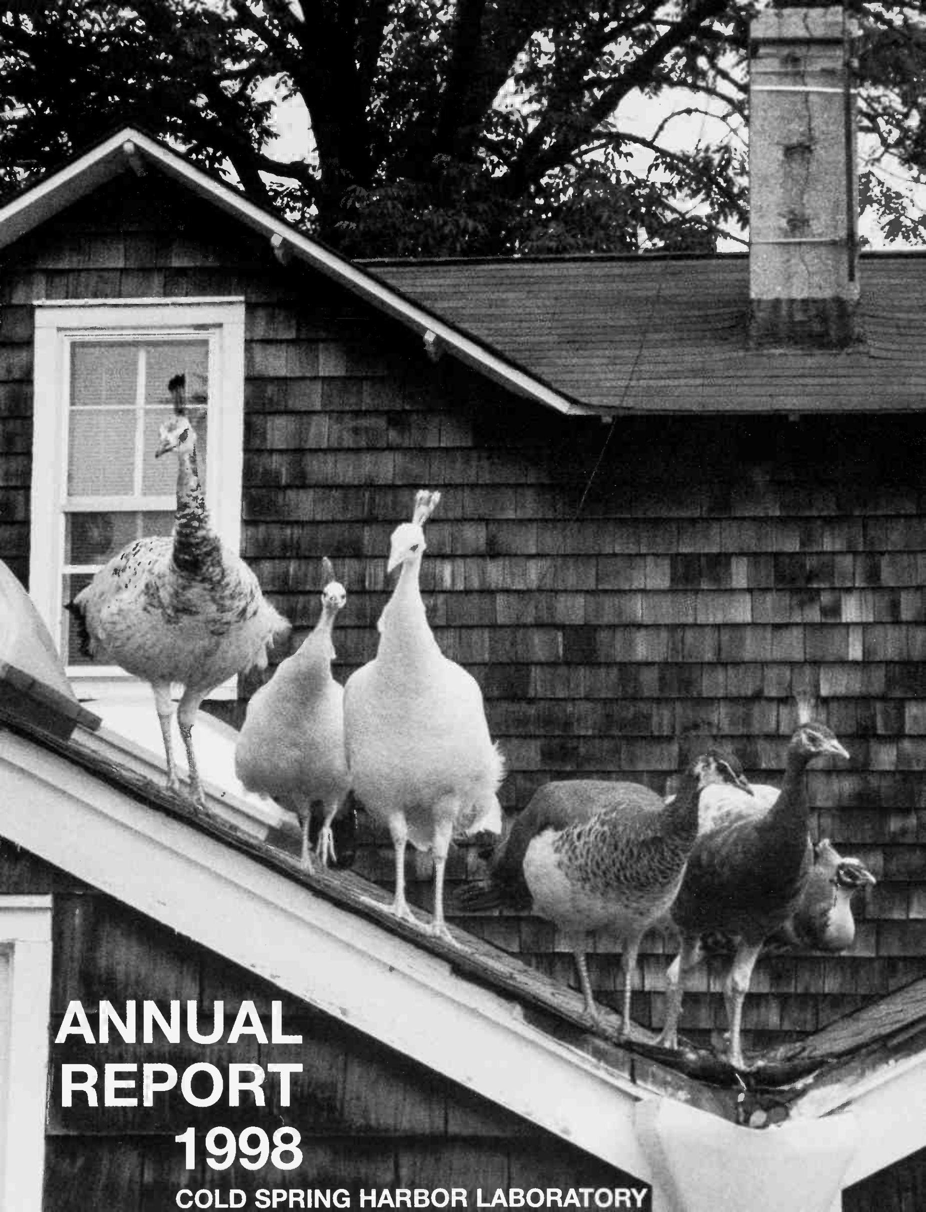
COLD SPRING HARBOR LABORATORY


ANNUAL
REPORT
1998
COLD SPRING HARBOR LABORATORY

ANNUAL REPORT 1998

©1999 by Cold Spring Harbor Laboratory

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

Website: http://www.cshl.org

Managing Editor Wendy Goldstein
Editorial staff Dorothy Brown, Cynthia Allen, Dawn McCoy
Nonscientific Photography Margot Bennett, Ed Campodonico, Bill Dickerson, Marléna Emmons
Desktop Editor Susan Schaefer
Cover designer Tony Urgo
Book designer Emily Harste

Front cover: Female gametophytes in whole-mount ovules of *Arabidopsis thaliana* are visualized by enhancer-trap-driven expression of a reporter gene encoding for the β-glucoronidase (GUS) protein. The enhancer-trap technique was developed at CSHL in 1995 by Rob Martienssen and Venkatesan Sundaresan, utilizing the "jumping genes" discovered by Barbara McClintock. (Image courtesy of Ueli Grossniklaus.)

Back cover: The pond (Marléna Emmons)

Section title pages: Marléna Emmons, Ed Campodonico,
Bill Dickerson, Sue Lauter

Contents

Officers of the Corporation/Board of Trustees v
Governance and Major Affiliations vi
Committees vii
Walter H. Page II (1915–1999) viii
Jane N. Page (1918–1998) xv

PRESIDENT'S ESSAY 1
DIRECTOR'S REPORT 6
Highlights 10
ADMINISTRATION REPORT 41

RESEARCH 45
Tumor Viruses 46
Molecular Genetics 71
Cell Biology 105
Structure and Genomics 127
Neuroscience 151
CSHL Fellows 177
Author Index 181

THE SCHOOL OF BIOLOGICAL SCIENCES 183
Dean's Report 184

COLD SPRING HARBOR MEETINGS AND COURSES 187
Academic Affairs 189
Symposium on Quantitative Biology 191
Meetings 194
Postgraduate Courses 224
Seminars 274
Undergraduate Research 276
Nature Study 278

BANBURY CENTER 279
Director's Report 281
Meetings 287

DNA LEARNING CENTER 319
Director's Report 321
Workshops, Meetings, and Collaborations 333

COLD SPRING HARBOR LABORATORY PRESS 337
Publications 338
Executive Director's Report 339

FINANCE 343
Financial Statements 344
Financial Support 348
Grants 348
Methods of Contributing 357
Capital and Program Contributions 358
Nancy and Edwin Marks Imaging Center Capital Campaign 359
The School of Biological Sciences Capital Campaign 360
Annual Contributions 361

LABORATORY STAFF 374

Top Row: E. Harlow, W.L. Matheson, W.S. Robertson, J.J. Phelan, Jr., W.E. Murray, J.L. Spingarn, J.R. Reese, C.E. Harris, J.A. Steitz

Middle Row: J. Kuriyan, L.N. Grace, W.D. Pidot, M. Ptashne, M.F. Gerry, L.B. Polsky, L.C. Quick, Jr., H.A. Dolan

Bottom Row: B. Stillman, W.R. Miller, D.L. Luke III, E.S. Marks, J.D. Watson

Not shown: C.T. Caskey, S. Hockfield, R. Jaenisch, L.M. Mendell, C.J. Sherr, H. Solomon

Officers of the Corporation

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

James D. Watson, Ph.D., *President*
Bruce Stillman, Ph.D., *Director*
G. Morgan Browne, *Administrative Director*

Board of Trustees

Scientific Trustees

C. Thomas Caskey, M.D., F.A.C.P.
Merck & Co.

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

Susan Hockfield, Ph.D.
Yale University

Rudolf Jaenisch, Ph.D.
Massachusetts Institute of Technology

John Kuriyan, Ph.D.
HHMI, Rockefeller University

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

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

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

Bruce Stillman, Ph.D.
Cold Spring Harbor Laboratory

Individual Trustees

Helen A. Dolan
Oyster Bay, New York

Martha Farish Gerry
Mill Neck, New York

Lola N. Grace
Glen Head, New York

Charles E. Harris
New York, New York

Edwin S. Marks
Kings Point, New York

William L. Matheson
Mill Neck, New York 11765

William R. Miller
New York, New York

William E. Murray
New York, New York

John J. Phelan
Mill Neck, New York

Whitney D. Pidot
Locust Valley, New York

Leon B. Polsky
New York, New York

Leslie C. Quick, Jr.
Palm Beach, Florida

William S. Robertson
Huntington, New York

James L. Spingarn
Great Neck, New York

Howard Solomon
New York, New York

James D. Watson, Ph.D.
Cold Spring Harbor, New York

Honorary Trustees

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

Robert Cummings
Glen Head, 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

Walter H. Page*
Cold Spring Harbor, New York

Wendy V.P. Russell
Oyster Bay, New York

*deceased

Governance and Major Affiliations

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 plus 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 community representatives 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
John J. Phelan

Building

Helen A. Dolan, Chairman
Arthur Brings
John P. Cleary, Esq.
Martha F. Gerry
Mary D. Lindsay
Jack Richards
Wendy V.P. Russell
Elizabeth Watson

Commercial Relations

John P. Phelan, Chairman
G. Morgan Browne
John P. Cleary
John P. Maroney
William R. Miller
Bruce Stillman, Ph.D.

Compensation

William R. Miller, Chairman
Lola N. Grace
Whitney D. Pidot

Development

Leslie C. Quick, Jr. Chairman
W. Dillaway Ayres, Jr.
G. Morgan Browne
Rick Cosnotti
Martha F. Gerry
Laurie J. Landeau, V.M.D.
Mary D. Lindsay
David L. Luke III
Wendy Vander Poel Russell
James L. Spingarn
James D. Watson, Ph.D.

Executive Committee

William R. Miller, Chairman
G. Morgan Browne
Lola N. Grace
Edwin S. Marks
John J. Phelan
Whitney D. Pidot
Leslie C. Quick, Jr.
James L. Spingarn
Bruce Stillman, Ph.D.
James D. Watson, Ph.D.

Finance and Investment

Lola N. Grace, Chairman
G. Morgan Browne
Charles E. Harris
William D. Keen
Edwin S. Marks
William R. Miller
William E. Murray
Leon B. Polsky
William S. Robertson
Howard Solomon

Nominating

Edwin S. Marks, Chairman
William R. Miller
Whitney D. Pidot
Bruce Stillman, Ph.D.

Robertson House

William S. Robertson, Chairman
Art Brings
G. Morgan Browne
Maude Robertson
Jan Witkowski, Ph.D.

Robertson Research Fund

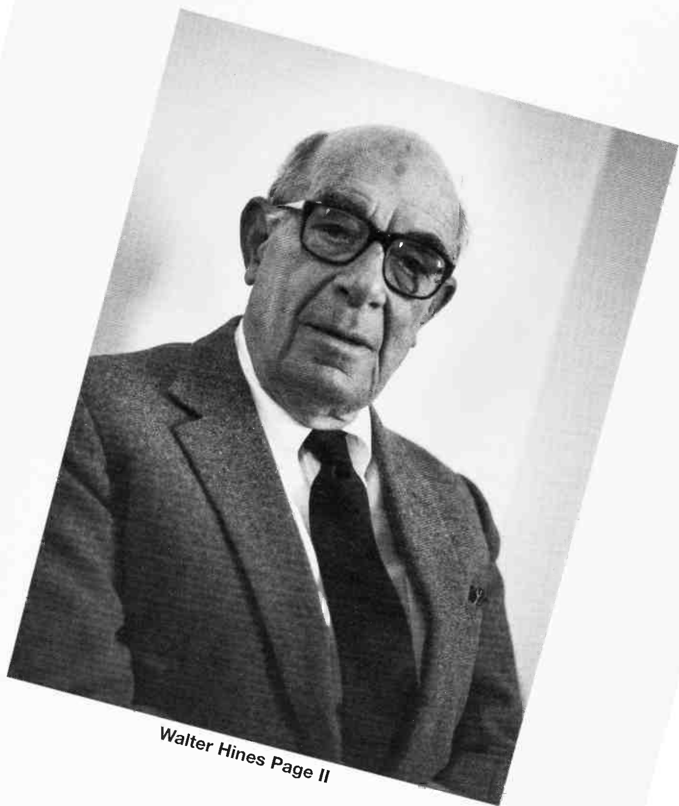
Carl Schafer, Chairman
W. Dillaway Ayres, Jr.
G. Morgan Browne
William R. Miller
Bruce Stillman, Ph.D.
James D. Watson, Ph.D.

Family Representatives

Katherine Ernst
Walter Meier, M.D.
William S. Robertson

Tenure and Appointments

C. Thomas Caskey, M.D.
Edward E. Harlow, Ph.D.
Susan Hockfield, Ph.D.
Rudolf Jaenish, Ph.D.
John Kuriyan, Ph.D.
Lorne M. Mendell, Ph.D.
Mark Ptashne, Ph.D.
Charles Sherr, M.D., Ph.D.
Bruce Stillman, Ph.D.



Walter Hines Page II

Walter Hines Page II (1915–1999)

Walter Hines Page II, whose home looked down on Cold Spring Harbor and out toward Long Island Sound, died on January 8, 1999 at the age of 83. A considerate, wise, and effective leader, he spent his entire working life as a banker. Following education at St. Bernard's, Milton Academy, and Harvard University, he joined J.P. Morgan & Co. After wartime service as a naval officer on a submarine chaser in the Atlantic, he steadily moved up Morgan's ranks, becoming a vice president in 1953 and vice chairman of the board in 1968. The latter role reflected his many major successes during the 1960s as head of the company's rapidly expanding international division. In 1971, Walter was promoted to Morgan's presidency and was chairman of its board when he retired at the end of 1979.

Walter's life was also closely associated with the Cold Spring Harbor Laboratory. Beginning in 1957, he was president of its parent body, the Long Island Biological Association, and starting in 1963, he served several long intervals on its Board of Trustees. Following his retirement from J.P. Morgan, he was for six years (1980–1986) our very able chairman.

Walter's roots on Long Island date to his distinguished North Carolina-born grandfather and namesake, Walter Hines Page (1855–1918). A journalist and editor, he founded with Frank Doubleday the publishing house Doubleday-Page, which in 1910 they located in Garden City, New York. Earlier in Boston, the first Walter Hines Page rose to become editor of *The Atlantic Monthly* and an adviser to the publishers Houghton Mifflin. Doubleday-Page achieved renown when it published Theodore Dreiser's novel *Sister Carrie*. The decision to publish this novel was made by Walter's grandfather, with Frank Doubleday later wanting to revoke the agreement. But its publication went ahead, albeit in a less sexually explicit form orchestrated by Dreiser's wife at the time, Sara. Soon after, Walter Hines Page founded *The World's Work*, a monthly magazine that placed great emphasis on the public responsibility of big business. He and Frank Doubleday also started *Country Life in America*, more immediately a financial success.

Walter's father, Arthur Wilson Page, joined the family firm in 1905 following his graduation from Harvard. He became its vice president in 1913, when his father was appointed American Ambassador to the Court of St. James by President Wilson. An extraordinarily well-liked ambassador, Walter Hines Page was to remain in London for the duration of World War I, dying just at its conclusion.

While with Doubleday-Page, Arthur Wilson Page bought an old homestead called County Line Farm in West Hills, several miles south of Cold Spring Harbor. The farm was adjacent to the estate of the distinguished New York lawyer Henry L. Stimson, who served as Secretary of State during both the first and second World Wars and as secretary of state during Herbert Hoover's administration. Walter's attachment to the Cold Spring Harbor region thus began in his boyhood years. Then he acquired his love of sailing boats, and it was from the Cold Spring Harbor Beach Club, which his father helped found, that he raced with children from the families whose estates dominated the lands around Cold Spring Harbor until the end of World War II. Among them was Jane Nichols, three years

Walter's junior, whose family's land looked down onto the lower mill pond and St. John's Church.

In January 1942, just after the United States entered World War II, Walter and Jane married. During the next six years, their three children were born—Jane Norton in 1943, Walter Hines, Jr. in 1945, and Mark Nichols in 1948.

As a youth, Walter learned from his father about the Biological Laboratory, which had been established in 1890 through gifts from Long Island's then preeminent family, the Joneses. Sited next to the Laboratory, along Cold Spring Harbor's inner harbor, were the turn of the century buildings of the Department of Genetics of the Carnegie Institute of Washington. The Department's mission was to broadly extend the applicability of the then newly rediscovered Mendelian laws of heredity. While the Department of Genetics labs had stable financial backing from the Institute, the Biological Laboratory was habitually underfunded and periodically faced extinction. Enlightened philanthropy from the owners of nearby Gold Coast estates came to the rescue in 1924 with the formation of the Long Island Biological Association (LIBA). The association's purpose was not only to keep the Biological Laboratory alive, but to expand its activities to include research as well as teaching. Arthur Page was LIBA's first treasurer, and in 1928, he became its president. By that time, his association with the Doubledays had ceased, and he was using his formidable personal qualities and great wisdom in behalf of AT&T, where he was vice president of public relations. He soon became a member of AT&T's board and remained in that position until just before his death at age 74.

In his role as LIBA's president, Arthur Page guided the Biological Laboratory through the difficult years of the Depression (at its depth, he secured a $1 donation from a then assistant secretary of the U.S. Treasury!). His most important contribution was in 1940, when the flames of World War II were soon to engulf the United States. Entirely on his own, he secured the agreement of Carnegie Institute officials to coordinate the efforts of the Biological Laboratory with those of the Department of Genetics. A Carnegie geneticist, Milislav Demerec, was appointed director of both institutions, leading to a period of 20 highly fruitful years. During that time, Cold Spring Harbor acquired worldwide renown through the Nobel Prize-winning research on maize by Barbara McClintock and on bacterial viruses by Alfred Hershey, Salvador Luria, and Max Delbrück.

A new period of great institutional instability arose at Milislav Demerec's retirement in 1960. Unable to find a suitable successor, the Carnegie Institute made the decision to gradually withdraw its support of science at Cold Spring Harbor. Fortunately, by this time, Walter Page had assumed the LIBA presidential mantle long held by his father. In this capacity, he helped bring into existence the Cold Spring Harbor Laboratory (CSHL), under whose organizational framework all science activities at Cold Spring Harbor would be managed. To help fill the financial gap created by the Carnegie Institute's withdrawal, Walter secured $25,000 pledges from several major universities and medical research institutions. In turn, representatives of these groups were appointed to a newly formed Board of Trustees. Walter Page, as LIBA's president, and several other prominent community members were also members of the Board.

During the next several years, Walter's responsibilities at J.P. Morgan increasingly limited the time he could devote either to CSHL or to LIBA in its new, nonoperational role as a mobilizer of community support. Seeing the need for a talented person to help him, Walter wisely chose Edward Pulling to take over as LIBA's Board chairman. Pulling would replace Nevil Ford, a banker who had held the position for many years. Having just retired

Walter H. Page, upon retiring as chairman of the CSHL Board of Trustees, 1986

Walter and Jane, 1970

Walter, Claire Landau, 1986

Ed Pulling, Jane and Walter Page, Joe Sambrook, David Livingston at the Sambrook Laboratory dedication, 1985

as headmaster of the Millbrook School, Ed was living on nearby land inherited from his wife's father, Russell Leffingwell, a banker who was Morgan's chairman between 1948 and 1950. Under Ed, LIBA became effectively led by its chairman, not its president. In the capacity of chairman, Ed assumed the place on our Board held for the previous five years by Walter Page.

Walter returned to the Board in 1972, when Charles S. Robertson, owner of a large Lloyd Harbor estate fronting on Cold Spring Harbor, was searching for an institute to which he could gift his estate. In contemplating an even larger second gift that would be designated for research support, Robertson wanted the assurance that Walter Page's financial acumen would be at the Lab's side if the proposed Robertson gifts became finalized. Happily, Walter agreed to come back onto our Board, and Charles Robertson, now at ease, provided $8 million for the creation of the Robertson Research Fund. Today, thanks in part to wise spending guidelines set in 1973 by Charles Robertson and Walter Page, the Robertson Research Fund totals some $80 million.

