Patricia Roberts
Douglas and Kathleen Robinson
Linda L. Roll
Elizabeth E. Roosevelt
Mrs. Julian Roosevelt
Mr. and Mrs. Stanley W. Roth
Emanuel J. Rubin, M.D.
Charlotte and Bob Rubin
Richard and Pamela Rubinstein
Mr. and Mrs. Leon A. Rushmore, Jr.
Ms. Charlene A. Russert
Mr. Irving Sager
Dr. Marilyn Moffat Salant
Mrs. Harold P. Salembier
Patricia P. Sands
Phyllis Satz
Mr. Allan Schmidlapp
Harvey A. Schneier, M.D.
William J. Scholan
Seymour H. Schpoont, Ph.D.
Jeffrey Schreiber
Mr. and Mrs. John E. Schwartzberg
Mr. and Mrs. Frederick E. Seymour
Dr. and Mrs. Edward M. Shepard
Col. and Mrs. Robert E. Sheridan
Mr. John D. Simpson
Mrs. William C. Simpson
Maxine F. Singer
Richard Slade
Marjory G. Smith
Smith & Jung Land Surveyors
Paula Sutker
Mr. and Mrs. William S. Smoot
Mr. and Mrs. James Sommermeyer
Mr. and Mrs. Charles E. Spicer
Dr. and Mrs. David Stacy
Mr. and Mrs. Thomas Staudt
John C. Stevenson
Mr. and Mrs. James M. Stewart
Mr. William M. Stiger
Dr. and Mrs. Joel Strom
Mr. and Mrs. Brian E. Swiggett
Mr. and Mrs. John W. Taylor
Mr. and Mrs. Daniel G. Tenney, Jr.
Mrs. Anne D.R. Thomas
George M. Thomas
Mr. and Mrs. Edward M. Thomas
Dr. and Mrs. Robert W. Tilney
Stanford W. Ting
Alexander C. Tomlinson
Mr. John J. Toscano, P.L.S./Carman-Dunne, P.C.
Mr. and Mrs. Bronson Trevor
Tri-State Consumer, Inc.
Mr. and Mrs. Stephen Van R. Ulman
Mr. and Mrs. Cornelius M. Ulman
Mr. and Mrs. Lawrence B. Van Ingen
Dr. and Mrs. Robert N. Van Son
Mrs. Beverly S. VanderPoel
Dr. and Mrs. Thornton A. Vandersall
Mr. and Mrs. Niklaus Vetsch
Arthur and Hattie Vogel

Dr. and Mrs. Gary I. Wadler, M.D.
Mr. and Mrs. Eugene D. Wadsworth
Mr. and Mrs. Colton P. Wagner
Dr. and Mrs. Peter Walker
Terry and Bob Walton
Dr. Heidi Wang
Florence and Eugene Warren
Mrs. Bradford G. Weekes
Richard and Janina Weghorn
Mr. and Mrs. L.B. Wehle, Jr.
Clifford M. Weingarten, M.D.
Mr. Jerry Weiss
Edward C.R. Whitcraft
Mary Louise Whitmarsh
Mrs. John C. Wilmerding
Catherine Bostwick Wilson
Mrs. T. Scudder Winslow
Margaret W. Wright
Marty Yurate

CSHLA CORPORATE AND FOUNDATION MATCHING CONTRIBUTIONS

Bristol-Myers Squibb Foundation, Inc.
Chase Manhattan Bank
Coca Cola Bottling Co.
Dow Jones & Company, Inc.
Florence Corporation
GreenPoint Financial
Guild Agency, Inc.
GreenPoint Financial
The Henry Luce Foundation, Inc
iStar Financial, Inc.
J.P. Morgan & Co. Inc.
The Lubrizol Foundation
Marsh & McLennan
Mellon Financial Corporation Foundation
Morgan Guaranty Trust Company
Morgan Stanley Dean Witter and Company
Newsweek
Pfizer Inc.
Phelps Dodge Foundation
The Prudential Foundation
Union Pacific Corporation

CSHLA Annual Fund

CSHLA Membership and Other Support

Angels, Benefactors, Patrons, Associates	$597,855.09
Friends, Basic	$71,093.86
Other*	$94,773.00
Total	**$763,721.95**