Several years later, Walter persuaded the Carnegie Institute to sell to CSHL at a very reasonable price 20 acres of land it had long ago purchased from Jones family members. This was an asset that Carnegie had initially held back from the newly formed Cold Spring Harbor Laboratory, believing that it might soon bite the dust. Now that our long-term survival was assured by Charles Robertson's great generosity, Walter informed the Carnegie management that we wanted to resolve the land question because we would soon need a portion of it for construction of a new building for our cancer research. Walter, who had once been on the Carnegie Board, went to Washington to see embryologist Jim Ebert, Carnegie's newly installed president. To our great relief, the visit was a complete success. Carnegie's asking price, which Walter immediately accepted, was $200,000, payable over five years with no interest. Acquiring the needed Carnegie land would in no way decrease the forward momentum of our cancer research.

Walter's negotiating skills again proved crucial to our long-term betterment when the Village of Laurel Hollow, seeing that the Lab's financial health was no longer precarious, asked us to cover village service costs that, as a tax exempt body, we were not legally bound to pay. Walter had the good sense to know that our community relations could only profit by our seeing that Laboratory activities did not prompt an increase in village taxes. On the other hand, he was firm in saying that we should never pay more than our village services actually cost. Luckily, common sense and good will prevailed, and both the village officers and CSHL officials were pleased with the final agreement.

The stage was thus set for the Laboratory to commence construction of several new buildings needed to maintain our preeminence in research and education. I shall always remember Walter telling me that every successful organization, be it a bank or a research institution, had to expand. Either we would get bigger or go into decline. This rule most certainly held for our Cold Spring Harbor Symposium, which continued to grow. If we could not provide a larger, better-equipped meeting site, our June Symposium and its attendant intellectual excitement would be forced to move elsewhere. Happily, Ed Pulling was soon to find a major donor who agreed to support the construction of a 360 seat brick-covered auditorium that would replace our 200-seat Bush Lecture Hall. The donor was Oliver Grace, newly moved into the community and a true capitalist, known as a tough, buccaneering business negotiator. In the past, he and Walter had pitted their skills against each other, but this was the time to make peace. Oliver was asked to join our Board, and just before Walter retired as chairman, the Oliver and Lorraine Grace

Auditorium was dedicated at the opening of our 1986 Symposium on Human Genetics.

Walter's support also proved crucial to Cold Spring Harbor's return to a major role in plant genetics research. The famous field, next to the library, on which George Shull had discovered improved yields from hybrid corn in 1908 and which Barbara McClintock had used to show the existence of jumping genes in maize 40 years later, was no longer suitable for agricultural purposes. To use the powerful new recombinant-DNA techniques to exploit Barbara's jumping-gene concept, we needed a new plot of good agricultural land that we could yearly count on. Walter led the negotiations that let us purchase for $700,000, 10 acres of land and assorted buildings on the Uplands Farm property, just recently deeded by his wife's mother to the Nature Conservancy.

With good field plots now assured, the time had come to construct a building specifically for plant research. Since the Page Motel, built in 1953 and named for Arthur's father, was to be soon torn down to make way for construction of the Beckman Laboratory, our new plant biology building, dedicated in 1987, was appropriately named the Arthur and Walter Page Laboratory.

To Walter, the many personal advantages of being the scion of an important, landed family demanded that he in turn be an enlightened public servant. His lifelong devotion to our Laboratory was only part of his effort to keep our civilization moving forward. He knew that J.P. Morgan Bank's great prominence meant that it also must work for the benefit of New York City's less-advantaged men and women. He was a strong backer of the Urban Coalition and made sure that significant Morgan Guaranty philanthropy was directed toward the education of minority students. He was also personally involved in seeing that educated minority members entered the banking community.

In so doing, Walter recognized that the social fabric of the United States had been changed for the better by World War II. To him, the promise of a productive future did not necessarily belong to those born well but to those who possessed inherent talents and were willing to work hard and effectively. He believed that talent should be encouraged, wherever it lies.

Even as Morgan's chairman, Walter took the 7:46 a.m. Long Island Railroad from Cold Spring Harbor each day into New York City. The Pages' home, located across from the Lab, and their Murray Hill townhouse reflected their view that money was best spent on simple comforts. Only through his long-beloved sloop, the *Barn Swallow*, could Walter be marked as more than a simple fisherman who knew when the blues were running. Never far from his heart were the waters of Cold Spring Harbor. That its inner harbor was not dredged to become another pleasure-boat-clogged Huntington Harbor and that Robert Moses never built a massive causeway along its eastern shore to Marshall Field's former lands owe much to Walter's gritty determination. We will long miss the many sensible ways he used his privileges and connections to our great betterment.

James D. Watson

Walter and Jane Page, 1958

Jane Norton Page (1918–1998)

Jane Norton Page's death on December 15, 1998, preceded that of her husband, Walter, by only 25 days. They had been together for 56 cherished years, having married just after Walter went on active duty as a naval officer in World War II. Jane's no-nonsense, yet always caring, manner complemented well that of her husband, whose tactful conversational openings often masked strong personal convictions. And when civic duty called, Jane and Walter could always be counted on to lead.

Born in 1918, Jane, like Walter, was the product of an intelligent, landed family with roots early into our country's history. Her father's family, the Nichols, had lived in Massachusetts since the 17th century. A distant forebear, Susanna Martin, a Quaker widow, was in 1692 convicted of witchcraft and hanged on Gallows Hill in Salem. During the Revolutionary War, Ichabod Nichols, who had captained a ship to St. Petersburg and the court of Catherine II, was expelled from their Quaker meeting for privateering against British shipping. Late in the 19th century, Jane's grandfather, John White Treadwell Nichols (1852–1920), who owned a textile mill, moved to New York and bought lands bordering the road from Cold Spring Harbor to Syosset. This land continued to be farmed by his descendants until after World War II.

Jane's father, George Nichols, carried on the family textile business. A prominent yachtsman, he maintained the family farming tradition at Uplands Farm, an estate off Lawrence Hill Road, where he and his family moved in 1924. Uplands Farm had been given to his wife by her father, the financier J.P. Morgan (1867–1943), whose Long Island estate was on Matinecock Point in Glen Cove. Morgan's summers were generally spent in England, to the north of London in Hertfordshire. There Jane Page, as a young girl, experienced the pleasures of the English country house life that New York's great wealth was trying to emulate, if not better, on Long Island's North Shore. In the summer of 1939, Morgan's chauffeur, in a casual conversation with a Lab scientist, gave assurances that war was not imminent because Mr. Morgan was still in England.

Jane Page's strong sense of civic responsibility was also present in her mother, from whom she took her first names. Jane Norton Morgan Nichols had two main interests in her later life—social welfare and land preservation. In her early 50s, she enrolled in the New York School of Social Work. Her certification from this program allowed her to be a better liaison between professional social workers and the boards of their community organizations. Later, following her husband's death, she became president of the Huntington Family Service League. It was her long residency at Uplands Farm, where she maintained a prize herd of Guernsey cows until the early 1960s, that led her to become concerned about the increasing pressures of development on open land. As she grew older, she derived great pleasure from the fact that the Nature Conservancy would later take possession of her estate and keep her beloved fields and forests perpetually free of development.

During the 1960s, Jane Page very much followed in her mother's steps, becoming a member of the Board of the Long Island Chapter of the Nature Conservancy and eventually serving as its president. In particular, Jane helped spearhead our local Nature

Summer of 1920

Jane Nichols (*lower right*) and her cousins with their grandmother in the summer of 1920.
Top: Jim Estabrook, Floyd Nichols, Mary Blake Nichols holding Nancy Taylor, Mollie Nichols, John Nichols
Bottom: David Nichols, John Estabrook, Laura Estabrook, Olivia Taylor, Jane Nichols

Conservancy's chapter in its acquisitions of selective, small parcels of woodland and open land that would serve as buffers between tracts of up-market family homes. Toward that end, she gave to the Conservancy the land across from St. John's Cemetery, on which her stable stood, to serve as a sanctuary. Later, she strongly opposed decisions made in the Conservancy's Washington office to discontinue acceptance of local lands that could not be part of much larger land parcels. Saddened, Jane realized that this decision meant that even more of her local community would be lost to suburban sprawl.

While her children were still in local schools, Jane began to champion community causes. In 1950, she became director of the Long Island Biological Association (LIBA). Two years later, she became the group's vice president, a position she held for six years. She effectively ran the committee that raised the funds that would permit the LIBA-owned James Lab to have a second floor.

Feeling it tactful to have only one Page as a LIBA officer, she left the Board in 1958, when her husband became LIBA's president. But she very much continued in our camp, hosting one of our annual Symposium dinner parties. I, like previous Lab directors, always took care to direct to their party several of the more lively symposium speakers. Over the past decade, the rebirth of plant genetics at Cold Spring Harbor Laboratory brought her much pleasure because she again could see Uplands Farm as a site for successful agriculture.

Outside our community, Jane's most cherished philanthropy was the Robert College of Istanbul in Turkey. During her college years at Bryn Mawr, she became fascinated by classical archeology, an interest heightened when she sailed to Turkey on a boat built for their family friend Paul Hammond, who, like her father, was an experienced yachtsman. Excited by the opportunities Robert College and the American College for Girls offered for high-level secondary education, Jane soon became a member of its Board of Trustees. Later, she also became actively involved with the Near East College Association.

In addition to sailing, a pleasure that took her and Walter to Maine's waters virtually every summer, Jane loved horseback riding. Unfortunately, as had been true for her mother, arthritis would dominate her later years. Never one to complain, Jane valiantly persisted in attending Robert College Board meetings, despite obvious periods of physical pain. The cruel stroke suffered by Walter in 1986, when they were sailing on Puget Sound, sadly meant they could not continue to be forceful community leaders. But even then, they remained strong backers of our local Whaling Museum, with Walter taking on its presidency.

The New Year's Day party that the Pages annually held for their friends in the community was a cherished event, bringing together individuals whose commitments to excellence on all fronts made them and their families important players in our nation's cultural and business fabric for almost a century. To our sorrow, Jane and Walter are now gone. So we must strive ever harder to see that the values they so long worked for stay with us.

James D. Watson

PRESIDENT'S ESSAY

For now more than 50 years, the postgraduate courses at the Cold Spring Harbor Laboratory have been without equal. The first one, the famous Phage Course, offered for 26 consecutive years, was initiated and taught in the waning days of World War II by the German-born theoretical-physicist-turned-biologist Max Delbrück. He saw his course—offered for three weeks during the summer—as a lifeboat for his six talented students, academic scientists who might otherwise begin to grow stale. The course gave them the opportunity to use bacterial viruses (phages) to come to grips with the essence of what was then still very mysterious: the gene. Although no long-term converts emerged from the first-year class, the Phage Course in subsequent years had many real payoffs. The summer of 1948, for example, saw Seymour Benzer and Gunther Stent doing their first phage experiments, and they never went back to their respective past worlds of physics and chemistry.

Encouraged by the teaching triumphs of the Phage Course, then-Director Milislav Demerec, in 1950, started a second course, on bacterial genetics. No one then could have imagined that we would be celebrating its 50th consecutive offering this year. Its success, in turn, inspired the creation in 1958 of our third course on animal cells and viruses. Equally influential, its early students included Purnell Choppin, later long to serve as the president of the Howard Hughes Medical Institute; growth factor discoverer and Nobel prize winner Stanley Cohen, and the clever Swiss interferon cloner, Charles Weissman. Today, our yearly course offerings, ranging from Yeast Genetics to Neurobiology, total 25, with the number bound to increase as biology grows ever more relevant to human life during the next century.

We always aim to teach ideas and experimental techniques that are not yet part of the academic teaching scene even in the best of universities. We therefore recruit teachers who have actually generated new ideas and techniques, and they choose the students from the respective applicant pools. To ensure that their choices are not influenced by financial cir-

cumstances, our student fees are low, covering only a small percentage of the true teaching costs. And when a given student has no means to cover even such reduced fees, we usually provide scholarship support. As a result, we are constantly seeking governmental and foundation monies to cover most of our true teaching costs.

Next fall, we shall be taking on an equally important academic challenge. Our long-dreamed-for opportunity of offering our own Ph.D. degrees is now a reality. Late last September, we received formal permission from the New York State Department of Education to create a degree-granting School of Biological Sciences. Although we have long served as a site for extensive Ph.D. thesis research, the actual degrees have been awarded by nearby universities—for the most part, by the State University of New York (SUNY) at Stony Brook. With large numbers of most valuable Stony Brook students continuing to do their thesis research here, an increasing number of students will have all aspects of their Ph.D. education take place here.

In helping plan our new Ph.D.-granting program, I have naturally looked back on my own education to try to pinpoint the crucial elements that led to my future success as a scientist. Not surprisingly, I see the very powerful influence of my parents. They both strongly encouraged my studies, seeing knowledge and its creation as the key factor that liberates human beings from much of illness, poverty, and superstition. To them, ultimate truth came from observation and experiments, never from personal revelations. Scholarly and cultural pursuits were the ways our Depression-limited family's money was most enthusiastically spent. At Christmas, books were increasingly favored over toys, starting with the child-oriented *Travelling with Birds*, given to me by my uncle and aunt on my eighth Christmas. It was instantly a cherished gift, with its focus on bird migration kindling an interest in biology that increasingly dominated my intellectual aspirations as I moved through adolescence.

Even before high school, I wanted to master the basic laws of nature, and I found Darwin's Theory of Evolution by Natural Selection a powerful liberating force in facing up to the extraordinary diversity of living forms that now inhabit the earth. Its importance to me steadily increased as I went through the University of Chicago and, with little hesitation, early on chose zoology as my major. That I had matriculated when just 15 in no way indicated unusual childhood precocity. Instead, it was a reflection of President Robert Maynard Hutchins' belief that the last two years of American high schools served no useful purpose. In particular, he faulted them for not successfully challenging human minds to think as opposed to learn facts, a task best left to trade schools. To prove his point, he created a program that let a small number of students enter the university after only two years of high school. I was one of those so chosen, most likely because I was a keen reader with much better than ordinary memory.

Not until my third year in college did I as a student feel at ease, knowing by then that it was the ideas, not the facts of the past, that led to good grades in Hutchins' College. Only with ideas in place could facts be prioritized. I was thus ready for intellectual zapping by the famous Austrian physicist Erwin Schrodinger, through his little, elegantly written, wartime book, *What is Life?* Here he posed the key question that had attracted me to biology but whose previous answers had left me wanting. Schrodinger's message was that the essence of life was its ability to pass genetic information from parental to daughter cells. Most importantly, this information must reflect the precise arrangement of atoms within the molecules of heredity located on chromosomes. To truly understand life, we must pursue genetics at the molecular level.

Suddenly, birds seemed objectives for outdoor fun, not for serious science, and I began anticipating going on to a graduate school that would broaden my education to include

chemistry as well as physics. But my first choice, Caltech, did not find me up to their standards, being apprised from my transcript that I was a straight-A student only in ecology courses. Happily, Indiana University only wanted to be sure that my main intellectual objective was no longer birds, and so in the fall of 1947, I was off to its main campus at Bloomington.

My two and a half years there as a graduate student were to transform me from a student into a scientist. Crucial to this process were Indiana's splendid courses. They gave me a sense not only of topics then currently exciting, but also of key earlier experiments that had put the gene at the center of heredity. Particularly inspirational during my first term were lectures on advanced genetics by the recent Nobel prize winner Herman J. Muller. They, in effect, were the story of his pursuit of the gene, starting with his undergraduate days at Columbia University. A year later, I took Felix Haurowitz's course on proteins and nucleic acids, the molecules most likely to carry genetic information. From him I learned that their highly complex structures were likely too complex for current X-ray crystallographic methods to soon give any meaningful take-home lessons for genetics. Instead, this was the time for me to master the ways of the phage world first introduced to me by Salvador Luria's fall 1947 virus course at Indiana University. By the time its final exam was over, I was totally hooked on phages and the possibility that in studying how they multiply, we were in essence studying how genes were copied. Although my resulting Ph.D. thesis, which Luria suggested I do on X-ray–inactivated phage, eventually went nowhere, I frequently went to the lab hoping my next experiment might have a real intellectual payoff.

By the spring of 1950, when I wrote up my thesis, I had mastered all the key phage publications of the past decade, by then knowing their factual details to a greater depth than did either Luria or his long-close, then Caltech-dominated, collaborator, Max Delbrück. Equally important, I felt myself a bona fide member of the phage group by having spent two long summers with it—first at Cold Spring Harbor (1948) and then at Caltech (1949). I also immensely profited from many school-year microbial genetics gatherings in Chicago hosted by Leo Szilard, the legendary developer with Enrico Fermi of the first atomic reactor. When I did not understand an argument or experiment, I had the courage to say so. By my last year with Luria, I was effectively on my own, generating several new ideas on how X-rays inactivate phages. Although all of those ideas soon proved to be off-base, I was having fun. Intellectual torture only came from having to write up minor results. Happily, Luria, in effect, wrote most of my thesis, enjoying his position as an immigrant in control of the English language. Later, I found I could write well when I had a message of importance.

By the time my graduate student days came to a close, I more than subconsciously knew that even the most elegant phage experiments were unlikely to reveal the gene at the crucial molecular level. Somehow, I had to move closer toward the chemistry of DNA. I thus applied for (and received) a National Research Council postdoctoral fellowship that would take me to the Copenhagen lab of Max Delbrück's biochemist friend Herman Kalckar. But soon after my arrival, I feared I had made a bad move. Herman's interests were enzymatic, and there was no way his experiments on how DNA precursors are made could help determine the structure of the gene. Soon I reverted to doing phage experiments in the Copenhagen lab of Ole Maaløe. My intellectual course only truly changed after a meeting on macromolecules that I attended with Kalckar in Naples. There, Maurice Wilkins presented his newly obtained X-ray diffraction diagram of the crystalline "A" form of DNA. Conceivably, within its myriad details were the clues to cracking the mystery of the gene. Learning soon of my change of heart, Salva Luria arranged for me to move to the small but powerful group of protein crystallographers at Cambridge University led by Sir Lawrence Bragg and Max

Perutz. Only 18 more months were to pass before Francis Crick and I found the extraordinarily informative double-helical structure of DNA. The basic structure of the gene was far simpler than we or anyone else could have predicted.

Looking back, I see my first graduate school years as teaching me the values of science and how experiments should be planned, executed, and interpreted. Most importantly, my days were not narrowly focused, and I spent much time learning how others were trying to approach the gene. To his great credit, Luria never saw my experiments as a means to further advance his own career and would not have put his name on my thesis publications even if they had proved major scientific announcements. Nor did he ever express displeasure when I stopped following his research suggestions and went in directions that he thought unlikely to succeed. He saw my need to move forward or backward on my own terms.

That I was then given such independence owed much to my tough undergraduate days at the University of Chicago. I now look back at it as an officers' training school of intellectuals. There, the utterance of an illogical argument inevitably resulted in verbal face slaps that seldom elicited the sympathy of others. No other biology student at Indiana had been so tested over such a hard intellectual obstacle course, and students there found it hard to accept that so much of the science around them would never yield meaningful answers to important questions.

After I joined the Harvard faculty, I tried as much as possible to handle my Ph.D. students as I had been looked after by Salva Luria and Max Delbrück. My students' inherent intelligence was a big plus in my being able to let them run at their own pace. By the time they were in the lab, they had listened to sufficient numbers of my lectures to know what I thought were the big problems to be solved over the next several years. They also knew what fields I thought had no effective chance of soon moving forward. Until the late 1960s, I gave an annual lecture on why developmental biology should be avoided until the basic facts about genes and their expression were better known.