MEMORIAL AND GIFT IN HONOR CONTRIBUTORS

Mrs. Silas Reed Anthony
ASPEC Engineering
Chaplin Bradford Barnes
Dr. and Mrs. Dino Belletti
Mr. and Mrs. Nicholas Biddle, Jr.**
Burt Building Materials Corp.
Mrs. Linda H. Calabrese
Rose D. Callahan
Mrs. Madeline S. Carver
Dr. and Mrs. Bayard Delafield Clarkson
Mr. Charles De Bare
DeSilva & Phillips, Inc.
Ms. Sybil K. Dukehart
Jean Eakins
Dr. and Mrs. N. Dennis Eryou
Mr. and Mrs. William Everdell
Mr. Bruce L. Fahlbusch
Mrs. Ruth S. Fahlbusch
Ferran Development Corporation
Mr. and Mrs. Allen A. Goldring
Susan and Andrew Goldstein
Ms. Kim Gronachan
Mrs. Hamilton Hadden
The Hershowitz Family
Mr. and Mrs. Philip R. Herzig
Meg and Bill Jacobs
Mary and Allan Jay
Mr. and Mrs. Holden Johnson, Jr.
Sarah F. Johnson-Houlihan
Dr. and Mrs. Alan M. Kisner
Mr. Ralph A. Lanza
Mr. and Mrs. John H. Leib
Mr. David L. Luke III
Mr. and Mrs. Kenneth MacDonald
Ethel and Bill Mahoney
Mansions & Millionaires, Inc.
Robert and Joyce Marcus
Mr. Robert M. McLane
Joan Medvecky and Family
Don and Liz Meyer
Mr. William H. Miller
Mr. Steven S. Miller
Mrs. Grinnell Morris
Brent and Monica Mowery
Ms. Johanna Northam
Mrs. Charles P. Noyes
Mrs. John H. Page
Mr. and Mrs. Arnold J. Palmer
Mr. and Mrs. Joseph Rizza, Jr.
Mr. and Mrs. William S. Robertson
Alison, James, Cecily and Jonathan Rose
Mrs. William F. Russell
Salzhauer Family Fund of the Jewish Communal Fund
Regina and Martin Salzman
Barbara Santos
Mr. and Mrs. Daniel S. Shapiro
Stephen A. Smiles, M.D.
Mrs. Cathy Cyphers Soref
Ruth Stanton/The Ruth and Oliver Stanton Foundation
Mrs. Abbey K. Starr
Mr. and Mrs. Larry Steiner
Marty and Carol Sternberg
Mr. Gordon D. Stott
Mr. Robert C. Vincent, Jr.
Mr. Charles W.B. Wardell, Jr.
Mr. and Mrs. Herbert G. Wellington, Jr.
Whaler's Cove Yacht Club, Inc.
Mrs. Ichabod T. Williams
Mr. and Mrs. Samuel D. Williams

IN MEMORY OF

Mr. Dennis Begley
Ms. Katharine Montgomery Biddle
Mrs. Toby Brooks
Dr. Peter Casadei
Mr. Charles Cassens
Mr. Robert L. Cummings
Mr. Alex Gordon
H.A. Julich
Mr. Robert Laffan
Mr. William Willis Reese II
Mr. Jack Richards
Dr. Jean Schmidlapp
Ms. Bonnie Schwartz
Ms. Marilyn Sheerin
Mr. Howard Spingarn
Mr. James Stein
Fred and Lotte Stern
Mr. Thomas E. Sullivan
David Thompson

IN HONOR OF

Andrea Blozen and Charles Hendrickson
Mr. Allen A. Goldring
Mr. John S.Grace
Mr. Ralph Guild
Mr. and Mrs. Oskar Lewnowski
Mrs. Terry O'Driscoll
Mr. Alan Pekchi & Edward Gauss
Mr. and Mrs. Steven and Eileen Schlussel
Mrs. Georgette Spingarn
Mr. James L. Spingarn

*Includes "Memorial" and "In Honor of Gifts"; Proceeds from Special Events; Annual Fund Endowment Income; and Nonmember Gifts.
**To Establish The Katharine Montgomery Biddle Fund For Research Into Mental Illness.

President's Council

The President's Council was formed seven years ago as a means of bringing together leaders from business, research, and biotechnology who are interested in science and the work at Cold Spring Harbor Laboratory. President's Council members contribute a minimum of $25,000 in support of the Laboratory's research fellowships, which allow promising young researchers to pursue their own high-level, independent research, rather than assisting in the laboratory of an established scientist. The following are members of the 2000 President's Council:

Mr. Abraham Appel
Mr. Roderick H. Cushman
Mr. Michel David-Weill
Mr. Stefan Englehorn
Mr. Jacob Goldfield
Dr. Leo A. Guthart
Mr. Charles E. Harris
Mr. Walter B. Kissinger
Mr. Thomas J. McGrath
Mr. Robert B. Menschel
Mr. Donald A. Pels
Mr. James H. Simons
Dr. Charles L. Stone, Jr.

Total	**$285,881**

The Harbor Society

One of the most personal and meaningful ways of saying, "I believe in Cold Spring Harbor Laboratory's mission," is to make a planned gift. A planned gift takes many forms such as a bequest in your will, a charitable remainder trust, or a gift of real estate, securities, life insurance, retirement plan or other asset.

The Harbor Society is a distinguished group of individuals, currently numbering 56, who have made planned gifts to the Laboratory and have given us permission to list their names. In 2000, the following individuals became members of the Harbor Society.

CONTRIBUTORS

Julia Antal
Mr. and Mrs. Edward A. Chernoff
Donald L. and Meleanor L. Deming
Dr. Jeffrey B. Shellan
Mrs. Georgette Spingarn
Mr. and Mrs. James L. Spingarn
Mr. and Mrs. Oliver Stanton

Undergraduate Research Program

The Undergraduate Research Program introduces college students to the skills they need to become research scientists. Approximately 25 participants work under the guidance of staff scientists for 10 weeks each summer on projects of mutual interest.

CONTRIBUTORS

Mrs. and Mrs. Cornelius N. Bliss III
Dorcas Cummings
Reverend Linda P. Hancock
Jephson Educational Trust
National Science Foundation
Mrs. Pamela P. Post
Mrs. Joan P. Tilney

ENDOWMENTS

Emanuel Ax Fund
Burroughs Wellcome Fund
Garfield Fellowship
H. Bentley Glass Fellowship
Libby Fellowship
Robert H.P. Olney Memorial Fund
Joan Redmond Read Fund
William Shakespeare Fellowship
Frederica von Stade Fund

Total	**$207,568**

Total All Annual Funds **$27,574,046**

LABORATORY STAFF

Bruce W. Stillman, Director and Chief Executive Officer
James D. Watson, President
Winship Herr, Assistant Director/Dean, Watson School of Biological Sciences
W. Dillaway Ayres, Jr., Chief Operating Officer*
G. Morgan Browne, Chief Financial Officer*
Lari C. Russo, Comptroller*

John P. Maroney, Director, Office of Technology Transfer/In-House Counsel
Terri I. Grodzicker, Assistant Director for Academic Affairs
Jan A. Witkowski, Director of Banbury Center
David A. Micklos, Director of DNA Learning Center
John R. Inglis, Executive Director of CSHL Press
David J. Stewart, Director of Meetings and Courses

FACULTY

Professors

Hollis Cline
David Helfman
Nouria Hernandez*
Adrian Krainer
Scott Lowe
Roberto Malinow
Robert Martienssen
David Spector
Nicholas Tonks
Timothy Tully
Michael Wigler

Associate Professors

Grigori Enikolopov
Gregory Hannon
Michael Hengartner
Tatsuya Hirano
Leemor Joshua-Tor
Ryuji Kobayashi
Yuri Lazebnik
W. Richard McCombie
Jacek Skowronski
Lincoln Stein
Arne Stenlund
Karel Svoboda*
Linda Van Aelst
Rui-Ming Xu
Jerry Yin
Michael Zhang
Yi Zhong

Assistant Professors

Dmitri (Mitya) Chklovskii
Shivinder Grewal
Masaaki Hamaguchi
Z. Josh Huang
David Jackson
Robert Lucito
Zachary Mainen
Vivek Mittal
Andrew Neuwald
William Tansey
Anthony Zador

Cold Spring Harbor Laboratory Fellows

Gilbert (Lee) Henry
Terence Strick
Marja Timmermans

Research Investigators

Neilay Dedhia
Natalia Peunova
Andrew Reiner
Mona Spector
Clifford Yen

Adjunct Professor

Bhubaneswar Mishra

Visiting Professor

Eli Hatchwell

Adjunct Associate Professors

Scott Powers
Craig Ogata*

Visiting Scientists

Vincent Colot
Anna Giulini
Edith Heard
Boris Kuzin
Tatyana Michurina
Vladimir Scheinker
Jacqueline Vitali
Ying-hua Zhu