For thesis topics, I tried to start my graduate students out on problems bound to yield sufficient data for a thesis but that nonetheless had potential big payoffs. I never intentionally assigned a topic that I thought had a low probability of success. Occasionally, I guessed wrong, suggesting to Volker Vogt, for example, that he purify the enzyme that removes formyl groups from the amino-terminal end of growing bacterial polypeptide chains. It proved an intractable objective, but he nevertheless received an on-time Ph.D. I did not see then, nor do I now, that the primary purpose of a Ph.D. is to pull off difficult science. Of course, both I and my students always hoped that their thesis research would lead to big conceptual breakthroughs. This, in fact, happened sufficiently often to keep most everyone's morale high. Although my first graduate student, Bob Risebrough, interrupted his studies for a year on an oceanographic ship, he returned to do extraordinarily important experiments during the late winter of 1960 that demonstrated the existence of messenger RNA.

By sharing my lab and its intellectual objectives with one or more already accomplished scientists, my students always had at least two mentors they could turn to for advice. For my first five years, both I and my students greatly profited from the many talents of the Swiss biochemist Alfred Tissiéres, then waiting for a senior professorship to open up in Geneva. Upon his departure, his lab space was taken over by Wally Gilbert, then makng the transition from theoretical physics to molecular biology. Upon Wally's promotion to tenure in biophysics, we continued to share growing lab space, and when one of us was otherwise involved or away for sabbatical periods, our students were not lacking for advice when they wished it.

My students always knew they were working primarily to advance their own careers, not those of Alfred, Wally, or me. Except for review-like papers, only our students' names decorated their manuscripts. Of course, Wally's and my applications for funds benefited heavily from showing how well our students had used our existing grant monies. It is my hope that this same atmosphere will prevail in our new graduate school, despite the many changes in the ethos of science since I left Harvard. While I was a professor there, the number of scientists was much smaller, and I spent little emotional time worrying whether my next grant would be funded. My main anxieties always related to whether our science was going well. Today, there are many, many more scientists, and the funding process is less personal and more undependable—even for good scientists. Anxieties about money are at least equal to those about whether our science is original or derivative.

Too frequently, predoctoral students are now seen as necessary cogs in the machinery of getting grants. With their salaries more often than not paid out of grants, they sense all too clearly that their respective mentors' grant objectives must succeed. It is thus essential that significant new endowment funds be raised for use by our School of Biological Sciences. Only when such funds are ensured will our students sense the freedom to move where their new knowledge tells them to go. I feel confident that these monies will be raised and that not too many years will pass before students from our school are seen helping lead biology to further improve the quality of human life.

April 1999 **James D. Watson**

DIRECTOR'S REPORT

This year, Cold Spring Harbor Laboratory witnessed one of the most significant events in its history: the establishment of our own School of Biological Sciences. With the ability to grant the degree of Doctor of Philosophy (Ph.D.), we expect to attract some of the world's brightest young people to the shores of Cold Spring Harbor for an experience that will change their lives and, we expect, ours. As we enter into a new era, the Watson School of Biological Sciences will have a broad impact on the future of the Laboratory, much like other new educational programs have in the past.

For much of its existence, CSHL has played two overlapping roles in biological science. One stems from the need in the late nineteenth century for a gathering place for people—primarily teachers—to practice and teach biology. Biology was at that time predominantly devoted to the study of the organism and its environment, so the bountiful resources of the shore of Cold Spring Harbor were a distinct attraction. With the help of the local philanthropic community, the Laboratory evolved into a center for both visiting researchers and year-round investigators, particularly with the addition of the Carnegie Institution of Washington laboratories in 1904, where genetics became the primary focus of research. Thus, the Laboratory became a place for teachers to learn during their summer breaks and for researchers to work year-round. In addition, local school-age children could come to the Laboratory to participate in a summer nature study program, making the most of the shores of Cold Spring Harbor, its surrounding springs, creeks, and fields, a program that still exists today.

During the height of the classical genetics period, the year-round research effort was complemented by the introduction of a formal meetings program (1933) and soon after the Second World War, with an advanced laboratory course (1945). Both of these programs set a new direction for the Laboratory, and it is these programs that greatly influence our current activities and make the Laboratory a wonderfully unique place. Principal among the modern benefits is the constant flow of visiting scientists who attend meetings and demonstrate and lecture in our advanced courses, thereby keeping us up to date with the latest results and technologies. In 1998, approximately 7000 scientists visited CSHL to attend 1 of the 20 meetings at our main campus, study in 1 of our 25 courses, or work in our labs as visiting scientists. We have thus long existed as a dynamic center for science by combining a focused and productive research program with a vibrant and world-renowned academic program.

There are many times when the two endeavors have dovetailed, with each program influencing the other. The animal cells and viruses courses that started in 1958 preceded the highly productive era of research at CSHL aimed at understanding how DNA tumor viruses induce cancer. Tumor virus research at CSHL in turn helped focus attention on the molecular biology of animal cells and led to major insights into how genes are expressed, how they replicate, and how the tumor viruses intersect with the host cell's division-control machinery to promote tumor growth. Similarly, the introduction of the neurobiology courses in the early 1970s promoted an environment in which we could no longer resist starting year-round research on learning and memory, a program that is currently under significant expansion due to its great success.

Forty years ago, the introduction of a summer Undergraduate Research Program broadened our educational program. With the goal of providing research opportunities to undergraduate students from throughout the United States and abroad, this program has brought

some of the brightest students to Cold Spring Harbor to experience both the research and the summer's advanced educational programs. This year, we had more than 400 applications and accepted 22 students, who then studied with our best researchers.

Learning about science is not just for the young; it is a life-long undertaking that will never escape the productive scientist. Attending research meetings is a form of continuing education that enables active scientists to keep apace with the rapid developments in research. In addition to the meetings on our main campus, we have a complementary program of smaller meetings at our Banbury Conference Center. It was very fortunate that we were graced with the addition of Charles Robertson's Banbury estate in 1976, where we were able to establish the Banbury Conference Center. Functioning as a biological think tank, the Center brings scientists together to discuss research, policy, funding, and ethics, as well as to learn about fields of biology unfamiliar to them. Today the Banbury Center facilitates the continuing education of scientists in a remarkably beautiful setting.

In the mid 1980s, the DNA Learning Center (DNALC) arose because of the need to reach out to high school students and their teachers and inform them of the exciting developments in DNA research. Biological discovery is moving at an unparalleled pace and has had, and will have, a broad impact on society as a whole. The DNALC therefore fulfills three essential goals: First, to educate young students in modern genetics so that the next generation can understand how modern biology can improve their lives; second, to expose talented students to biological research at an early age, so that some will be inspired to pursue a career in biological science or medical research; and third, to make it possible for teachers to use the DNALC and its resources to keep abreast of developments in biology, so that they are teaching relevant biology to their students. The DNALC is another one of the Laboratory's educational success stories, and it serves as a model for similar centers throughout the United States and abroad. As a recent part of this effort, an exciting genetics Internet site, inspired by Jim Watson in collaboration with David Miklos, Jan Witkowski, and John Kruper, and with funds from the Josiah Macy, Jr., Foundation, saw significant development in 1998. The first sections on genetics were released in January 1999 (http://vector.cshl.org/dnaftb). This site is for everyone who wishes to learn about biology, not just high school students.

With the extensive teaching opportunities we have offered in the past, CSHL has in a very unique way emerged as a powerhouse of science education. The establishment at the end of 1998 of the School of Biological Sciences represents another significant milestone and a major addition to our educational mission and soon will have an impact that will be as prominent as the other educational activities. We now maintain an almost continuous educational slate, ranging from early school years to the most advanced biology for active scientists. Importantly, the graduate school will establish a more intimate connection between our research program and its scientists and our educational program.

When Jim Watson arrived as Director in 1968, he brought along a few students to do their thesis research at CSHL, contributing to the successful DNA Tumor Virus program. But they were formally aligned with universities such as Harvard, Columbia, and New York University. Beginning in the mid 1970s, students from the nearby State University of New York at Stony Brook began working at CSHL. The first arrival was Jim Manley, who graduated in 1978 and is now chair of the Department of Biological Sciences at Columbia University. The number of students coming from Stony Brook increased steadily, initially through our strong relationship with the Stony Brook Department of Microbiology, which was then headed by Arnold Levine, a former Laboratory trustee and now president of the Rockefeller University, and more recently by Eckard Wimmer, also a former CSHL trustee. With the establishment of the multi-institutional graduate program in genetics in the early 1980s, which brought together scientists at Stony Brook, CSHL, and the Brookhaven National Laboratory, there were even more opportunities for students to work at CSHL.

A particularly important event in the evolution of the interactions between Stony Brook and CSHL was the collaboration between Stony Brook's Nicholas Muzyczka, director of Stony Brook's Genetics Graduate Program, and Winship Herr, who spearheaded the involvement of CSHL scientists in the recruiting and teaching students. The interactions between Stony Brook and CSHL flourished and expanded to include students from the graduate programs in molecular and cellular biology, pharmacology, and, most recently, neurobiology. CSHL scientists are also members of the Medical Scientist Training Program that trains M.D./Ph.D. students at Stony Brook. In 1998, we had 57 graduate students from Stony Brook working at the Laboratory, making up a sizable fraction of our total research scientists. As we established our own graduate school, maintaining and even strengthening the interactions between Stony Brook and CSHL were of the utmost importance. These interactions not only promote the training of graduate students, but they are the basis of the relationship between our two institutions and thereby broaden the intellectual environment on Long Island, a highly desirable goal for all involved.

Throughout this period, there had often been discussions about whether CSHL should establish its own graduate program, but they were usually tabled because we were busy venturing into new research fields. In addition, many people quite rightly questioned whether our own program might change the nature of the Laboratory. This was a very appropriate question to ask. Eventually, however, the notion that we start a graduate school at CSHL emerged as one of the principal topics for discussion, encouraged by the Board of Trustees under the strong and enthusiastic leadership of David Luke. As a result, we embarked on a journey that, I believe, will have as much of an impact on the Laboratory as the starting of the postgraduate courses did in 1945.

CSHL has always enjoyed the ability to be flexible in its programs and not having to follow what people elsewhere have done. This key thread was woven into the plans for the new graduate program. Beginning from scratch, Winship Herr again took on a major new challenge to lead the design of a graduate program with CSHL faculty that was appropriate for the modern era of biology and that would serve us well far into the future. He also had to guide our application through the New York State Board of Regents' stringent approval process while not compromising on our desire to be innovative. In both of these, he and the Laboratory were spectacularly successful.

Incorporating the experiences of our diverse faculty who have studied in many different countries and graduate programs, the design of our graduate school took shape. Several important principles emerged that guided the thinking behind the design process and that became part of the graduate school curriculum. Many graduate students in the United States spend far too long in graduate school (up to seven years in some cases), so the program was designed to take about four to four and a half years. For the highly motivated students we expect to attract, this time frame should be more than adequate to embark on a life-long journey as a scientist. (I was fortunate to have completed a successful Ph.D. degree in three years and thus come to Cold Spring Harbor as a postdoctoral fellow having just turned 25. But I was trained well enough to appreciate all that CSHL had to offer.)

To ensure that our students can maximally benefit from the exciting environment at CSHL, we have incorporated a two-tier mentoring system. Students will have an academic mentor from the faculty, who will guide their intellectual and individual development, in addition to a research mentor to guide their thesis research. The relationship between a student and a research advisor is particularly unique and affects both people and their careers in significant ways. Indeed, it is a very special relationship that develops. But there are potential conflicts because the research advisor's research goals often have little to do with the education of a student. Moreover, the very nature of graduate thesis research focuses the student on a very narrow, albeit important problem. The addition of a second mentor for each student who will meet with and guide the student in all aspects of science should counter these possible con-

flicts. That mentor will guide the overall education of the student, particularly in areas of science not related to the research thesis.

Together, the student and academic mentor will discuss science and address problems that might arise. Although such discourse may also come from the research mentor (and I hope it will), the academic mentor will provide another point of view. I was aided greatly by having a marvelous thesis advisor in Alan Bellett, but also a second mentor, Ban Younghusband, who gave me the good advice to come to CSHL from Australia, against the advice of others. Ban urged me to come here because we got to know each other very well, and he therefore knew what would be good for my future. He turned out to be right.

The shorter training period necessitates a way of teaching that is more intense and different from what many graduate programs use, where students spend one to one and a half years in formal course work. It is my observation that following this, students often think that they do not have to continue to learn about science outside their thesis field. But those who take this attitude are doomed to mediocre science.

The innovative curriculum of the new school was designed to teach students in an initial 14-week course how to learn, think, and expand their own horizons. By exposing students to what they need to know in the first semester, we do not expect them to become experts in all subjects. Rather, we expect that the course will open doors and that they will then seek out more information on their own, with their mentors' guidance. To reinforce the concept of continuing education, short courses over the entire four years of the program will provide opportunities for students to learn about new areas, particularly taking advantage of the CSHL advanced courses. There, scientists do not become instant experts in the field taught in a course, but they are exposed to what is currently possible, enabling them to continue the learning process when they return to their own laboratories.

The graduate program involves a significant commitment on behalf of our faculty and the students, so we have initially limited student numbers to approximately five per year. But the small number will also allow us to address other important aspects of the program. First, because we seek high-quality students who will bring a vital energy to the program, recruiting must be highly selective. Second, so as to not have a negative impact on the current research budget, we will endow the graduate school with sufficient funds so that every student will have a guaranteed stipend, in addition to coverage of research costs and other expenses. This will have the added advantage that the students will have more flexibility in selecting the topics for their thesis research because they will not have to rely solely on funds from research grants that have other aims.

There was an obvious choice for the name of the school. Jim Watson has made pioneering contributions to research and to CSHL, but he has also made many significant contributions to education. As a teacher at Harvard, he developed perhaps the most influential textbook in modern biology, *Molecular Biology of the Gene*. This book greatly influenced later texts, including the popular *Molecular Biology of the Cell*, which Jim also helped write. He was a driving force in the establishment of the DNA Learning Center and its programs, and greatly expanded the postgraduate courses at CSHL during his tenure as director. Jim has been uncompromising on quality, innovation, and enthusiasm as a leader in education. I was therefore most pleased that when David Luke and I asked Jim whether we might name the school the Watson School of Biological Sciences, he said yes, although somewhat reluctantly.

None of this would be possible if it had not been for the dedication and very hard work of Winship Herr. He has guided the development of the curriculum, organized the faculty, and set a high standard for the graduate school. The Laboratory owes him a great debt. It is therefore most appropriate that Winship was named the first Dean of the Watson School of Biological Sciences at the November meeting of the Board of trustees.

HIGHLIGHTS OF THE YEAR

Each of the existing divisions of the Laboratory had a busy and successful year with exciting developments on many fronts, and expansion of our research and education efforts into new arenas. In addition to the formation of the new Watson School of Biological Sciences, new research initiatives in neurobiology were under way. We also began an expansion of our research facilities on our main campus and at a nearby technology center, and we worked with New York State to develop a nearby Biotechnology Park. Coupled with our existing activities, these initiatives will ensure that the Laboratory remains at the forefront of research and education as we enter the next millennium.

Research Highlights

The research program at Cold Spring Harbor Laboratory continues to be strong and productive. Advances have occurred in cancer research; the neuroscience program has continued to grow; and plant biology has persisted as a powerful cornerstone of research. The bioinformatics initiative reported last year has proven to be an invaluable asset to the Laboratory and has been expanded, in terms of its scientific staff and the development of advanced courses on the use of computers to solve biological problems. There also continues to be important research on basic cell biology that advances our knowledge of the most fundamental aspects of life.

Cell Biology

Last year, Tatsuya Hirano and his colleagues reported the discovery of condensins, protein complexes that work to physically compact chromosomes in preparation for cell division. This year, Hirano and his colleagues reported the identification of cohesins, other protein complexes that keep together the two parts of newly replicated DNA (sister chromatids) until they are split and transported into two daughter cells during mitosis. Condensins and cohesins both contain a subset of related components called the SMC (structural maintenance of chromosomes) proteins. The two SMC protein complexes help to ensure that the genetic information encoded in DNA that has been replicated segregates evenly during cell division. Not surprisingly, these proteins are conserved in bacteria and eukaryotes, which include human cells, and it is remarkable that such important proteins have, until recently, eluded cell biologists.

N. Hernandez

During the period between one cell division and the next, cells perform essential functions as part of tissue. One of the most important functions is the process of controlling which genes are expressed and which are silenced. This was the topic of this year's Symposium (described below). Nouria Hernandez studies how a particular class of genes within the genome is transcribed into small nuclear RNA molecules (snRNAs). The snRNAs play several roles in the cell, but their main job is to organize and participate in the complex machinery that processes the RNA that is transcribed from other genes to produce the messenger RNA (mRNA) that will then produce proteins. Like regulatory mechanisms for all genes, many pro-

teins bind to each snRNA gene promoter (the regulatory region of a gene), thereby controlling the expression of the snRNA genes.

In her studies on the mechanisms of gene control in human cells, Nouria and her collaborators have characterized $SNAP_c$, a molecular complex made of five protein subunits that helps to direct transcription of genes that encode snRNAs. Recent results have demonstrated that $SNAP_c$ cannot bind to the promoter in the absence of an activator protein that binds to DNA adjacent to the $SNAP_c$ binding site. $SNAP_c$ and the activator cooperate to trigger gene transcription by direct protein-protein interactions. The activator is a protein called Oct-1, an activator of other genes—including those in the herpes simplex virus family—and the subject of research in Winship Herr's laboratory. The observation that $SNAP_c$ and the activator cooperate was striking because Nouria had shown previously that $SNAP_c$ also binds to another protein that binds to gene promoters, the TBP protein, and the two cooperate to activate gene transcription. The protein-protein interactions between Oct-1 and $SNAP_c$ and between $SNAP_c$ and TBP both involve the inactivation of regions of the proteins that prevent promoter DNA recognition. Thus, Nouria and her colleagues may have uncovered one of the mechanisms—if not the mechanism—of how activators of gene transcription cooperate to control the expression of genes in cells.

Cancer

The breast cancer tumor suppressor gene *PTEN*, identified in 1997 by Michael Wigler's lab at CSHL in collaboration with Ramon Parsons of Columbia-Presbyterian Medical Center, has now been shown to be involved in many types of cancer. From the sequence of the protein, it was suspected that the gene product would prove to be involved in removal of phosphate groups from substrate proteins. A collaboration between Mike Wigler's lab and Nick Tonks' lab, then, was a natural.

Nick studies protein phosphorylation and its role in signal transduction, the process whereby extracellular signals are transmitted into the cell to control all manner of events. In fact, Nick helped pioneer studies of dephosphorylation, the removal of phosphate groups on tyrosine residues in proteins. A protein's condition (phosphorylated or dephosphorylated) helps to determine whether it is active or inactive. Phosphorylation and dephosphorylation regulate approximately one third of all proteins in a cell.

To pursue the function of the PTEN protein, Tonks and postdoctoral fellow Mike Myers collaborated with Javor Stolarov of the Wigler lab and Peter Downes of the University of Dundee to determine that the *PTEN* gene does encode a regulator of phosphorylation. But it turned out to be a poor enzyme that used proteins containing phosphates. After much investigation, its key target for tumor suppression was found not to be a phosphoprotein, but rather a membrane phospholipid called PIP_3. PIP_3 functions in a variety of cellular responses related to cancer, including signaling from cell surface receptors to control whether a cell survives or undergoes cell death, or apoptosis. Apoptosis is the cellular equivalent of a suicide mechanism that, when triggered by serious DNA damage or external signals, causes a cell to self-destruct. Apoptosis is a natural self-defense mechanism against cancer; it rids the body of abnormal cells and prevents their replication.