Senior Fellows

Michael Myers
Edward Stern

Postdoctoral Fellows

Carlos Aizenman
Harsh Bal
Vivekanand (Vivek) Balija
Andres Barria-Roman
Benjamin Boettner
Katherine Braun
Paula Bubulya
Athanasios (Tom) Bubulya
Ingrid Bureau
Mary Byrne
Isabel Cantallops
Luca Cartegni
Douglas Conklin
Olivier Cuvier
Marc Davenne
Ramana Davuluri
Elisa De Stanchina
Robert Del Vecchio
Michael DeWeese
Joseph Donovan
Eric Drier
Jian Du
Joshua Dubnau
Dominik Duelli
Veronica Egger
Ingrid Ehrlich
Viola Ellison
Eric Enemark
Jose Esteban
Gerardo Ferbeyre
Robert Filipkowski
Lisa Foa
Jordan Fridman
Toshiyuki Fukada
Michael Greenberg
Hui-Fu Guo
Kurt Haas
Frances Hannan
Michelle Hastings
Andreas Herbst
Hiroyuki Higashiyama
Edward Hofmann
Sangeet Honey
Junjiro Horiuchi
Susan Janicki
Jianping Jin
Eric Julien
Natarajan Kannan
Alyson Kass-Eisler
Catherine Kidner
Jae-Yean Kim
Peter Krasnov
Prakash (Patrice) Kumar
Patrice Lassus
Wen-Chuan (Athena) Lin
Ana Losada
David MacCallum
Stephen Macknik
Miguel Maravall
Carla Margulies
Bruce May
Shanmugam Mayilvahanan*
Juan Mendez
Tzu-Ching (T.C.) Meng
Olaf Merkel
Jinrong Min
Jun-chi Nakayama
Masashi Narita
Esther Nimchinsky
Kenichi Noma
Takashi Odawara
Thomas Oertner
Pol O'Fearghail
Michael Packer
Helena Palka
Xiaokang Pan
Geraldine Pawlak
Shannon Pendergrast
Antonella Piccini
Emmanuelle Querido
Pablo Rabinowicz
Jyotishankar (Jyoti) Raychaudhuri
Michael Regulski
Francesc (Xavier) Roca
Michael Ronemus
Edward Ruthazer
Bernardo Sabatini
Noriko Saitoh
Cyril Sanders
Clemens Schmitt
Arndt Schmitz
Kenneth Seidenman
Lidia Serina
Yu Shen

*Positions approved November, 2000, and effective January 2001.
†Employee of the Howard Hughes Medical Institute.

Yi-Jun Sheu
Wun Chey Sin
Tatjana Singer
Maria Soengas
Christian Speck
Yuri Stasiv
Armen Stepanyants
Takuya Takahashi
Joshua Trachtenberg
Naoshige Uchida
Anne Vaahtokari
Erik Vollbrecht
Thomas Volpe
Yalin Wang
Guanming Wu
Shouzhen Xia
Zhenyu Xuan
Ryohei Yasuda*
Qing-Shuo Zhang
Zhiguo Zhang
Lifang Zhang
TongTong Zhang
Xinyang Zhao*
Xuemei Zhao
Jun Julius Zhu
Ahmad Zia*
Karen Zito

Graduate Students

Jannik Andersen
Anitra Auster
Nilanjana (Nila) Banerjee
Emily Bernstein
Andrea Calixto
Michelle Carmell
Amy Caudy
Santanu Chakraborty
Kenneth Chang
Bidisha Chattopadhyaya
Hsu Hsin (May) Chen
Brian Chen
Jeffrey Christensen
Michelle Cilia
Laureen Connell
Ahmet Denli
Sujit Dike
Yi-Chieh (Nancy) Du
Elena Ejkova
Farida Emran
Rebecca Ewald
Lavina Faleiro
Eve-Ellen Govek
Ira Hall
Jill Hemish
Brian Hill
Jennia Hizver
Ping Hu
Ajit Janardhan
Ashkan Javaherian
Kendall Jensen
Michelle Juarez
Flavio Kamenetz
Manpreet Katari
So Young Kim
Edward Kim
Jason Kinchen
Ying Ying Koh
Sungwoo Lee
Soyoung Lee
Arlindam Lee
Guillaume Lettre
Zheng Li
Shih-Chieh (Jack) Lin
Zachary Lippman
Marco Mangone
Michele McDonough
Troy Messick
John Mignone
Stuart Milstein
Masafumi Muratani
Zaher Nahle
Patrick Paddison
Thomas Pologruto
Irina Pugach
Patrick Reilly
Emiliano Rial Verde
Jose Rodriguez
Andrew Samuelson
Ashish Saxena
Laura Schramm
Yvette Seger
Stephanie Shaw
Hang Shi
Songhai Shi
Michael Stebbins
Marcela Tello-Ruiz
Elizabeth Thomas
Niraj Tolia
Jiayuan (James) Tong
Thomas Tubon
Kathryn Tworkowski
Joseph West
Joanna Wysocka
Ming Ming Zhao
Jun Zhu

Research Programmers

Gengxin Chen
Naoya Hata
John Healy
Jerzy (George) Kakol
Michael Morgan
Aleksandar Poleksic
Ravi Sachidanandam
Steven Schmidt

Research Associates

Robert Gasperini
Lisa Manche
Linda Rodgers
Patricia Wendel
Cristina Yordan

Lab Facility Managers

Carmelita Bautista
Diane Esposito
Sang Young Kim
Timothy Mulligan
Spencer Teplin
Ming Wang
Regina Whitaker

Laboratory Manager

Martha Daddario

Scientific Administrators

Sadie Arana
Patricia Bird
Carol Haff-Gear
Idee Mallardi
Carol Marcincuk
Anita Matthes
Barbara Matusik
Lorraine McInerny
Jane Reader
Carol Sekunda*
Madeline Wisnewski

Research Assistant

Tomasz (Tomek) Swigut

Laboratory Technicians

Aysha Ahmed
Joan Alexander
Esteban Araya
Juana Arroyo
Deborah Aufiero
Ariana Bahret
Joshua Baker
Jody Barditch
Benjamin Bartelle
Matthew Blennau
Sabrina Boettcher
Kimberly Bronson
Anthony Buglino
Barry Burbach*
Don-Michael Cunnius
Nancy Dawkins-Pisani
Melissa de la Bastide
Margaret Falkowski
Eugenia Friedman
Scott Gossweiler
Lori Grady
Susan Granat
Karen Greenwood
Inessa Hakker
Michiko Hirano
Lenka Hurton
Wei Jiang
Lisa King
Kristin Kirchoff
Kathy Kuit
Kimberly LaVine
Stacy Lopez
Beicong Ma*
Mila McCurrach
Jennifer Meth
Beth Miller
Barbara Mish
Lidia Nascimento
Aigoul Nourjanova
Peter O'Brien
Ximena Opitz-Araya
Andrew O'Shaughnessy
Ronnie Packer
Kimberly Pennino
Raymond Preston
Yi Qin
Michael Riggs
Steven Rodriguez
Dongyon (Donna) Roh
Christine Rosenthal
Seth Rostan
Simone Salghetti
Lee Santos
Ravi Shah
Joseph Simorowski
Patrick Smith
Lori Spiegel
Yuling Sun*
Julie Thomas
Jennifer Troge
Klara Velinzon
Marie Vil
Jennifer Walls
Jeanne Wiggins
Priscilla Wu
Denise Wurtz
Guoping Xiao
Mianjia (Jennifer) Ye
Lewis Yesner
Zhuang Yuan
Xiaoqun (Catherine) Zhang*
Ying Zhang
Hui Zhou
Theresa Zutavern

Media Makers/Laboratory Aides

Maria Belvis
June Blanchford
Patricia Brady
Jack Brodsky
Florinda Chaves
Terrance (Rodney) Chisum
Janice Douglas
Salvador Henriquez
Marcela Lara

*Employee of the Howard Hughes Medical Institute.

Yang (Nancy) Liu
Vincent Meschan
Rulan Shen
Margaret Wallace
Xiu-Xia Zhang

Research Administration

Denise Roberts
Maureen Bell

Watson School of Biological Sciences

Lilian Gann
Janet Duffy

DEPARTMENTS

Comptrollers Office and Accounting

Lari Russo, Comptroller
Sharon Bense
Jennifer Blovsky
Karine Boyce
Guido (Guy) Cozza
Mary Ellen Goldstein
Laura Guterman
Elizabeth Janow
William D. Keen
Patricia Maroney
Alison McDermott
Carlos Mendez
Mary Ann Miceli
Elizabeth (Betsy) Panagot
Kari Pavlovsky
Patricia Urena
Noreen Vance
Barbara Wang