The finding that the PTEN protein dephosphorylates PIP_3 is the first to indicate that mutations in a gene encoding a lipid phosphatase are directly linked to cancer. Mutations in *PTEN* occur in breast cancer, glioblastoma, prostate cancer, and endometrial cancer. In addition, mutations in *PTEN* are found in several disorders predisposing to cancer, including Cowden's

Syndrome, Lhermitte-Duclos disease, and Bannayan-Zonana syndrome, all of which are characterized by the formation of multiple benign tumors and an increased incidence of malignant tumors.

Several labs at Cold Spring Harbor continue to study apoptosis. Because apoptosis is a natural defense against cancer and causes the death of cells with serious genetic defects, it has become clear that the deregulation of apoptosis contributes to cancer. Yuri Lazebnik's lab is studying the molecular machinery that effects apoptosis and the signals that trigger it. His goal is to determine how this cell death mechanism—present in all cells—can be selectively reactivated to destroy cancer cells.

Yuri is following up on the observation that expression of certain oncogenes can either induce apoptosis or sensitize cells to the stimuli that cause apoptosis. It intrigues him that some of the cellular events that cause cancer may also be used to kill cancer cells.

All cells have the apoptotic machinery, but healthy cells lack the signals to trigger it. Oncogene expression naturally generates a signal that can activate apoptosis. When this signal is interrupted and fails to reach the apoptotic machinery, cancer cells can survive and become resistant to chemotherapeutic drugs. Yuri's plan is to develop a way to recouple the oncogenic signal and the apoptotic machinery in order to kill the otherwise apoptosis-resistant cells.

To understand the molecular mechanisms that link oncogene-induced signals and the apoptotic machinery, Yuri studies caspases. This family of cysteine proteases—enzymes that cleave, or cut, other proteins—comprise an essential component of the apoptotic machinery. Caspases are activated at the onset of apoptosis and they cause cell death by cleaving several proteins in a coordinated manner. Yuri's objective was to determine how oncogenic transformation triggers apoptosis, specifically to identify which caspases become activated.

Yuri's lab developed a cell-free system to study the apoptotic machinery and its activation by oncogenes. Through the use of this system, Yuri's lab has identified the caspase that is activated by oncogenes, caspase-9. What occurs in tumor cells, however, is that other mutations somehow prevent or bypass the activation of caspase-9.

Scott Lowe also studies apoptosis and has made significant contributions to the field with his work on the tumor-suppressor gene *p53* and its role in programmed cell death. Scott and CSHL adjunct investigator David Beach have identified another way in which tumor-suppressor genes, such as *p16* and *p53*, can prevent cancer. The p53 protein has long been recognized as an important tumor suppressor, because it plays a vital role in programmed cell death. When cells lack active p53, programmed cell death (including oncogene-dependent apoptosis) does not occur and cells are robbed of an important defense mechanism against cancer. In their recent studies, Scott and David discovered that cells carrying the normal forms of the tumor suppressor genes *p16* and *p53*, as well as the oncogene *ras*, are prevented from becoming tumor cells by another mechanism. They found that the proteins p16 and p53 can also induce cell senescence—a state in which cells cease to divide but do not die.

Then in July, Scott, in collaboration with Charles "Chuck" Sherr, a Howard Hughes Medical Institute researcher at St. Jude's Children's Research Hospital in Memphis, Tennessee, reported that another gene, *p19 (arf)*, also functions like a tumor suppressor. *p19 (arf)* plays an important role in the pathways that lead to programmed cell death and senescence, the same pathways that are triggered by the normal *p53* gene. Together, Scott's recent studies indicate that two genes, *p16 and p19* (which are physically overlapping in the genome of a cell), defend against cancer in different ways. *p16* works with *p53 (arf)* to induce cell senescence, and *p19 (arf)* works with *p53* to induce programmed cell death as well as senescence. Both pathways can prevent tumor growth, and the loss of either pathway can promote tumor progression.

In recent studies, Scott and his colleagues have shown that APAF-1 and caspase-9 are downstream from the *p53* gene in the p53-dependent apoptosis pathway. Thus, loss of *p53*, which occurs in more than half of all cancers, or of *p19 (arf)*, which is mutated in many of the other cancers, eliminates a major signaling pathway to the cell death machinery in tumor cells. This may explain why these tumor cells eventually become resistant to chemotherapeutic drugs that are used to treat cancer by inducing cell death. Tumor cells lacking the proteins encoded by these genes cannot signal the cell death machinery to kill the cells; thus, the tumor continues to grow. It now seems clear that we now must find ways around this defect in tumor cells.

Greg Hannon's laboratory studies another aspect of oncogene-dependent cell death. He is looking for mutations that might inactivate this defense mechanism and allow the formation of a tumor and has developed a method for identifying such genes. A visiting scientist in Greg's lab, Roberta Maestro from the CRO in Aviano, Italy, identified a gene called *twist* using the screening method that Greg developed. The Twist protein may help to regulate diverse developmental processes; it was already known that mouse embryos lacking the *twist* gene die before birth. In 1998, Greg's lab found that Twist protects cells in culture from apoptosis. Because of these observations, Greg suspected that the *twist* gene might be a part of the *p53* pathway. Indeed, a variety of experiments confirmed that the Twist protein could interfere with activities of p53 that are essential for tumor suppression.

G. Hannon

The observation that the Twist protein might affect the p53 tumor suppressor pathway prompted Greg and his collaborators to search for mutations in the *twist* gene in human cancers. They found that *twist* is frequently expressed in rhabdomyosarcomas, a cancer of the bone, soft tissues, or connective tissue (e.g., tendon or cartilage) that is the most common soft-tissue cancer in children. It begins in the soft tissues of muscle and is thought to derive from skeletal muscle precursor cells that fail to complete terminal differentiation. The *twist* gene is not expressed in differentiated muscle cells, but in 50% of rhabdomyosarcomas, they found that *twist* expression is maintained.

In collaboration with Larry Kedes of UCLA, Greg and Roberta have demonstrated a likely role for Twist in this tragic childhood disease. The Twist protein interacts with a component of the p53 pathway, called p300, a gene transcription regulator. They also have provided the first evidence that Twist can disable cells' ability to commit programmed cell death, thus promoting the development of cancerous growth.

DNA Microarray Technology

One of the most powerful new techniques in modern biology, called DNA microarrays, or DNA chip arrays, emerged from recent studies on yeast at Stanford University. During the past year, we have been working to merge this method with techniques developed at the Laboratory that were designed to identify mutations in human cancers. Mike Wigler has entered into a collaboration with Larry Norton, M.D., of Memorial Sloan-Kettering Cancer Center in New York City, to use DNA chip technology to look for genes that are mutated in breast cancer.

To use chip technology, researchers place fragments of different DNA samples on a glass microscope slide in a grid of very high density. They expose the slide to labeled DNA, or a complementary DNA (cDNA) copy of mRNA, which hybridizes with the DNA on the glass slide, and then analyze the amount of hybridization in each spot on the slide. The results are color-coded,

so that the most active genes (with the greatest degree of hybridization) are colored red, and genes that are repressed (hybridized the least) are colored green. Alternatively, the experiment can be set up so that genes that are overrepresented in cancer cells are labeled red and genes that are missing in the tumor cells are labeled green. Mike and his associates are using the method to compare the genes in healthy versus cancerous breast tissue and at various stages of breast cancer progression.

This year, thanks to a $300,000 grant from the Lillian Goldman Charitable Trust through The Breast Cancer Research Foundation in New York City, Mike installed state-of-the-art microarray equipment in his Demerec Laboratory. His lab will now be able to analyze tremendous numbers of DNA samples from breast cancer tissue. The emerging DNA chip technology should make possible the development of better methods for the diagnosis and treatment of breast cancer, because it will allow researchers to design treatment aimed at the specific mutations present in each patient.

Just as Mike uses the microarray technology to study gene activity at various points in disease progression, Bruce Futcher is using it to locate and characterize genes whose activity is determined by the stages of the cell division cycle in yeast.

Bruce is collaborating with David Botstein and Patrick Brown of the Stanford University School of Medicine. In 1998, they used DNA microarray technology to find, characterize, and analyze yeast genes whose activity is regulated by the cell division cycle. In just four months, the two labs located and characterized about 800 yeast cell-cycle-regulated genes. During the past 15 years, many scientists had identified and characterized only 103 cell cycle(regulated genes in yeast, revealing the power of the new technology.

Microarray technology has yielded an unprecedented increase in the rate of accumulation of data. The microarrays used in Bruce's cell cycle experiments contained 6000 discrete DNA fragments, each of which represents one of the approximately 6000 genes in yeast. Michael Zhang, one of our bioinformatics experts, is using the power of computational analysis to sift through the enormous amounts of data.

Knowing the set of 800 yeast genes that are regulated by the cell cycle has provided a wealth of information that is contributing to a more complete understanding of cell division. For example, researchers now have an overview of the different kinds of biochemical processes that change with cell division. They can study the clusters of dozens or hundreds of coregulated genes that cooperate in these processes and probe the molecular mechanisms that allow the different clusters of genes to be turned on one after another in an orderly way. Researchers can begin to identify the functions of uncharacterized genes by analyzing the known functions of genes in the same coregulated cluster. The combination of biochemical studies and microarray analysis is destined to lead to an ever-greater understanding of the vital cell process of cell division in yeast—and in higher organisms.

Through the acquisition of a small campus in nearby Woodbury—a building of 60,000 square feet located on 12 acres—the Laboratory has positioned itself to further exploit and develop microarray technology. The property was previously owned by the American Institute of Physics and will provide the Laboratory with much-needed space for expansion of high-technology research, such as genome sequencing and microarray research.

In structural biology, Leemor Joshua-Tor is continuing her studies of the three-dimensional structure and activity of the enzyme bleomycin hydrolase (BH), which is similar in humans and yeast. (Researchers discovered human bleomycin hydrolase [hBH] in the 1970s when they realized that it detoxifies the anticancer drug bleomycin and causes resistance to the drug.) Leemor has found that yeast BH interacts with its substrate (the bleomycin molecule) in an unusual way.

Six molecules of BH come together to form a barrel-shaped structure with a channel in the center. One end of each molecule of the enzyme projects toward the central channel and into the active site region of the enzyme. This spatial configuration allows the enzyme to interact specifically with bleomycin.

Leemor, in collaboration with Stephen Johnston of the University of Texas-Southwestern Medical Center, recently solved the three-dimensional structure of hBH, in its wild type and a mutant form. Knowing the three-dimensional structure of a molecule often provides important clues about its function. Although the structure of hBH was predictably similar to that of yeast BH, Leemor's lab found a striking difference in the human molecule's electrostatic charge: The inner channel of the yeast BH has a strong positive charge, but the same region of the human enzyme has a slight negative charge.

Like the enzymes that Yuri studies, bleomycin hydrolase is a protease—an enzyme that cleaves other proteins. BH deactivates bleomycin, the anticancer drug, by cutting each bleomycin molecule at a particular place. Although most proteases determine the cleavage site on their targets (usually proteins) by finding a specific point in the target molecule's amino acid sequence, Leemor found that the point at which BH cleaves its target is determined not by an amino acid sequence, but by the relative positions of the negative and positively charged ends of the substrate. The protease acts like PacMan, eating the substrate protein away from its end.

Neuroscience

Surprisingly, in 1998, several labs reported a possible link between bleomycin hydrolase and Alzheimer's disease. A group led by Robert Ferrell and John Lazo at the University of Pittsburgh found that individuals with two copies of a particular allele (type) of the BH gene were four times more likely than the average person to develop sporadic Alzheimer's disease. Then, Carmela Abraham of Boston University and her collaborators purified BH from the brains of individuals with Alzheimer's disease while looking for amyloid precursor protein (APP), a protein that is processed to yield β-amyloid, a component of the plaques that occur in the brains of Alzheimer's patients.

Leemor's lab is currently studying the connection between BH and APP processing, specifically the relevance of a BH polymorphism suspected of playing a role in this process. In addition, her lab is continuing its studies of the protease's effect on the anticancer drug bleomycin and ways to ameliorate the resulting drug resistance in order to preserve the drug's anticancer activity.

Roberto Malinow, a senior neurobiologist who was recently appointed by the Board of Trustees to the Harrison Chair of Neuroscience, has also discovered a possible link between his research and Alzheimer's disease.

Scientists at other institutions have shown that mutations in the presenilin 1 gene (*PS1*) are associated with the most common cause of familial Alzheimer's disease. Roberto's lab is studying mice that carry a mutant form of the *PS1* gene—the same mutation that some human Alzheimer's patients carry. These mice provide an experimental model for studying certain molecular aspects of Alzheimer's disease. (Although the mice do not display the behavioral changes characteristic of Alzheimer's patients, this is not unexpected, as the same mutation in humans is present at birth, yet 30 or more years typically pass before the onset of the behavioral manifestations of the disease.)

Roberto's lab studies long-term and short-term potentiation (LTP and STP)—a strengthening of synaptic communication between neurons that occurs when neurons are repeatedly

stimulated at high frequency. Roberto's lab found that LTP and STP are notably—and unexpectedly—elevated in brain cells of the *PS1* mutant mice. Surprisingly, synaptic inhibition, which suppresses LTP and STP as well as the firing of electrical impulses, was also enhanced in the mutant mice. This increased synaptic inhibition may represent a compensatory homeostatic response to the increased ability to produce LTP and STP. Roberto's lab then further inhibited synaptic activity in these PS1 mutant brain cells by applying the drug benzodiazapine, which returned synaptic potentiation (LTP and STP) to normal levels.

Coincidentally, clinicians in Sweden recently reported that the regular use of the benzodiazapines Xanax and Valium correlated with a markedly reduced incidence of Alzheimer's disease in the patients studied. Although the mechanism of the drugs' action is known as it relates to their traditionally prescribed use as anti-anxiety agents, it was not clear how these drugs affected the likelihood of developing Alzheimer's disease. Roberto's studies of the relationship between the molecular biology of the *PS1* gene and the activity of the bezodiazepines may reveal a greater understanding of ways to prevent or treat Alzheimer's disease.

Roberto's lab is also continuing to study factors involved in neuronal plasticity, i.e., the ways in which neurons change their activity or shape during development or as a result of learning, trauma, or other events. The researchers have developed improved tools for studying the activity of brain cells and the connections between them, called synapses. One new method developed by the Malinow lab involves the introduction, using a viral vector, of recombinant proteins whose movement in cells and at synapses can be monitored by electrophysiology and imaging techniques. By attaching these proteins to specific molecules important for learning and the formation of memories, Roberto can track the movement and activity of important nerve cell factors. The studies also utilize the sophisticated two-photon laser-scanning imaging system that Karel Svoboda brought to CSHL in 1997, as well as high-resolution electron microscopy, to track the movement of other important proteins in the brain during learning.

Karel, in collaboration with Roberto and postdoctoral fellow Mirjana Maletic-Savatic, used the new imaging system to observe and document the effect of synaptic stimulation on the shape of brain neurons. Karel custom-built a two-photon laser-scanning microscope to study living nerve cells in brain slices from rat hippocampus, a region of the brain that is important for the formation of memories. The researchers stimulated axons from one group of nerve cells that form synapses on a small dendritic branch of a neighboring cell—a strong but highly localized stimulus. They found that the stimulus triggers the growth of new dendritic protrusions called filopodia. Growth of filopodia begins within 20 minutes after the stimulation and continues for at least 40 minutes more. The new filopodia may ultimately form new synapses, an important phenomenon during development and learning and memory. By comparing stimulated and unstimulated dendritic regions, the researchers found that growth of filopodia evoked by strong electrical stimulation was limited to regions of the dendritic tree close to stimulated synapses.

Mirjana, Roberto, and Karel also found that the growth of dendritic filopodia requires activation of *N*-methyl-D-aspartate (NMDA) neurotransmitter receptors at the synapses under study. Other studies had shown that activation of these receptors is required for the formation of memories and for normal brain development. In the recent experiments, the blockade of NMDA receptors prevented stimulation-induced growth. Thus, synaptic transmission, and activation of NMDA receptors in particular, is required to produce this kind of dendritic growth.

In the cells affected by the synaptic stimulation, the growth of filopodia subsided about 45 minutes after the stimulus ended, but the filopodia remained, and some formed bulb-like tips which suggests that they may become functional synapses. Karel and Roberto will continue to study the structure of the outgrowths, as well as their signal transduction properties.

Meanwhile, Jerry Yin and postdoctoral fellow Marcia Belvin at the University of California at Berkeley have made a fascinating observation that suggests how and when memories are formed. Neuroscientists have long suspected that important brain functions take place while people are sleeping. Jerry and Marcia found that the activity of CREB, a protein that Tim Tully and Jerry Yin previously showed is important in memory formation, displays circadian, or 24-hour cyclical, patterns of activity. The Yin lab is trying to determine the functional connection between the nighttime peak in CREB activity and memory formation. Given what is known about memory formation in mammals, it is likely that some aspect of memory consolidation occurs at night, probably during sleep.

Advanced Neuroscience Imaging

In an exciting expansion of the Laboratory's neuroscience program, close on the heels of the establishment of the Beckman Neuroscience Center in 1992, an advanced neuroscience imaging facility is being constructed on the main campus just west of James Laboratory. The new building, named the Nancy and Edwin Marks Building, will house advanced imaging facilities for use in studies of the living brain—a great advance for neurobiology research. Karel Svoboda will use and further develop state-of-the-art laser-scanning microscopy to extend his ongoing studies of neuronal activity and synaptic plasticity in individual neurons in functioning brains of live animals. In addition, scientists from around the world will take courses in the Marks Building to learn how to use this sophisticated new technology. The imaging facility is scheduled to open in August 1999.

In another expansion of existing neuroscience facilities, the Laboratory is replacing the old visitors' cabins just north of the Beckman Neuroscience Center with a small building dedicated to computational neuroscience. The new facility, made possible by Laboratory Trustee William Murray, will be known as the Samuel Freeman Building. There scientists will use the latest computerized tools to model how the brain works.

Plant Biology and Plant Genetics

In April, Ueli Grossniklaus and his colleagues reported that *MEDEA*, a gene in the widely studied model plant *Arabidopsis*, is important for the maternal control of plant embryo development. If a plant embryo (in a seed) has inherited one mutant copy of the *MEDEA* gene from the mother plant, cells in the embryo grow excessively and the embryo dies. But if the embryo inherits one mutant *MEDEA* gene from the father plant, it lives and functions normally. Because of this difference, researchers call *MEDEA* a "maternal-effect gene," meaning that it dictates certain behavior (in this case, cell division and embryo development) only if the gene comes from the female parent. Ueli found that the function of the *MEDEA* gene product in plants resembles the *Polycomb* group of genes in animals, which are important for controlling cell proliferation and for mediating the expression of traits inherited from one parent.

Also in plant genetics, the Laboratory's role in the global *Arabidopsis* Genome Sequencing Project continues to go well. Dick McCombie's sequencing expertise and Rob Martienssen's work with the gene traps he developed with Sundaresan Venkatesan have proved to be an invaluable contribution not only to the sequencing effort, but also to the determination of gene function in this small, flowering plant. Rob continues to do research on other aspects of plant genetics as well, and he recently made an important discovery about the movement of intracellular proteins across cell membranes.

The May 1998 Genome meeting occurred in the wake of Craig Venter's surprising announcement of plans to complete the sequence of the human genome on an accelerated schedule. (Venter now heads the Celera Corporation in Rockville, Maryland.) Most of the leaders in human genome research were in attendance, which resulted in lively discussions about—and considerable media interest in—Craig's plan. The Laboratory's goal in these efforts is to produce a timely, accurate body of data that is made available to all researchers.

The Laboratory also plans to expand its genomics research program. A significant portion of the recently acquired building in high technology center in nearby Woodbury will be dedicated to genome sequencing projects. This expansion of the existing program will enable CSHL to more quickly play a role in other sequencing projects.

Broadhollow Bioscience Park

The long quest to establish a suitable biotechnology park within close proximity to the Laboratory has finally been fulfilled. The new facility will allow start-up biotech companies based on CSHL research as well as research from other academic centers and companies to locate nearby and to maintain easy scientific interactions and collaboration. John Cleary, who has been a member and president of the CSHL Association and a member of the Laboratory's Board of Trustees, was instrumental in securing substantial backing for the park from Governor George Pataki and New York State, as well as the support of many local business and political leaders.

The park is located on 20 acres of land adjacent to the State University of New York (SUNY) Farmingdale campus on nearby Route 110 in Farmingdale. The Broadhollow Bioscience Park will be supervised by a separate not-for-profit organization that will be controlled by Cold Spring Harbor Laboratory and SUNY Farmingdale. Completion of the facility will enhance the transfer of innovations and discoveries in basic research to the private and public business sectors, and thereby enhance the utility of our research efforts.

Watson School of Biological Sciences

As described above, another long-standing initiative came to fruition in 1998 when the New York State Board of Regents voted in November to approve accreditation of Cold Spring Harbor Laboratory as a Ph.D.-granting institution. The Laboratory is now in the process of raising funds to endow the school.

In the initial application for accreditation, the school was named the Cold Spring Harbor Laboratory School of Biological Sciences. In January, the state approved the new name for the graduate school at Cold Spring Harbor: The Watson School of Biological Sciences, named for the Laboratory's long-time Director and now President James D. Watson.

To supplement housing for Watson School students, the Laboratory recently purchased a grand old house on land adjacent to Laboratory property on Route 25A, facing the inner harbor. The house was constructed in the 1700s, and it once served as the general store and post office for Cold Spring Harbor. The house was also home to many generations of the illustrious Long Island Jones family, including Jones descendant and former CSHL Trustee Townsend Knight. The building, located at 222 Main Street in Cold Spring Harbor, was recently named the

Knight House

Knight House in honor of Townie and will serve as housing for graduate students attending the Watson School of Biological Sciences. But due to its considerably run-down condition, the house will first undergo considerable restoration.

Symposium LXIII

Transcription, the copying of one strand of DNA into a complementary RNA sequence, was the topic of the 63rd CSHL Symposium, Mechanisms of Transcription. For five days, from June 3 to June 8, Grace Auditorium was filled with talk of promoters, transcription factors, and chromosome structure. In attendence were the best-known scientists in the field, as well as many younger ones.

On Sunday evening, as is customary, the Laboratory welcomed neighbors and friends for the annual public Dorcas Cummings Lecture. Ronald M. Evans, a Howard Hughes Medical Institute investigator at the Salk Institute, discussed "The Molecular Biology of Fat: Weighing the Risks," a subject of broad interest to a general audience.

Dr. Evans described the role of hormones, the signaling molecules secreted by glands and other tissues. Hormones coordinate the activities of organs and tissues by regulating gene activity. His group discovered a hormone called 15-deoxy-Δ12,14-prostaglandin J2, which directs muscle and fibroblast cells to become fat cells. This hormone is now being used to treat type II diabetes. Evans advocated a diet with fewer saturated fats, such as a Mediterranean-style diet which is rich in legumes and grains.

Jim and Liz Watson invited Australian artist Lewis Miller to attend the annual Symposium as artist-in-residence. Miller sketched visiting scientists at meeting sessions, breaks, and at the Symposium picnic. A collection of his marvelous framed sketches now appears along the staircase in Blackford Hall.

Sketch by Lewis Miller in Grace Auditorium during the Symposium

Jim Watson's CSHL Anniversaries

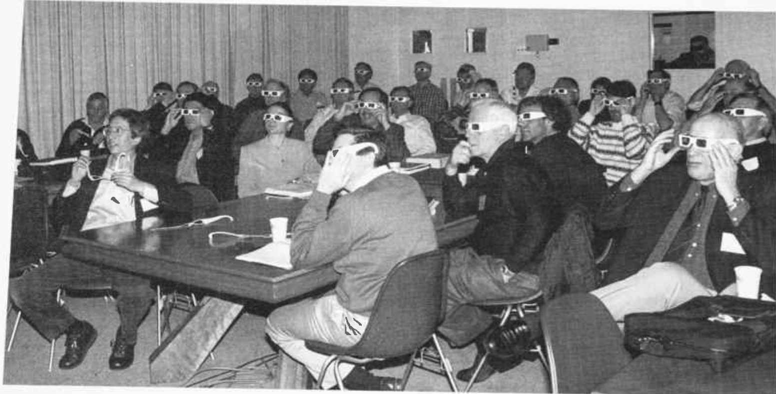
In addition to a milestone birthday, 1998 marked two special anniversaries for Jim Watson. It has been 50 years since his first visit to Cold Spring Harbor Laboratory as a graduate student doing summertime research, and 30 years since he became director of the Laboratory. To commemorate these events, the Laboratory held a special late-winter conference called *Pathways to Cancer*. CSHL alumnus and Trustee Ed Harlow, of Massachusetts General Hospital and Harvard University, Jan Witkowski, and I, were coorganizers.

The meeting included sessions entitled "Cancer Genetic Pathways," "Cancer Cell Growth Controls," and "Cancer Pathways"—a discussion of research from the cell to the clinic. But the highlight of the meeting came on Thursday evening when the BBC film *Life Story* was shown in Grace Auditorium. What an extraordinary experience it was to see Jim Watson stand before us offering offered his thoughtful comments on the movie that was based upon his 1968 best-selling book *The Double Helix*. The showing of the movie in the context of a modern meeting on biology highlighted how far we have come since those exciting days in 1953.

Banbury Center

From Molecules to Brains—The J.P. Morgan Meeting

The unusually broad topic of the 1998 annual Executives' Seminar weekend, sponsored by J.P. Morgan, was *Imaging: From Molecules to Brains*. Carlos Bustamante described his work using the atomic force microscope with which he images single molecules and tries to "see" how a DNA molecule is synthesized. Paul Sigler described his X-ray crystallography studies of the



Banbury meeting participants watch cells in three dimensions.

structures of large protein assemblies, and Mark Ellisman showed some remarkable three-dimensional pictures of cells taken with a powerful electron microscope. Bruce Rosen and John Gabrieli discussed their studies of the brain. We are indebted to Sandy Warner and David Deming for their continuing support of this unique occasion.

Horse Genetics and the Performance of Thoroughbreds

The 1998 Banbury Center meeting on horse genetics was stimulated by CSHL Trustee and race horse owner Charles Harris. It was his belief that the performance of racing thoroughbreds has fallen and that this decline might be related to inbreeding. Horse geneticists, scientists who study horses and humans, and thoroughbred owners and breeders considered this question and offered an in-depth analysis of the current state of the genetic map of the horse as well as ways to promote genetics research on the horse. Such policy issues have been raised at previous Banbury meetings and have been influential in stimulating funding of research in this area.

Finding Individual Differences in Human Disease Genes

One of the most important advances in human genetics came in 1980 with the development of a new method for identifying individual differences in human genes that are linked to specific diseases. The new strategy, based on small but common variations called single nucleotide polymorphisms (SNPs), has generated a great deal of excitement. Many unresolved issues remain, however, and the Banbury meeting, *Large-scale Discovery & Genetic Applications of SNPs*, tackled some of them: how to find these variations, how many will be needed for effective mapping, and how to use them. The group also discussed interesting and controversial questions of intellectual property.

Robertson Research Fund

Since 1973, the Robertson Research Fund has supported Cold Spring Harbor Laboratory's scientific program. Its balance is now almost $80 million, up from approximately $8 million in 1973. Once again this year, Robertson funds supported labs in each of our primary fields of research—cancer, neurobiology, and plant genetics.

In 1998, Robertson funds supported cancer research by providing start-up support for new bioinformatics investigators Andy Neuwald, Andy Reiner, and Lincoln Stein and research program support for established scientists David Helfman, Michael Hengartner, Nouria Hernandez, Tatsuya Hirano, Scott Lowe, Ryuji Kobayashi, Adrian Krainer, Jacek Skowronski, David Spector, Nick Tonks, and Rui Ming Xu. In neurobiology, the fund supported research in the labs of Karel Svoboda, Holly Cline, Grisha Enikolopov, Alcino Silva, Jerry Yin, and Yi Zhong, and furthered plant research by Erich Grotewold, Hong Ma, Rob Martienssen, and David Jackson.

The Robertson Research Fund also helps to support many postdoctoral researchers, graduate students, and scientific seminars.

In addition, the Marie H. Robertson Memorial Fund, dedicated to neuroscience, gave support to Grisha Enikolopov and Yi Zhong.

Board of Trustees

David L. Luke III completed a more than 12-year tenure with the Board of Trustees, and the Laboratory remains deeply indebted to him for his outstanding leadership during a time of significant growth and improvement. Very few people have devoted as much time and effort to the Laboratory as has David. His guidance has ensured that we remain a dynamic and growing institution, and the generosity of David and his wife, Fanny, has reflected their love of the Laboratory. I am most pleased that David has agreed to continue to serve the Laboratory as chair of the campaign to endow the Watson School. In November 1998, David retired as Chairman of the Board and was elected Honorary Trustee. In April 1999, he was feted at a dinner in his honor.

It is our great fortune that William Miller has stepped into David's shoes as our new Chairman of the Board. Bill, retired Vice Chair of Bristol Myers Squibb and Co., served as David's deputy and has already made major contributions to the Laboratory.

The following members of the Board of Trustees also completed their terms in 1998: John R. Reese, Arnold J. Levine, J. Anthony Movshon, and Joan A. Steitz. David H. Koch completed his term on the Board as well, and to him we are indebted for a Watson School of Biological Sciences fellowship that will endow one student per year. Thomas A. Saunders III stepped down from the Board due to other commitments, and John Cleary completed his term on the Board as he completed his term as President of the CSHL Association. During John's tenure as Association President, the organization expanded considerably and increased its support of the Laboratory's programs. John was also very helpful in the creation of the Graduate School and Broadhollow Bioscience Park and is still making major contributions as Broadhollow's chairman. The Laboratory is grateful for the guidance and input of every Trustee.

We are pleased to welcome the following individuals, who joined the Board in 1998: Charles E. Harris, chairman and CEO of the public venture capital firm Harris & Harris Group, Inc.; Leslie C. Quick, cofounder of one of the first and largest discount stock brokerage firms, Quick and Reilly, Inc.; Howard Solomon, president and CEO of the pharmaceutical company Forest

Laboratories; Susan Hockfield, professor of neurobiology at Yale University School of Medicine and dean of the Yale University Graduate School of Arts and Sciences; Rudolf Jaenisch, M.D., member of the Whitehead Institute for Biomedical Research and professor of Biology at Massachusetts Institute of Technology; Charles J. Sherr, M.D., Ph.D., Howard Hughes Medical Institute investigator and professor at St. Jude's Children's Research Hospital; and James Spingarn, senior vice president of investments at Gruntal & Co. located in Great Neck, New York, and president of the CSHL Association.

At the November 7 meeting of the Board of Trustees, the playground of the Mary D. Lindsay Child Care Center was dedicated to Honorary Trustee Wendy Vander Poel Russell. Mrs. Russell has a long and rich history with CSHL; in fact, as a small child she participated in the Lab's Nature Study program. More recently she was instrumental in securing on-site child care for CSHL.

A champion fund-raiser at the Laboratory, Wendy has been tireless in her devotion to various projects over the years, including those for Grace Auditorium, the Beckman Neuroscience building, and the Mary D. Lindsay Child Care Center. She was instrumental in establishing the Corporate Advisory Board as a supporting body for the DNA Learning Center and has been a consistent and ardent supporter of the CSHL Association Annual Fund. She began serving on the Board of Trustees in 1984 and has since served four 3-year terms including those as Secretary in 1985–1987 and 1992–1997. She has served on the Board's Development, Executive, Finance & Investment, Banbury, Building, and DNA Learning Center committees and has been a long-time member of the CSHL Association.

It was our honor to name the playground for her. What more cheerful recognition could we have offered to such an upbeat and dynamic lady?

Sadly, one of the newer members of the Board—and a long-time member of the CSHL Association—passed away in July. Mrs. Vernon L. Merrill, who assumed the position of president of the CSHL Association in February, had been an active and generous supporter of the Laboratory since 1985. She, too, was an ardent proponent of the Laboratory's initiative to establish on-site child care, and served on the Building, Development, and Executive committees for the Board of Trustees. Mrs. Merrill lost her long and valiant battle with breast cancer on July 13. Her enthusiastic involvement will be deeply missed, and the Laboratory extends deepest condolences to her husband, Robert, and their family.

V.L. Merrill

CSHL Association

Following the death of Mrs. Merrill, CSHL Association member James Spingarn assumed the role of acting president of the Association. He has since been elected President. Jim has been a most enthusiastic supporter of the Laboratory's research programs and public education efforts.

On February 1, the CSHL Association held its Annual Meeting and hosted Judah Folkman as its keynote speaker. Dr. Folkman is a senior associate in surgery and the director of the Surgical Research Laboratory at Boston's Children's Hospital and Harvard Medical School. His research on anti-angiogenic factors has attracted much attention during the past year. His work is directed at stopping the development of new blood vessels (angiogenesis) in tumors as a way to shrink and eradicate them. His proposed therapy, using compounds such as endostatin and

Dr. Folkman

angiostatin, has proven successful in controlling tumors in mice. Dr. Folkman's talk was entitled "Anti-angiogenic Therapy."

On April 18, the Association hosted the Blue Hill Troupe musical group for a most successful fund-raiser. The group performed a variety of Gilbert and Sullivan operettas and joined concertgoers for a spectacular dinner in Blackford Hall following the performance.

Association members Jim and Carol Large hosted an enjoyable cocktail party at their home in Locust Valley in October in honor of the Association's major donors. Thanks to the warm evening weather and a beautiful backyard patio, much of the mingling and conversation between scientists and Laboratory supporters took place outdoors.

Everyone at the Laboratory was saddened to learn of the death of a cherished Association member and friend of the Laboratory, Mrs. Edna Davenport. Edna became involved with Cold Spring Harbor Laboratory in early 1970 when she and her husband, retired Pfizer Pharmaceutical executive John Davenport, donated generously to the Laboratory with the intention of helping initiate the tumor virus research program in James Laboratory. Their initial gift enabled the Laboratory to execute a much-needed expansion of James Lab, and after John passed away in 1988, Edna's continued generosity became a cornerstone of support for the Laboratory's young scientists through the CSHL Association. While we sense a terrible loss in Edna's passing, we are even more sympathetic to her son Peter and daughter Linda Spire and their families for their loss.

The Blue Hill Troupe musical group

DNA Learning Center

The DNA Learning Center experienced record growth in 1998, with income increasing by 59%, to $1,393,100. The greatest single source of growth in 1998 was funding from the Josiah Macy, Jr., Foundation to create "DNA from the Beginning," an online, animated primer on genetics. Targeted at the level of a bright teenager, the site uses a number of multimedia elements and tools—animation, video clips of scientists and historians, archival photographs, audio glossary, interactive quizzes, and powerful navigation tools—to let users master genetics at their own pace and according to their own learning style. The first installment of DNA from the Beginning was a resounding success, with more than 10,000 log-ins during its first eight weeks on the World Wide Web. Four additional releases are planned in 1999–2000.

Cold Spring Harbor Laboratory Press

In 1997, the productivity of CSHL Press was improved through management restructuring and investment in technology. These gains were consolidated in 1998 and resulted in improved financial performance.

The journals, *Genes & Development* (*G&D*), *Genome Research*, and *Learning & Memory*, all improved in several ways. Scientists submitted more and better manuscripts for publication and each journal published more pages than ever before. *G&D* led the way with a 30% increase in the number of submitted manuscripts. *G&D* continues to rank in the top ten of all journals in the life sciences, in terms of the number of citations reported by the Institute for Scientific Information. All three CSHL journals are published online as well as in print, and online users now have access to several improved services, including E-mail alerting services. The three journals either maintained or improved their subscription base, a significant achievement in a period of continued change in library purchasing habits and increased electronic distribution of information.

The CSHL Press published 14 new books in 1998. The most widely anticipated was *Using Antibodies*, a techniques manual by Laboratory alumni Ed Harlow (currently a Trustee) and David Lane. A complete update of their 1988 *Antibodies: A Laboratory Manual*, the new book, with its innovative design and improved binding, seems likely to attain the classic status of its predecessor. Two other former Laboratory staff also revised a highly successful book: Ray Gesteland and John Atkins, in collaboration with Nobel prize winner Tom Cech, edited a second edition of the influential monograph *The RNA World*. A collection of essays by Max Perutz, entitled *I Wish I'd Made You Angry Earlier*, attracted glowing reviews and brisk sales and is now being translated into several languages. Other strong titles included *At The Bench*, a quirky introduction to life in the laboratory by Kathy Barker, and the 1997 Symposium volume *Pattern Formation during Development*, which brought together excellent science from widely differing areas of investigation. These lead titles, and a high-quality backlist, increased book sales by 25% over the previous year.

The Laboratory's well-established publishing activities for professional scientists expanded in 1998 to incorporate an ambitious new program for creating undergraduate textbooks. Opportunities to reshape university teaching in a variety of fields have been identified, and, led by newly appointed Senior Editor Alex Gann, teams of talented, innovative authors have begun work on these projects. The acquisition of the Meier House in Lloyd Harbor, and its conversion for use as a writing center by textbook authors, has provided an extraordinary asset in building

this program, which has potentially far-reaching consequences for the future growth of the Press.

For several years, that growth has been hampered by space limitations and by the distribution of the staff at three sites: Urey Cottage and the Library on the main campus, and a warehouse in Plainview. The pending renovation of the Woodbury property should allow the Press to consolidate many of its activities while retaining a base of editorial activities at the Laboratory's main campus.

Major Gifts

Private support has been a cornerstone of the Laboratory's research program for many years, but it is particularly important for special projects like building construction and renovations or the creation of graduate school. Government grants for such initiatives, regardless of their worthiness, are scarce.

The initial campaign to endow the Watson School of Biological Sciences was quite successful. Contributions received in 1998 included $5,068,568 from a donor who wishes to remain anonymous, $885,000 from Mr. and Mrs. Leslie C. Quick, Jr., and $548,748 from Mr. and Mrs. David L. Luke III. The William Stamps Farish Foundation gave $350,000 and the Seraph Foundation gave $50,000. Additional gifts came in after the new year, and the public phase of the capital campaign will begin in April 1999.

The Nancy and Edwin Marks Building will house our new brain imaging program and allow for expansion of the Laboratory's strong neuroscience program, including creating space for more teaching labs. This project has also received substantial support. The Marks Family Foundation gave $1,261,531; the Booth Ferris Foundation gave $100,000; The Weezie Foundation donated $100,000; the Estate of Sophie Rubenfield gave $57,731; the Fairchild Martindale Foundation gave $50,000; and Mary D. Lindsay made a gift of $50,000. We have added previous gifts from Thomas Saunders and David Koch to this project.

In support of neuroscience research, the Lita Annenberg Hazen Foundation gave $200,000 to the Lita Annenberg Hazen Fund for Neurobiology, and Mr. and Mrs. William L. Matheson gave an additional $50,220 in support of their previous gifts that established the Matheson Fund for Neuroscience. The Seraph Foundation gave $27,000 for neuroscience research in Grisha Enikolopov's lab, as well as $23,000 for cancer research in Yuri Lazebnik's lab.