Art

Michael Ockler
James Duffy
Irwin Mendlinger
Philip Renna

Banbury

Jan Witkowski, Director
Katya Davey
Eleanor Sidorenko
Beatrice Toliver

Central Administration

Maureen Berejka
Delia King
Charles Prizzi
Roberta Salant
Judith Smith

CSHL Press

John Inglis, Executive Director

Acquisition

Alexander Gann
Elizabeth Powers

Advertising

Ann Delfox
Marcie Ebenstein

Books: Editorial

Janet Argentine
Mary Cozza
Judith Cuddihy
Maryliz Dickerson
Joan Ebert
Kaaren Janssen
Inez Sialiano

Journals: Editorial

Laurie Goodman
Margaret Calicchia
Dawn Canfora
Heather Cosel-Pieper
Arlen Feldwick-Jones
Emily Pei-hsin Huang
Michele McDonough

Production

Denise Weiss
Patricia Barker
Dorothy Brown
Elaine Cantwell
Daniel deBruin
Stephanie MacCallum
Mala Mazzullo
Nora Ruth
Susan Schaefer
Anne Wenzel
Peri Zeenkov

Fulfillment Distribution

Guy Keyes
Kathleen Cirone
Katya Gurbeloshvili
Geraldine Jaitin
Jacqueline Matura
Joan Pepperman
Mary Sabala
Sharon Story
Joanne Thomas
Christopher Vacca
Kathleen Watras

Finance

Nancy Hodson
William D. Keen

Marketing

Ingrid Benirschke
Kathryn Fitzpatrick

Development

Richard Cosnotti, Chief Development Officer
Jill Barton
Lynn Cannon
Annette Gangitano
Gordon Hargraves
Donna Harrington
Julie Sutherland
Vincent Torti

DNA Learning Center

David Micklos, Director
Joan Alexander
Veronique Bourdeau
Scott Bronson
Elna Carrasco
Shirley Chan
Susan Conova
Maureen Cowan
Judy Cumella-Korabik
Nancy Daidola
Uwe Hilgert
Susan Lauter
Amanda McBrien
Raymond Preston
Danielle Sixsmith
Chun-hua Yang

Facilities

Arthur Brings, Chief Facilities Officer
Christopher Hubert
Peter Stahl
Leslie Allen
Russell Allen
Marlene Rubino
Frank Russo
Barbara Santos
Charles Schneider
Daniella Zullin

Carpentry and Painting

Peter Schwind
Edwin (Scott) Bennett
Michael Demers
Paul Edwards
Romulo Estrella
John Meyer
Joseph Pirnak
Wilson Ramones
John Schindler
Benjamin Veneable III
Harry Wozniak

Custodial/Housekeeping

Lawrence Giordano
Carol Bowden
Dessie Carter
Ming-Kai Chan
Leo Franz
Robert Giarraputo
Steven Goerler
Leo Greene
Joseph Houser
Louis Hunter
Joseph Judge
Christina Lazar
Patricia Le Strange
Sandra Lopez
Susanne MacPherson
Patricia McAdams
Janeth Mejia
Dora Merlino
Maria Mosquera
Mary Muno
Wayne Pav
Ronald Romani
Claudia Schmid
Danuta Slodkowska
Steven Tang
Yew Teo
Curtis Williams

Electrical

James Decker
Louis Jagoda
Jeffrey Goldblum

Environmental Health and Safety

John Pisciotta
Paul Cannava
Randal Jones
Steven Sallese

Equipment Design Fabrication and Repair

Clifford Sutkevich
Robert Eifert
George Newell III
James Robey

Grounds

Daniel Miller
Drew Comer
Alonso Diaz
Paul Draser
Jeffrey DuPree
Joseph Ellis
Edward Haab
Matthew Hoyer
Christopher McEvoy

John Murphy
Hector Roman
Andrew Sauer
Stanley Schwarz
Anthony Terrizzi
Przemyslaw (Przemek) Wysocki
Yuliana Yevoushok

Harris Research Support Facility

Lisa Bianco
Kelly Brings
Amy Burrows
Jodi Coblentz
Charlene De Poto
Lea Mamet
Cindy Schreibman
Cheryl Sunderland
Halina Swidzinski
Ryszard Swidzinski