Support from breast cancer advocacy groups has continued to be quite helpful to our cancer research program. In 1998, Mike Wigler's lab received a $300,000 grant from The Lillian Goldman Charitable Trust through the Breast Cancer Research Foundation and our long-time supporter, 1 in 9: the Long Island Breast Cancer Action Coalition, donated $100,000 to the Michael Scott Barish Human Cancer Fund. Both donations, along with $25,000 from the Huntington Breast Cancer Action Coalition and $4,600 from the Long Island Foundation for the Elimination of Breast Cancer, will be applied to the development of the DNA microarray facility.

The renovation of the old Power House and Carpentry Shed, to make room for the Laboratory's growing Public Affairs, Development, and Human Resources departments, was subsidized in part by a $200,000 gift from the William and Maude Pritchard Charitable Trust and a $200,000 gift from the Estate of Vernon L. Merrill. The Development conference room will be named for Mrs. Merrill, and the building will be named in honor of former Chairman of the Board of Trustees, and now Honorary Trustee, David L. Luke III and his wife Fanny.

The Stone Foundation gave $60,000 in support of the Mary D. Lindsay Child Care Center; the Gladys Brooks Foundation donated $57,000 for the purchase of microfilm equipment for

the Library; and the Joseph G. Goldring Foundation continued support to Bruce Stillman's laboratory through a gift of $50,000 for two postdoctoral fellows. In addition, Alan and Edith Seligson once again gave $35,000 in support of one postdoctoral researcher, as they have since 1990. The Goldring support went to Kate Simpson and Bill Henry and the Seligson fellowship supported Howard Fearnhead in his final year as a postdoc.

President's Council

The President's Council was formed five years ago in an effort to bring together a small group of leaders from business, research, and biotechnology who are interested in science and in the work at Cold Spring Harbor Laboratory. Members of the President's Council contribute $25,000 or more to support research and educational programs at the Laboratory. The funding helps the Laboratory attract top young scientists fresh from their Ph.D. or M.D. studies. The fellowships allow promising young researchers to pursue their own high-level, independent research, rather than assisting in the laboratory of an established scientist.

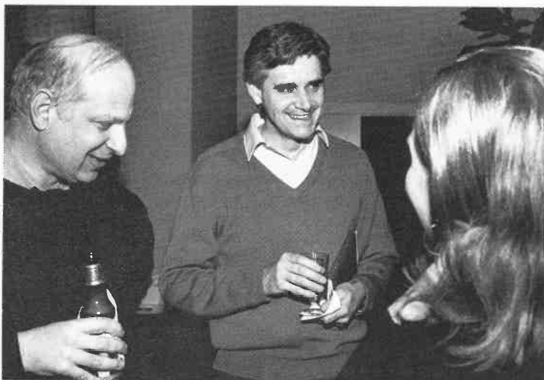
The 1998 meeting of the President's Council, held May 15–16, focused on the topic of human evolution and began with lunch on Friday at the President's House. The luncheon was followed by an afternoon lecture by CSHL scientist Karel Svoboda on imaging neuronal function in the intact brain. The keynote speaker, Dr. Roger Lewin, a member of the science and engineering technology development company AEA Technology in Oxfordshire, U.K., and a collaborator of renowned Kenyan anthropologist and conservationist, Richard Leakey, described what archaeology is able to tell us about evolution. The speakers on Saturday were Dr. Sean Carroll of the University of Wisconsin, Dr. Mark Stoneking of Pennsylvania State University and Dr. Michael Hammer from the University of Arizona.

The following are members of the 1998 President's Council:

Abraham Appel, Appel Consultants, Inc.
Peter Bloom, General Atlantic Partners, LLC
James Conneen, A. T. Hudson & Co.
Theodore N. Danforth, Oxford Bioscience
Michel David-Weill, Lazard Freres & Co.
Stefan Englehorn M.D.
Leo A. Guthart, ADEMCO
Charles E. Harris, Harris & Harris Group, Inc.
Walter B. Kissinger, WBK Associates
Thomas J. McGrath, Simpson Thacher & Bartlett
Donald A. Pels, Pelsco, Inc.
George B. Rathmann, ICOS Corporation
Hubert J. P. Schoemaker, Centocor, Inc.
James H. Simons, Renaissance Technologies Corporation
Sigi Ziering, Diagnostic Products Corporation

Gavin Borden Visiting Fellow Lecture

This annual event was initiated in 1995 to honor scientific textbook publisher Gavin Borden, who died in 1991 of salivary gland cancer, in an effort to carry on the mission that was so dear to him—the education of graduate students. Each year, the visiting Gavin Borden Fellow gives



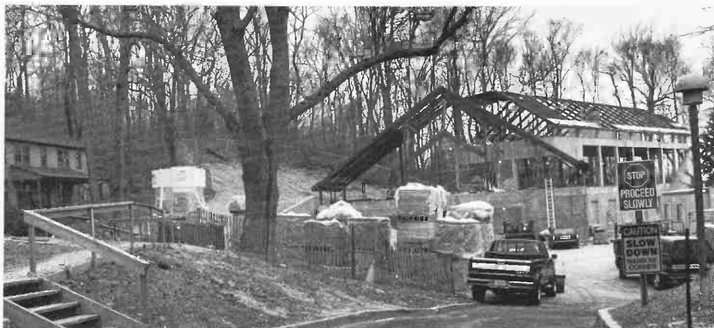
M.W. Kirschner, B. Stillman

a lecture to students and spends time dining and talking with them about science and careers in science.

The Gavin Borden Fellow in 1998 was Marc W. Kirschner, chair of the Department of Cell Biology at Harvard Medical School and a prominent contributor to science policy issues. His talk on March 23 was entitled "Proteolysis Control of the Mitotic Cycle."

Building Projects

Construction of the Nancy and Edwin Marks Building, on the hill to the north of James Laboratory, began in early 1998. It will contain a state-of-the-art brain imaging and research facility, together with a new teaching laboratory. The building is scheduled to be in operation by the fall of 1999.



Nancy and Edwin Marks Building site

Power House renovations

Renovation of the Laboratory's old Power House, built on the waterfront in 1913, and the adjacent former Carpentry Shed began in 1998. By the sping of 1999, the buildings were converted into office space for the Departments of Public Affairs, Development, and Human Resources. The new complex is called the David and Fanny Luke Building in honor of long-time Laboratory supporter and retired Chairman of the Board, David L. Luke III and his wife.

In November, members of the facilities staff removed the old guest cabins north of the Beckman Neuroscience Center to make room for the construction of the new Samuel Freeman Building. The new building will house facilities for computational neuroscience, an important component in the further expansion of neuroscience program. We were saddened to see the historic (albeit utilitarian) cabins come down, but it was not possible to preserve them for relocation due to their extensive deterioration.

Historic Guest Cabins

Undergraduate Research Program (URPs)

The 1998 summer Undergraduate Research Program, through which students live and work at the Laboratory for 10 weeks during the summer, consisted of 23 students (12 men and 11 women) from nine countries. They were chosen from among 459 applicants from 39 countries. The students receive room and board on Laboratory grounds as well as a stipend.

The objectives of the program are to provide students with a greater understanding of the principles of biology, to instill in them an awareness of major topics of investigation, to help them develop intellectual tools necessary in modern research, to expose them to the process of research, and to allow them to meet people involved in that research.

The URP program received support this year from the C. Bliss Memorial Fund, the Burroughs Wellcome Fund, the Jephson Educational Trust, the National Science Foundation, the Dorcas Cummings Fund, and the URP endowment (which includes proceeds from the Emanual Ax Fund, the Garfield Fund, the Glass Fund, the Libby Fund, the Olney Fellowship, the Shakespeare Fellowship, and the Von Stade Fellowship).

Partners for the Future (PFF)

Each year, the Laboratory selects several outstanding high school seniors to work on original research projects in a laboratory under the supervision of a scientist-mentor. The students are expected to spend a minimum of ten hours per week at the Lab, beginning in October, and to present scientific summaries to an audience of scientists, teachers, parents, and Laboratory administrators at the conclusion of the program in March. They gain valuable research experience and are paid a small stipend for their efforts.

The participants for the 1998–1999 school year were Francis Browne of Cold Spring Harbor, Cold Spring Harbor High School (mentor: Michael Hengartner); Mariza Daras of Manhasset Hills, Herricks High School (mentor: Michael Weinreich); Peter Hallock of Holtsville, Sachem High School North (mentor: Jean-Philippe Vielle Calzada); Rachael Neumann of Woodbury, Syosset High School (mentor: Grigori Enikolopov); Allison Scheff of Islip, Islip High School (mentor: Robert Filipkowski); Diane St. Fleur of Brentwood, Brentwood High School (mentor: Guy Birkenmeier).

Outreach

Outreach initiatives through the Department of Development include the Next Generation Initiative (NGI), a series of talks and tours designed to inspire an interest in basic research among individuals in their 30s and 40s. The Young President's Organization provides similar experiences for young leaders of industry and companies, and the Harbor Society is a small group of Laboratory supporters who have contributed to the Laboratory's planned giving program.

The Harbor Society

The Harbor Society gained six new members (or member couples) in 1998: Jim and Jan Eisenman, Lois Learned, Ed and Betty Palmer, Sam and Ann Parkinson, John and Joyce Phelan, John and Hope Reese, and one who wishes to remain anonymous. The Harbor Society

is a group of distinguished individuals, now numbering 44 (see report in the Financial Section) who have included the Laboratory in their estate planning. We are most grateful to these individuals and families for their generosity.

Public Education

The Laboratory continues to participate in Project WISE, a program to promote the entry of women into science and engineering. The program is run by SUNY Stony Brook and is designed to benefit high school students.

Once again, for the fourth year, the Laboratory hosted a group of Japanese exchange students and their teachers who were visiting Cold Spring Harbor High School in July. The Laboratory also continues to host the West Side School Science Nights, which are now open to all local elementary schools. Lectures this year included of Leemor Joshua-Tor talking about "Proteins in 3-D" in January; Michael Hengartner discussing "A Matter of Life and Death: How Cells Die, and Why That's A Good Thing" in March; and Jean Philippe Vielle-Calzada on "Fertilization Tales: Doing It With or Without Tails" in May.

The "Great Moments in DNA Science" lecture series is still a big hit with high school audiences. In April, Linda Van Aelst talked about "The Role of Cell Signaling in Cancer." In May, Jan Witkowski spoke on "Ian Had a Little Lamb: The Cloning of Dolly," and Karel Svoboda presented "Imaging Neurons in Action."

Breast Cancer Support

Our long-time supporter 1 in 9: The Long Island Breast Cancer Action Coalition donated $100,000 to the Laboratory for breast cancer research at its annual gala in October. The money was applied toward the new DNA microarray facility being developed by Michael Wigler. Also at the gala, 1 in 9 presented the Laboratory with an Olympus BX-40 microscope, which was donated by Dan Biandi, a vice president at Olympus America, through a breast cancer research fund-raiser held by Chameleon Hair Design in Port Jefferson.

The Laboratory also gained the support in 1998 of three other breast cancer activist groups: the Huntington Breast Cancer Action Coalition, the Breast Cancer Research Foundation, and the Long Island Foundation for the Elimination of Breast Cancer.

Huntington Breast Cancer Action Coalition founder and president Karen Joy Miller and vice president Marcy Usdan-Hyman made a $25,000 donation to Mike Wigler's lab at the Coalition's annual gala in October for use in the development of the DNA microarray technology. This was the group's first major research grant award and we are quite honored to have been the recipient.

Mike Wigler's breast cancer research program also received a $300,000 grant from The Lillian Goldman Charitable Trust through the Breast Cancer Research Foundation, a New York City-based organization led by president Evelyn H. Lauder. This major grant enabled the Wigler lab to purchase the expensive equipment necessary for the development of the DNA microarray technology. This technology is being used to search for new genes that are mutated in breast cancer.

The Long Island Foundation for the Elimination of Breast Cancer became a CSHL supporter in 1998 with gifts totaling $4,600. The Laboratory is pleased to have the support of this local grass-roots organization and looks forward to cultivating this and other new relationships with local activists who are so vital to raising funds and awareness for this important issue.

Special Events

Grace Auditorium was put to good use in 1998 not only for scientific conferences, but also for a host of cultural events. In March, Cablevision held the New York premier of the award-winning HBO mini-series "From the Earth to the Moon." Participants included astronaut Buzz Aldrin; actor Bryan Cranston, who played Aldrin in the HBO production (and is now famous for his role in "Saving Private Ryan"); Andrew Chaikin, author of the book on which the HBO production was based; Cablevision executives; and retired Grumman Aerospace engineers who designed and built the Apollo aircraft.

Also in March, Patrick Cunningham, Professor of Animal Genetics at Trinity College in Dublin, Ireland, gave a public lecture on horse genetics and the history of horse breeding. All of today's thoroughbred horses descend from a handful of stallions imported to England from North Africa and the Middle East back in the 1600s. In fact, about one third of the genes in current thoroughbreds can be traced to three top stallions of that century, and a full half of the genes in today's thoroughbred horses can be traced to ten seventeenth-century stallions. Dr. Cunningham, who was attending a Banbury Conference, has been studying genetics and animal breeding for more than 20 years. He initiated studies of thoroughbred breeding in the 1970s.

In May, Laboratory staff and visitors enjoyed a poetry reading entitled "the single secret," a phrase borrowed from an e.e. cummings poem. Performers Jane Lapotaire and Paul Jesson of England's Royal Shakespeare Company were in New York City for a series of performances of Shakespeare's "Henry VIII" at the Brooklyn Academy of Music. Their show for the Laboratory included an entertaining assortment of poetry by British and American writers including William Shakespeare, John Donne, Dorothy Parker, and e.e. cummings.

In October, during the meeting on Gametogenesis, several visiting scientists participated in a public forum about cloning. Moderator Dr. Anne McLaren, principal research fellow of the Institute of Cancer and Developmental Biology at the Wellcome CRC Institute, in Cambridge, England, led panel members Dr. Brigid Hogan, investigator at Howard Hughes Medical Institute and professor of Cell Biology at Vanderbilt University Medical School in Nashville, Tennessee;

News 12's Doug Geed interviewing astronaut Buzz Aldrin

Paul Jesson and Jane Lapotaire, in the Single Secret

Dr. Ryuzo Yanagimachi, professor in the Department of Anatomy and Reproductive Biology at the University of Hawaii Medical School (who had recently reported the production of 50 cloned mice); and Dr. Anthony Mahowald, professor and chairman of the Department of Molecular Genetics and Cell Biology and chairman of the Committee on Developmental Biology at the University of Chicago in Illinois.

Then, in November, Alan Kay, fellow and vice president of Research and Development at Walt Disney Company, discussed "Origins of the Personal Computer—and Beyond." Dr. Kay's "Dynabook" is considered to be the forerunner of the personal computer. He also invented the forerunner of today's Macintosh and Windows interfaces, and led one of several groups that together developed modern workstations, the Smalltalk programming language, and the EtherNet—the technology used today by the Internet. He was a member of the University of Utah Advanced Research Project Agency (ARPA), a federal Department of Defense research team that developed three-dimensional graphics, and he participated in the development of ARPANet, which became the Internet in the 1970s. Before joining the Walt Disney Company, Dr. Kay also worked for Xerox Palo Alto Research Center, Atari, and Apple.

Mark Ptashne and Irina Muresanu

Concerts in Grace this year included the following: May 9, Mary Phillips, mezzo soprano and Ted Taylor, pianist; May 23, Alex Velinzon, violinist and Inessa Zaretsky, pianist; May 30, Dmitri Berlinsky, violinist, and Elena Baksht, pianist; June 8, Irina Muresanu and Mark Ptashne, violinists; August 22, David

Korevaar, pianist; August 29, the Harold Betters Jazz Quartet; September 5, Jennifer Fratuschi, violinist, and Benjamin Loeb, pianist; September 12, Anton Barachovsky, violinist, and Sonya Ovrutsky, pianist; and September 26, Bion Tsang, cellist, and Benjamin Loeb, pianist.

The Banbury Center also served as a forum for public events. In February, Lloyd Harbor resident Dick Opsahl talked to community members about "Running on Everest: A Personal Account of a Marathon Starting at Everest Base Camp," and in November the Laboratory offered a Tax & Estate Planning Seminar at Banbury.

Long-term Service

On July 9, several employees celebrated long-term anniversaries with the Laboratory at a poolside dinner at Robertson House on the grounds of the Banbury Center. Jim and Liz Watson celebrated the 30 years that have passed since Jim became the Laboratory's director in 1968. I presented Jim with a beautiful, abstract lead crystal sculpture engraved with the image of Ballybung and "With Deep Appreciation, 30 Years of Dedicated Service, 1998." In addition, he received a three-tiered, custom-made base for the sculpture created by Long Island sculptor John Roper. Liz was presented with a framed print of the collage of dedication booklet covers that appeared in the tribute to Jack Richards that Liz edited in 1997. The calligraphy inscription read "30 Years of Dedication, Our Sincerest Gratitude, 1998." Liz's advice about the adaptive re-use and historic preservation of Laboratory buildings, and her 1991 book, *Houses for Science*, are enduring and most valuable contributions to Cold Spring Harbor Laboratory.

(*Back row*) W. Herr, C. Schneider, B. Stillman
(*2nd row*) M. Simkins, J. Wiggins, J. Watson, M. Wigler, P. Wendel
(*3rd row*) D. Jusino, M. Falkowski, J. Ebert, C. McEvoy, M. McBride
(*Front row*) P. Maroney, B. Toliver, L. Hyman

Laura Hyman joined the Library 25 years ago as a part-time library assistant when the Library staff consisted only of Susan Cooper and her volunteers. Since that time, Laura has become business manager for three departments—Development, Public Affairs, and the Library—as well as the Laboratory's art curator. Laura has selected, obtained, framed, arranged and supervised the hanging of most artwork in the Laboratory's many buildings.

Senior scientist and cancer researcher Michael Wigler celebrated his 20-year anniversary with the Laboratory. Mike came to Cold Spring Harbor in 1978, and three years later he codiscovered the first human oncogene, *ras*, now the object of much study at CSHL and elsewhere. Laboratory technician Jeanne Wiggins, Banbury administrative assistant Beatrice Toliver, Banbury grounds foreman Christopher McEvoy, Facilities' draftsperson/estimator Charles Schneider, and payroll administrator Patricia Maroney also celebrated 20-year anniversaries.

Assistant Director and Dean of the Watson School of Biological Sciences Winship Herr celebrated his 15-year anniversary with the Laboratory. Winship came to Cold Spring Harbor Laboratory in 1983 as a postdoc in Joe Sambrook's lab. The following people also celebrated 15-year anniversaries: CSHL Press project coordinator Joan Ebert, laboratory technician Margaret Falkowski, shipping and receiving foreman Daniel Jusino, meetings registrar Michaela McBride, personnel manager Merilyn Simkins, and laboratory technician Patricia Wendel. Connie Hallaran, who has been running the Laboratory's bookstore since its inception, also celebrated a 15-year anniversary. She now works under the auspices of Barnes & Noble.

Administrative Staff Changes

In April, W. Dillaway Ayres joined the Laboratory as associate administrative director. Dill earned a B.A. from Princeton University, where he majored in English literature, and an M.B.A. in finance from Columbia University Graduate School of Business Administration. He gained extensive experience in corporate planning and finance at several companies, including Capital Cities/ABC Inc. and American Express Company, and was an investment banker at Veronis, Suhler & Associates Inc., which specializes in television and radio broadcasting. Most recently, he was cofounder, executive vice president, and chief financial officer of Business and Trade Network (BATNET), which provides Internet services to national and international associations.

In July, after working for several months as a consultant, Deborah Barnes became director of Public Affairs. Deborah earned a Ph.D. in biology from Georgetown University and did postdoctoral research at the Children's Hospital at Harvard Medical School. She taught high school biology for five years, was a news writer for the journal *Science*, taught science writing for the Johns Hopkins University Graduate Program, and for seven years was editor of *The Journal of NIH Research*. Deborah has initiated a public lecture series about cancer and the production of a video about CSHL tentatively entitled "A Year in the Life of the Lab."

Late in 1998, soon after the Watson School of Biological Sciences became a formal entity, Lilian Gann was recruited as Assistant Dean. Lilian received her Ph.D. from the University of St. Andrews in Scotland for her research on adenovirus transcriptional regulation. After postdoctoral studies at Memorial Sloan Kettering Cancer Center, Harvard Medical School, and the Imperial Cancer Research Fund (ICRF) in London, she became administration manager at the ICRF and earned an M.B.A. In her role as administration manager, she helped direct the graduate student and postdoctoral programs. Most recently, Lilian was director of cancer support services at CancerBACUP, a U.K. charity that helps people with cancer. Lilian, too, worked as a consultant before assuming her full-time position in early March 1999.

Changes in Scientific Staff

Departures

Hong Ma left to become an associate professor at Pennsylvania State University in College Park. Akila Mayeda accepted an appointment as assistant professor at the University of Miami School of Medicine, in the Department of Biochemistry and Molecular Biology. Elly Nedivi left our neuroscience program to accept a position as assistant professor in Brain and Cognitive Sciences at the Massachusetts Institute of Technology, in Cambridge. Alcino Silva is now an associate professor in the Department of Neurobiology, Psychiatry, and Psychology at University of California, Los Angeles, and Erich Grotewold went on to become an assistant professor in the Department of Plant Biology at Ohio State University.

New Arrivals

Shivinder "Shiv" Grewal, who studies chromosome dynamics and epigenetic control of gene expression, was recruited to CSHL as an assistant investigator. Shiv comes from the National Cancer Institute's Frederick Cancer Research and Development Center. Lincoln Stein was recruited as an assistant investigator in bioinformatics. Lincoln specializes in database integration and management as it applies to biological data accessible via the Internet. He was formerly the director of bioinformatics at the Whitehead Institute for Biomedical Research/MIT Center for Genome Research.

We also have two new research scholars this year. Both were students at Eton College in Windsor, U.K., and are now living at Ballybung with the Watson family while they get experience working in the laboratory. Robin Holden is studying transcription in Winship Herr's lab, and Indraneil "Neil" Mahapatra is working in the lab of Holly Cline. Neil is cloning a family of genes encoding proteins that are involved in axon pathfinding in the developing brain.

CSHL Fellow

Marja Timmermans joined the Laboratory in June as a CSHL Fellow. The CSHL Fellow program provides an outstanding opportunity for talented scientists who have recently received their Ph.D. or M.D. degrees to establish strong independent research programs. Each CSHL Fellow has a lab and technician, as well as access to all of the resources of Cold Spring Harbor Laboratory.

Marja earned her Ph.D. in the lab of Jo Messing, at the Waksman Institute at Rutgers University in Piscataway, New Jersey. Marja is a maize geneticist who has focused on discovering and understanding the genes involved in maize leaf development. She has studied two such genes, *leafbladeless 1* and *rough sheath 2*, which are required for leaves to grow into their proper shapes.

Promotions

Hollis Cline was promoted to investigator. Holly came to the Laboratory in 1994 as part of our developing neuroscience initiative, and since that time, her work in neuronal development has been outstanding. Investigator is the highest research position at the Laboratory, comparable to

full professor status at an academic institution. Rather than tenure, though, the investigator appointment provides "Rolling 5" status—the contract is renewed each year for five years.

Michael Hengartner, Tatsuya Hirano, Yuri Lazebnik, Scott Lowe, and Linda Van Aelst were each promoted to associate investigator.

Masaaki Hamaguchi, of Mike Wigler's lab, was promoted to assistant investigator. Masaaki, who was formerly a gastrointestinal surgeon in Japan, came to do postdoctoral research in the Wigler lab after deciding to make the change from clinical medicine to basic research.

Visiting Scientists

Seven visiting scientists joined us this year: Yoshitaka Azumi of Nagoya University in Japan spent time in Hong Ma's lab; Simona Ceccarelli of the University of Milan was working in Peter Nestler's lab; Jyoti Raychaudhura of Lincoln Hospital in the Bronx and Winthrop University Hospital in Mineola, New York, joined Yuri Lazebnik's lab; Shern Chew of the University of Cambridge worked in Adrian Krainer's lab; Jyotsna Dhawan of Boston University spent time in David Helfman's lab; Costaicis Frangou of the University of Portsmouth in the United Kingdom worked in Greg Hannon's lab; and Benjamin Horwitz of the Technion Israel Institute of Technology spent three weeks in Hong Ma's lab as part of an ongoing collaboration.

Several other visiting scientists also wrapped up their stays at the Laboratory. Paula Enrietto, who was working with Tom Marr, returned to a staff scientist position with Tom's company, Genomica, in Boulder, Colorado. Eli Hatchwell completed his studies in Mike Wigler's lab and returned to his clinical work at Southampton University in the United Kingdom; and Mirjana Maletic-Savatic left Roberto Malinow's lab to finish her residency at SUNY Stony Brook School of Medicine's Department of Neurology.

Postdoctoral Departures

The Laboratory has long been a springboard from which many scientists launch their research and academic careers. In 1998, the tradition continued with the following postdoctoral researchers moving on to other positions.

Concurrent with Alcino Silva's move to UCLA, many of his lab members accepted new positions as well. Nikolai Fedorov is now an assistant researcher with Alcino in the Department of Neurobiology at UCLA; Masuo Ohno is a visiting assistant professor there. Three of Alcino's CSHL postdocs have relocated to parallel positions at UCLA as well: Ype Elgersma, Paul Frankland, and Jeffrey Kogan. Karl Peter Giese, of Alcino's lab, accepted a position as lecturer at University College in London.

Three postdoctoral researchers from Hong Ma's lab moved with Hong to the Pennsylvania State University Biotech Institute. Hiroyasu Onaka, Yixing Wang, and Ming Yang will all continue their postdoctoral research there.

From Nick Tonks' lab, Anton Bennett accepted a position as assistant professor at Yale University School of Medicine; Michael Gutch became a manager at Electronic Publishing Company in New York City; and Andrew Garton is now a senior scientist at OSI Pharmaceuticals in Uniondale, New York.

From the Stillman lab, Chun Liang accepted a position as assistant professor at the Hong Kong University of Science and Technology, and Alain Verreault went on to become a lab head at the Imperial Cancer Research Fund Clare Hall Laboratories in Potters Bar, United Kingdom.

Jianzhong Ding and Yu Liu both moved on from Rui Ming Xu's lab—Jianzhong to become an engineer with Aeroflex Laboratory Inc., in Setauket, New York, and Yu as a postdoctoral researcher at the Hong Kong University of Science and Technology.

Andrea Doseff from Yuri Lazebnik's lab accepted a position as research scientist at Ohio State University in Columbus; Jeffrey Dickinson from Tim Tully's lab went on to become a programmer at Zeneca Pharmaceuticals in Wilmington, Delaware; Cameron Gray of Karel Svoboda's lab went to Philadelphia to pursue other interests; and Fei Guo from Erich Grotewold's lab is continuing his postdoctoral research in Erich's new lab at Ohio State University's Department of Plant Biology.

Ruiping Liu moved from Peter Nestler's lab to a position as research scientist with 3-D Pharmaceuticals, Inc., in Exton, Pennsylvania; Yi Liu went from Winship Herr's lab to the position of Research assistant professor at the University of Southern California School of Medicine; Martin Lock moved from Jacek Skowronski's lab to continue his postdoctoral studies at the University of Pennsylvania, Bensalem; and Tom Misteli moved from David Spector's lab to the National Cancer Institute to become a principal investigator there.

Indrani Rajan from Holly Cline's lab is continuing her postdoctoral research in the Department of Pathology at the University of Washington in Seattle; Brandt Schneider moved from Bruce Futcher's lab to become an assistant professor at Texas Tech Medical School in Lubbock; Yung-Chih (Judy) Wang went from David Helfman's lab to the Ferring Research Institute, Inc., in California as a research scientist; Bill Henry finished up in Nouria Hernandez's lab and moved on to a position as assistant professor at Michigan State University; and Mark Curtis completed his Ph.D. in Rob Martienssen's lab, and, after a brief period as a postdoctoral fellow at CSHL, moved to the United Kingdom to pursue research in private industry.

Graduate Students

Eleven graduate students completed their Ph.D. degrees at the Laboratory this year. From Alcino Silva's lab, Ofelia Carvalho is now a staff research scientist with Alcino at UCLA Department of Neurobiology, and Pin (Adele) Chen is continuing her graduate studies in the Silva lab at UCLA (although she is expected to receive her degree from SUNY Stony Brook).

From Nouria Hernandez's lab, Ethan Ford went on to do postdoctoral research in the Department of Biochemistry and Molecular Biology at Oregon Health Sciences University in Lagunitas, California, and Debra Morrison went on to do postdoctoral research at Mount Sinai Ruttenberg Cancer Center in New York City. Craig Hinkley completed his studies and went to embark on postdoctoral studies in Bill Henry's lab at Michigan State.

Degui (Charlie) Chen from David Helfman's lab went on to do postdoctoral research at UCLA; Richard Freiman went from Winship Herr's lab to do postdoctoral research with former staff scientist Bob Tjian of the University of California at Berkeley; Anthony (John) Iafrate completed his Ph.D. in Jacek Skowronski's lab and went on to complete clinical training for his M.D. at SUNY Stony Brook; and Kenneth LaMontagne went from Nick Tonks' lab to do postdoctoral research with Judah Folkman at the Children's Hospital in Boston.

Peter Rubinelli finished up in Hong Ma's lab and went on to do postdoctoral research at Ohio State University in the Department of Biochemistry and Plant Biology; Hua Tu went from Mike Wigler's lab to a postdoctoral research position at Tularik, Inc., in San Francisco; and Qiang Wu of Adrian Krainer's lab left to do postdoctoral research at Harvard University's Department of Molecular and Cell Biology.

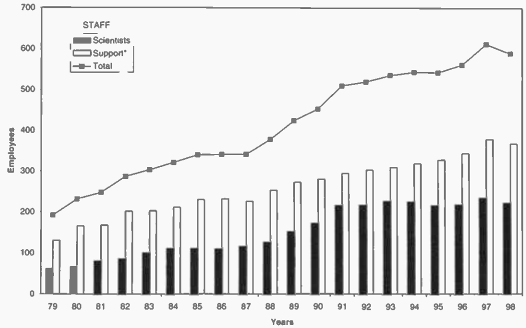
Twenty Years at CSHL

At the time of writing, it has been 20 years since I came to Cold Spring Harbor Laboratory from Australia, initially as a postdoctoral fellow for two years. Much has changed during those years, yet the Laboratory remains a vibrant and exciting place to work. I am pleased to have been welcomed into this marvelous community with such warmth.

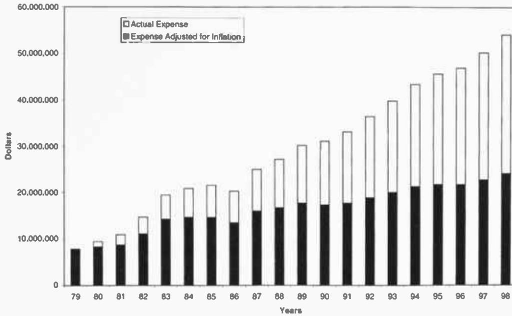
This year happens to be one of the busiest on record, and all the staff at the Laboratory must be congratulated for their efficiency and dedication. Because of the combined effort of all our staff, CSHL remains one of the most exciting addresses in science, making my life interesting every day.

On a personal note, I was most happy to learn at the end of the year that in the 1999 Australia Day honors list (January 26th), I was named as having received the Order of Australia (AO). This high national honor from my homeland is particularly pleasing as it recognizes the contributions to science and humanity at large by an Australian scientist who happens to be working in the United States, emphasizing the international nature of modern biomedical research.

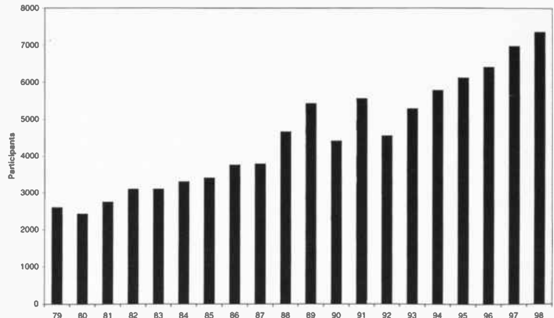
May 1999 **Bruce Stillman**



OPERATING EXPENSE



MEETINGS & COURSE PARTICIPANTS



ADMINISTRATION

With much anticipation, the Laboratory in 1998 advanced a broad series of initiatives in education and research that will shape our institution in the 21st century.

Foremost among these is the Watson School of Biological Sciences, which received approval in October from the New York State Board of Regents and by year end was recruiting its first class of Ph.D. candidates. We also established new courses at the postgraduate level and continued to expand the program of scientific meetings held on the main campus and at Banbury Center. The DNA Learning Center introduced a remarkable new genetics Internet site, DNA from the Beginning. Designed initially for high school students, the site will be further developed for the general public. The Cold Spring Harbor Press launched a new venture in textbook publishing at the college freshman level.

In research, the Laboratory began programs in neural imaging, bioinformatics, gene sequencing and function, and DNA microarray technology. All are designed to augment our traditional focus on cancer, neuroscience, and plant research.

Construction of new buildings for imaging and bioinformatics began on the main campus and will be completed this summer. A major renovation of the old powerhouse and carpentry shop is under way. When completed, it will provide office space for our expanding Human Resources, Development, and Public Affairs Departments. We purchased an historic Cold Spring Harbor residence across the harbor and are renovating it for use by our first class of graduate students, who will arrive in the fall. In Cold Spring Harbor Village, the DNA Learning Center completed plans for a badly needed expansion of its laboratory and computer teaching space.

A few minutes' drive to the south in Woodbury, the Lab purchased an 11-acre site and a 60,000-square-foot building from the American Institute of Physics. It will be renovated as a new home for the Cold Spring Harbor Laboratory Press and as a high-technology center for research on gene sequencing and function and DNA microarray technology. Space will also be available there for behavior studies and housing of new mouse lines.

During 1998, New York State approved $15 million in funding for a new biotechnology park, which will be located on State University of New York (SUNY) land in Farmingdale, just south of Woodbury. This will be the home for young biotechnology companies that are developing commercial applications for new technology developed at the Laboratory and other institutions. Because of the lack of appropriate facilities on Long Island, such companies have located in states such as Massachusetts, South Carolina, California, and Washington, which have aggressively encouraged biotechnology clusters near academic institutions. Now such companies will have the opportunity to locate in close proximity to the center of academic excellence represented by Cold Spring Harbor Laboratory, SUNY Stony Brook, and Brookhaven National Laboratory.

The park will be managed by a new nonprofit corporation, Broad Hollow Bioscience Park, Inc. A majority of the corporation's directors will be appointed by CSHL, the remainder, by SUNY Farmingdale. John Cleary, who in 1997 completed terms as president of the CSHL Association and as a trustee of the Laboratory, is Chairman of the Board of the new corporation. Construction will begin this spring and should be completed in 2000.

Strong finances are an important foundation from which to launch new initiatives, and,

therefore, it is pleasing to report that Cold Spring Harbor Laboratory had its best financial year ever in 1998. Revenues, at $53,639,000, were 10% larger than in 1997, while expenses increased by 8%, to $48,970,000. There was a surplus of $476,000 after depreciation of $3,443,000 and establishment of a reserve of $750,000 for the coming start-up costs of our new imaging and bioinformatics programs. These results are much better than had been anticipated in our annual budget. All major areas of operations—the main Lab, the CSHL Press, Banbury Center, and the DNA Learning Center—achieved break-even or better results, thus reaching a key goal established at the beginning of the year. There was a positive cash flow from operations of $4,669,700, which, as in the past, will be used to maintain and modernize our capital plant, purchase scientific equipment, and support new programs.

Our strong financial position was primarily due to our scientists' success in obtaining federal grants; the strength of our meetings, courses, and other educational programs; higher royalty and investment income; improved operations at the CSHL Press; and very careful cost control by administrative departments. The strong financial status of the Laboratory was recognized at year-end with a high A+1 rating from Standard & Poor's, which enabled us in early 1999 to issue $42.2 million of tax-exempt Civic Facility Refunding and Improvement Bonds through the Nassau County Industrial Development Agency. Underwritten by J.P. Morgan Securities Inc., the bonds provide $15.2 million for the new Woodbury High Technology Center and $27 million to refinance outstanding debt. The A+1 rating permits the new issue to be backed by a liquidity facility from Morgan Guaranty Bank at half the cost of the previously needed letter of credit.

Despite the great volatility experienced in financial markets during 1998, the Laboratory's endowment again increased substantially as a result of good market return and new additions. The endowment consists of the Robertson Research Fund and the Cold Spring Harbor Fund; the latter now includes the funds being raised for our new graduate school. At year-end, the market value of the endowment was $167,044,000, a 12-month increase of nearly $23 million, or about 16%. The funds, which continue to be invested in a balanced mix of equities, fixed-income and short-term instruments, are managed by a team of investment professionals—Essex Investment Management Company, LLC; Miller Anderson & Sherrerd (now a subsidiary of Morgan Stanley Dean Witter); U.S. Trust Company; and the Vanguard PRIMECAP Fund. In a year in which it was extremely difficult for managers to exceed benchmark indices, Vanguard and Essex were able to do so—in the case of Essex, by a very handsome margin. Over many years, the Laboratory's conservative policy, under which it draws 4% per annum from the endowment, has played an important role in the remarkable growth of the funds. The 4% drawdown is based on an average of the market value of the endowment at the end of each of the past three years. In consideration of the extraordinary strength of recent equity markets, the drawdown was held even below the 4% target in 1998 and 1999.

In 1992, the Board of Trustees established the Science Fund as the component of the endowment into which we place all equity positions and a portion of the royalties we receive for intellectual property licensed to new companies founded in whole or in part on the basis of CSHL technology. The Science Fund is intended for the future support of science, and no drawdown is currently being taken in order to encourage its growth. At year-end, the Science Fund was valued at $7,456,000, with a number of its equity positions very conservatively stated. In mid-2000, an important patent will expire, royalties from which have been providing significant funding for Lab research. The Science Fund will be helpful in bridging any temporary period while new sources of funding develop.

During 1998, our administrative departments successfully managed an extraordinary array of new projects with skill and good humor, despite tight budgets, staff limitations, and a shortage of working space.

John Maroney, director of Technology Transfer, and his department facilitate the transfer of cutting-edge basic research at the Laboratory to the commercial sector. Last year, two new companies, Genetica and Orion, were formed to pursue gene discovery in the fields of cancer and plant research. The Lab has equity participations in both companies. The Technology Transfer Department has also been instrumental—with New York State and SUNY Farmingdale—in planning and organizing the Broad Hollow Bioscience Park until a full-time manager can be recruited. In the meantime, preliminary discussions have been conducted with prospective tenants who have already spoken for more than half the initial space to be available.

The Facilities Department, led by Art Brings, manages each year to meet the heavy demands of schedules and budgets for construction, renovations, and maintenance while keeping the Lab productive and ensuring that it is also a showcase of style and natural beauty. Our grounds, under the care of Dan Miller, have never looked better. Grants Management, Human Resources, and Purchasing, managed by Susan Schultz, Cheryl Sinclair, and Phil Lembo, respectively, can be counted on to cope well with constantly increasing demands on their departments. Roberta Salant, administrative assistant to John Maroney and me, is of great help to both of us and meets the often-complex needs of our Board of Trustees and its committees with efficiency and grace.