Mechanical Services

Robert Collins
Gregory Beal
William Bishop
Robert Dickerson
Wesley Dreusike
Jeffrey Klaverweiden
Lane Smith
John Sundman

Security

Robert Gensel
Frank Carberry

Shipping and Receiving

Lawrence Giordano
Daniel Jusino
Kevin Donohue
Renee Le Strange
Joseph Marcello
Christopher Sutkevich

Food Service

James Hope, Director
Laurin Abbatiello
Andres Alarcon
Carlos Alfaro
Jeannette Amato
Jaime Bonilla
Francis Bowdren
Guiditta Carino
William Carmona
Rosa Ciurleo
Joan Doxey
Julie Ehrlich
Barbara Gordon
Jennifer Gordon
Quintin Irizarry
Pierre Jean-Baptiste
Scott La Torre
Oscar Lastra
Jesus Magana
Thomas McIlvaine
Maureen Morrow
Frederik Munter
Ronald Padden
Carlos Perez
Teodosio Perez
Jorge Ramirez
Susana Roman
Kimberly Sneed
Greogory Stanton
Suzanne Tavernese
Cathy van Deurs-Smith
Roger Vereen
Leslie Wenzel

Grants

Susan Schultz, Director
Sandra Altieri
Wendy Crowley
Maureen Davies
Carol DuPree
Patricia Kurfess
William Mahoney
Karen (Dee) McKeon
Kathleen Wall-Rooney

Human Resources

Cheryl Sinclair, Director
Marjorie Berko
Jennifer Donovan
Jan Eisenman
Kim Gronachan
Sandra Neuwald
Barbara Purcell
Merilyn Simkins
Zoe Stylianoudakis
Rita Wienclaw

Information Services

Gerald Latter, Director
James Bense
John Diffley
Robert Dusel
Salvatore Familia
James Gergel
Vsevolod (Simon) Ilyushchenko
Derek Johnson
Mark Kilarjian
Robert Pace
Barbara (Bobbie) Peters
Salvatore Serafino
Russell Strine
David Trimboli
John Uhl
Peter Van Buren
Lee Wilbur
Anthony Zanello
Steven Zielinski

Library

Ludmila Pollock, Director
Paula Absiognio
Clare Bunce
Krista Gruber
Helena (Leigh) Johnson
Danielle Kovacs
Ramah McKay
Claudia Zago

Meetings and Courses

David Stewart, Director
Edward Campodonico
William Dickerson
Donna Dykeman
Michael Glaessgen
Andrew Mendelsohn
Andrea Newell
Jonathan Parsons
James Parsons
Mary Smith
Margaret Stellabotte
Andrea Stephenson
Diane Tighe
Nancy Weeks
Jenna Williams
Barbara Zane

Public Affairs

Jeffrey Picarello, Director
Margot Bennett
Miriam Chua
Lynn Hardin
Laura Hyman
Peter Sherwood
John Verity

Purchasing

Philip Lembo, Director
Margaret (Peggy) Brock
Carol Brower
Susan De Angelo
Karen Fabian
Bruce Fahlbusch
Christopher Oravitz
Susan Rose
Leonard Sposato
Wanda Stolen

Technology Transfer

John Maroney, Director
Barry Braunstein
Carol Dempster

Research Staff Departures during 2000

Adjunct Professor

David Beach

Associate Professor

Bruce Futcher

Visiting Scientists

Christine Berthier
Daniel Bogenhagen
Hilde Grasmo-Wendler
Nathalie Pavy
Fumio Shiobara

Postdoctoral Fellows

Guy Birkenmeier
James Chong
James Dezazzo
Nicholas Edgington
Ludwig Englemeier
Francisco Ferrezuelo
Andrew Groover
Mariko Hayashi
Yasunori Hayashi
Daniel Hoeppner
Jianzhong Jiang
Keiji Kimura
Zhixin Lin
Hong Liu
Tohru Mizushima
Michael Murray
Jairaj Puthenveettil
Keiichi Shibahara
Javor Stolarov
Suzanne Tharin
Venugopal Valmeekam
Daniel Vaughn
Michael Weinreich
David Weissmann
Herman Wijnen
Jianlong (Jim) Zhou
Jian Zhu

Graduate Students

Eric Gillitzer
Tina Gumienny
Susan Harrington
Shirly Pinto
Julia Polyakova
Alex Rai
Bjorn Schumacher
Setareh Sepehri
Beth Trumbull
Xiaohong (Helena) Yang
Lee Zou