No area of the Laboratory has been growing faster than the need for information services. Its requirements permeate everything we do in science, education, and administration. Virtually all the buildings on our main campus and our six remote locations are now part of the Lab-wide computer network. Gerry Latter, director of Information Services, and his staff deserve great credit for bringing the Lab to the cutting edge of data management.

Very important to the smooth functioning of the Laboratory is our financial infrastructure, which is headed by Controller Bill Keen and Assistant Controller Lari Russo. "Best-ever" financial years are the result of the efforts of many people, but they also are very dependent on good planning and budget control. We can count on Bill and Lari to provide such services. Equally important is their ability to successfully take on and complete time-consuming major projects such as the Lab's recent $42-million bond issue.

A priority of the past few years has been to add a new depth of professionalism to administrative areas that will be particularly important to the Laboratory in the years ahead. We have made substantial progress in this regard.

The Lab's Development Department is currently challenged with raising some $67 million to help fund our new initiatives and to substantially increase the level of annual support for research. Rick Cosnotti, our chief development officer for 18 months, is making remarkable progress on this task. More than half the $32 million endowment planned for the Graduate School has been raised, and in 1998, both of the Laboratory's annual funds—the Association and the DNA Learning Center Corporate Advisory Board—established new records.

Deborah Barnes, our director of Public Affairs for nearly a year, is producing a new video encompassing a year in the life of the Lab. It will be an effective tool for development and for recruiting graduate students. In cooperation with *Newsday*, Deborah has introduced a new public lecture series on cancer. The first lecture, given in March by Richard Klausner, director of the National Cancer Institute, was a cautiously optimistic explanation of the current status of cancer research. The Laboratory's scientists, and Dr. Watson in particular, have been

featured broadly in the national media. Deborah and chief science writer Wendy Goldstein are making good progress on their mission to make Cold Spring Harbor Laboratory and its science more visible nationally and internationally.

At the close of 1998, Margaret Henderson, director of the Library, left the Laboratory due to her husband's new job responsibilities and two growing children at home. We miss her and hope she will visit often. Margaret's replacement, Ludmila Tsytlenok-Pollock (Mila), joined us in early April. Mila has a master's degree in library science, 19 years of experience in the field, and seemingly boundless energy. Before coming to the United States in 1993, she was head of the Reference Department of the State Central Scientific Medical Library in Moscow, Russia. Since 1993, she had been director of the Medical Library at South Nassau Community Hospital on Long Island. Now she will be dealing with the space limitations of the Lab's 94-year-old Carnegie Library, keeping abreast of the changes brought about by electronic publishing, and meeting the needs of our new graduate students. She will plan the organization and preservation of the personal archives of Dr. Watson and the voluminous records of the 110-year existence of the Laboratory, which encompass much of the history of molecular biology and genetics.

W. Dillaway Ayres will soon complete his first year as associate administrative director. Dill has been deeply involved with me in the planning of our new initiatives and has been of much help with many of the Lab's day-to-day concerns. Working closely with Gerry Latter and all the department heads, he has been instrumental in guiding a major capital project that entails the purchase and installation of new, Lab-wide business-management software and accompanying hardware. The new system, being purchased from Lawson Software, will change most of the accounting, reporting, approval, and operational methodology at the Lab. It should greatly improve our productivity and be a solid foundation for future operations. Dill is also chairman of our Year 2000 Committee and is well along in ensuring that the Laboratory will not be seriously affected by any technical problems associated with the arrival of the new century.

As noted elsewhere in this report, the annual meeting of the Board of Trustees this past November marked the retirement of David L. Luke III as chairman of the Board and John R. Reese as treasurer. Each had completed the maximum number of years in office permitted by our by-laws. Their guidance and leadership in matters of finance and many other areas of Laboratory activity have been invaluable. Fortunately, in their respective successors, William R. Miller and Lola N. Grace, we have two individuals in whom we have a similar level of confidence.

Looking ahead to the fast-approaching millennium, we are reminded that it is challenges and opportunities such as those described in this report that make Cold Spring Harbor Laboratory such an exciting place for all who come in contact with it—our employees, supporters, and the world of science. The future looks every bit as promising as the past.

May 1999

G. Morgan Browne
Administrative Director

RESEARCH

Row 1: A. Poleksic, A. Neuwald; B. Hill, T. Swigut, M. Greenberg, J. Skowronski, N. Shohdy; L. Molz

Row 2: D. Jackson, Z. Yuan (*top*); S.-Y. Kim, W. Tansey (*bottom*); J. Polyakova, A. Samuelson, S. Lowe; K. Svoboda; M. Myers

Row 3: A. Schmitz; D. Spector, P. Mintz; E.-E. Govek, M. Marin, M.-M. Zhao, M. McDonough, L. Van Aelst, B. Bottner, A. Schmitz

Row 4: C. Chen, G. Hannon; L. Stein

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, SV40, herpes simplex virus, and human and simian immunodeficiency viruses. A reading of the reports in this section reveals a robust research program.

- Arne Stenlund and Bruce Stillman study DNA replication of papillomavirus and SV40, respectively. The Stillman laboratory complements studies of SV40 DNA replication with studies of cellular DNA replication in human and yeast cells.
- Winship Herr and Adrian Krainer study the control of gene expression, particularly the control of gene transcription and pre-mRNA splicing.
- William Tansey, new to the section in 1997, has added to this section a new dimension in the study of gene expression control. His laboratory is studying how protein stability is regulated, 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.

James Laboratory Entrance

TRANSCRIPTIONAL REGULATION

W. Herr D. Aufiero Y. Liu T. Tubon
R. Babb V. Meschan K. Wu
C. Hinkley J. Reader J. Wysocka
R. Holden P. Reilly X. Zhao
S. Lee

We are interested in the mechanisms of transcriptional control 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, 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 is focused 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 and Oct-1, and how do they influence HSV infection?

Enhancer Function

C. Hinkley, T. Tubon, X. Zhao

Our current studies on how transcriptional regulators activate the basal transcriptional machinery focus on two areas: (1) the response of the basal transcriptional machinery to activators, and (2) how transcriptional activation domains discriminate among basal transcriptional machineries bound to different core promoter elements. In the first line of investigation, we continue to study the function of basal transcription factors in vivo through the use of altered-specificity interactions among these factors, which allows us to circumvent the endogenous transcriptional machinery and thus study the activity of mutant basal factors in vivo. In the second line of investigation, we study transcription in vitro to dissect how transcriptional activation domains can distinguish among basal factors bound to different core promoter elements.

For the latter studies, we use two types of promoters: (1) promoters that direct the synthesis of mRNAs by RNA polymerase II and contain a TATA box core promoter element and (2) promoters that direct the synthesis of small nuclear RNAs (snRNAs) by either RNA polymerase II as in the case of the U2 snRNA or RNA polymerase III as in the case of the U6 snRNA. snRNAs are involved in RNA processing and their corresponding promoters contain a dedicated core promoter element called the PSE. The TATA box of the mRNA-type promoters binds the basal transcription factor complex TFIID, whereas the PSE element of snRNA gene promoters binds the basal transcription factor complex $SNAP_c$ studied by the Hernandez laboratory. We study how transcriptional activation domains discriminate between the mRNA- and snRNA-type promoters.

Some years ago, Dr. Carol Greider, formerly of Cold Spring Harbor Laboratory, and her collaborators isolated a novel "small-nuclear-RNA" gene that is not involved in RNA processing, but instead is involved in chromosome telomere maintenance—this is the RNA component of telomerase. Together with the Greider laboratory and Dr. Villeponteau and colleagues of Geron Corporation, we have characterized the structure of the promoter for this gene to determine whether it has an "mRNA-" or "snRNA-" type structure.

Owing to the mechanism by which DNA is replicated, the ends of chromosomes would become progressively shorter during successive rounds of replication if they were not actively maintained. One way

chromosomal ends are maintained is by de novo addition of short G-rich repeats to their 3′ ends by telomerase. Telomerase contains a single RNA molecule and multiple protein components, which are essential for activity. In organisms as diverse as ciliates, yeast, and vertebrates, the telomerase RNA sequence contains a "template" region complementary to the sequence of the telomeric repeats. It is responsible for both aligning the telomere end and serving as a template for addition of the telomeric repeats. In all organisms analyzed prior to our studies, this template region was embedded in the telomerase RNA sequence.

In ciliates, the telomerase RNA gene is transcribed by RNA polymerase III and contains a promoter reminiscent of U6 snRNA genes. To characterize vertebrate telomerase RNA gene transcription, we mapped the mouse telomerase RNA 5′ terminus and determined the sequence of the human and mouse telomerase RNA gene promoter sequences flanking the telomerase RNA transcriptional start site. The human and mouse telomerase RNA gene sequences are related, but, in contrast to the ciliate genes, they reveal sequence elements typical of RNA polymerase II mRNA-type promoters—they contain TATA and CCAAT box sequences—and not a PSE element typical of the promoters of genes encoding the snRNAs involved in RNA processing.

Further to our surprise, although humans and mice are closely related, our studies showed that the position of the transcriptional start site relative to the template-encoding sequence is very different in humans and mice: Whereas the human transcriptional start site lies 45 bp upstream of template-encoding sequence, the mouse transcriptional start site lies only 2 bp upstream of the template-encoding sequence. Thus, the mouse telomerase RNA is unusual among all telomerase RNAs analyzed in that the template sequence is located very near the 5′ end of the RNA.

These results suggest that the mammalian telomerase RNA sequences 5′ of the template sequence are not essential for telomerase function. They may, however, be important to modulate the function of telomerase. In vitro, human telomerase is more processive than mouse telomerase. Perhaps, the sequences 5′ of the template sequence in the human telomerase RNA help stabilize the correct folding of the telomerase RNA or aid in interactions either with other telomerase components or with the chromosome substrate. Such interactions may afford greater processivity to the human enzyme.

Viral *Trans*-activation

C. Huang, R. Babb, L.K. Wu

We continue to study the structure and function of VP16 and members of the VP16-induced complex. In collaboration with Drs. Y. Liu, W. Gong, and X. Cheng, formerly of Cold Spring Harbor Laboratory, we have determined the three-dimensional crystal structure of the core region of VP16 that is sufficient for VP16-induced complex assembly.

VP16 has multiple roles during the HSV infection cycle. It is synthesized late during infection where it is incorporated into the tegument of the virion with other viral proteins. Upon de novo infection, VP16 is released into the cell where it associates with HCF and Oct-1 on HSV immediate-early promoters. Thus, VP16 is a multifaceted protein that, at different times during the HSV infection cycle, interacts with either viral proteins, as in the virion, or cellular proteins, as in the VP16-induced complex.

VP16 is a modular protein of 490 amino acids. Its potent transcriptional activation domain is located within the carboxy-terminal 80 amino acids, whereas sequences sufficient for VP16-induced complex assembly encompass much of the remainder of the protein. Comparison of the HSV VP16 sequence with those of the related proteins from other herpesviruses reveals that the region sufficient for VP16-induced complex assembly is conserved, whereas the sequence of the transcriptional activation domain is not conserved. The X-ray crystal structure of the free form of the conserved VP16-induced complex-forming region reveals a novel, seat-like protein structure. Sequences involved in DNA-sequence recognition and productive virion assembly are structured, but sequences involved in associating with HCF and Oct-1 are disordered, suggesting that VP16 undergoes conformational changes during VP16-induced complex assembly.

Cellular Functions of HCF

Y. Liu, 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 and Oct-1 to initiate HSV gene expression during lytic infection, we

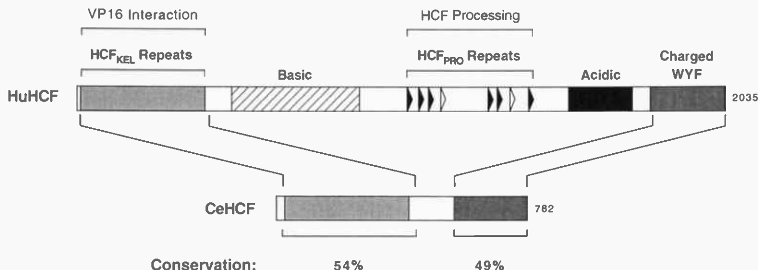


FIGURE 1 *C. elegans* HCF displays uneven sequence similarity to human HCF. Schematic structure comparison of human and *C. elegans* HCF. Conservation represents the percentage of identical residues between *C. elegans* and human HCF in the regions indicated. Charged/WYF is the region enriched in charged and large hydrophobic residues.

hypothesize that HCF 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 and Oct-1, and how these roles may influence HSV infection. We focus primarily on the cellular functions of HCF because HCF has been highly conserved during evolution and is involved in cell proliferation.

Human HCF is synthesized as a large approximately 2000-amino-acid precursor protein, which is proteolytically cleaved to generate a family of associated amino- and carboxy-terminal polypeptides. Only the amino-terminal 380 residues of HCF, however, are required to associate with VP16 and to stabilize VP16-induced complex formation. This region of HCF is not only involved in interaction with VP16, but also necessary to promote cell proliferation.

This past year, in collaboration with M. Hengartner at Cold Spring Harbor Laboratory, we reported that HCF has been conserved during metazoan evolution. Extracts from insect cells and the worm *Caenorhabditis elegans*, but not from yeast, can stabilize VP16 association with human Oct-1. We showed that *C. elegans* expresses a functional homolog of human HCF that can associate with and activate VP16. As illustrated in Figure 1, however, the pattern of sequence conservation between human and worm HCF is uneven. Amino-terminal sequences involved in cell proliferation and interaction with VP16 and carboxy-terminal sequences of unknown function are conserved, but sequences required for mammalian HCF processing are not present in *C. elegans* HCF. These results suggest that HCF possesses a highly conserved role in metazoan cell proliferation which is targeted by VP16 to regulate HSV infection. The precise mechanisms, however, by which HCF functions in mammals and worms appear to differ.

PUBLICATIONS

Herr W. 1998. The herpes simplex virus VP16-induced complex: Mechanisms of combinatorial transcriptional tegulation. *Cold Spring Harbor Symp. Quant. Biol.* **63:** 599–607.

Hinkley C.S., Blasco M.A., Funk W.D., Feng J., Villeponteau B., Greider C.W., and Herr W. 1998. The mouse telomerase RNA 5′-end lies just upstream of the telomerase template sequence. *Nucleic Acids Res.* **26:** 532–536.

In Press

Liu Y., Hengartner M.O., and Herr W. 1999. Selected elements of herpes simplex virus accessory factor HCF are highly conserved in *Caenorhabditis elegans*. *Mol. Cell. Biol.* (in press).

RNA SPLICING

A.R. Krainer L. Cartegni L. Manche T.-L. Tseng
S. Chew A. Mayeda Q. Wu
M. Hastings M. Murray J. Zhu
H.-X. Liu

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, which requires 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. 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 laboratory has focused on the identification, purification, and molecular characterization of protein factors that are necessary for the catalysis of splicing and/or for the regulation of alternative splice site selection.

CHARACTERIZATION OF HUMAN SR PROTEINS

The SR proteins constitute a large family of nuclear phosphoproteins required for constitutive pre-mRNA splicing and for the recognition of splicing enhancers present on at least some exons. These factors also have global, concentration-dependent effects on alternative splicing regulation, and this activity is antagonized by members of the heterogeneous nuclear RNP (hnRNP) A/B family of proteins. The SR proteins have a modular structure that consists of one or two RNA-recognition motifs (RRMs) and a carboxy-terminal RS domain. We recently demonstrated that a subset of SR proteins shuttles rapidly and continuously between the nucleus and the cytoplasm, although at steady state, only the nuclear population is detected by immunofluorescence. The difference between shuttling and nonshuttling SR proteins is due to the RS domain, although shuttling also requires RNA binding mediated by one or more RRMs. Shuttling SR proteins apparently exit the nucleus bound to the exons of spliced mRNAs. Differential modulation of hnRNP A/B and SR protein shuttling by kinases may serve to regulate alternative splicing in response to signal transduction pathways. The finding that some but not all SR proteins shuttle suggests that these proteins may have roles not only in nuclear pre-mRNA splicing, but also in mRNA transport, in cytoplasmic events, and/or in processes that involve communication between the nucleus and the cytoplasm. One of these events is mRNA decay triggered by premature nonsense codons, a process that is somehow linked to the position of the in-frame nonsense codons relative to the introns. When the mature transcripts are translated in the cytoplasm, protein tags may indicate the positions of the spliced exon junctions, and we have proposed that shuttling SR proteins could be the tags. J. Zhu is currently investigating this hypothesis.

We have continued the functional characterization of SR proteins with respect to their roles in enhancer-dependent splicing and in alternative splicing regulation. H.-X. Liu developed a randomization and iterative selection protocol based on biochemical complementation with single SR proteins to define the types of sequences that can function as exonic splicing enhancers in the presence of each of three different SR proteins. In collaboration with M. Zhang here at the Laboratory, short consensus sequences corresponding to enhancers specific for each SR protein were defined. These studies defined new classes of splicing enhancers, of which the well-studied purine-rich elements are only a small subset. In collaboration with G. Screaton (John Radcliffe Hospital, Oxford), A. Mayeda mapped natural enhancer elements specific either for SF2/ASF or for SC35 with-

in exons of human immunodeficiency virus (HIV) and IgM pre-mRNAs. This study demonstrated the presence of enhancers in constitutive exons and also revealed the existence of an exon silencer in HIV that suppresses the action of SC35 but not of SF2/ASF. In collaboration with X.-D. Fu (University of California, San Diego), we showed that the individual RRM modules of SR proteins appear to be responsible for the specific recognition of individual enhancer and silencer elements by each SR protein, whereas their RS domains can be interchanged without affecting substrate specificity. S. Chew obtained evidence suggesting a role of enhancer elements and SR proteins in the second catalytic step of splicing. This was seen both by observing a kinetic effect on both steps of splicing of an enhancer-dependent pre-mRNA and by use of a bimolecular exon ligation assay, in which second-step *trans*-splicing required a 3′ exon containing an enhancer.

STRUCTURE AND FUNCTION OF hnRNP A/B PROTEINS

hnRNP A/B proteins, exemplified by hnRNP A1, antagonize SR proteins to promote exon skipping and distal alternative 5′ splice site use. These proteins consist of two RRMs and a carboxy-terminal glycine-rich domain. A proteolytic fragment of hnRNP A1 lacking the carboxy-terminal domain is known as UP1. The crystal structure of human UP1 was determined here at the Laboratory by R.-M. Xu in collaboration with our group, and simultaneously by a team at Yale. Recently, B. Chabot (University of Sherbrooke) showed that UP1 and/or hnRNP A1 is also involved in telomere length regulation in vivo and that this effect is likely direct, involving binding of the protein to single-stranded telomere overhangs. R.-M. Xu has now solved the crystal structure of UP1 bound to single-stranded telomeric DNA. A. Mayeda and L. Manche have continued our efforts, in collaboration with R.-M. Xu and with S. Munroe (Marquette University), to investigate the mechanism of action of hnRNP A1 using functional assays with A1 variants bearing mutations designed on the basis of the structure and of phylogenetic conservation data. The observed functional cooperativity between the RRMs is borne out by the structure, as is the more critical role of RRM2, which can function when duplicated to replace RRM1. Although RRM1 and RRM2 fold almost identically, their nonequivalent roles may be due to a few differences in surface residues, to residues involved in interactions between the RRMs, and/or to residues involved in dimerization induced by nucleic acid binding. These possibilities are now being investigated.

ROLE OF THE TYPE-2C PROTEIN PHOSPHATASE PP2Cγ IN PRE-mRNA SPLICING

To identify activities involved in human pre-mRNA splicing, M. Murray developed a procedure to separate HeLa cell nuclear extract into five complementing fractions. One of these fractions was extensively purified by assaying for reconstitution of splicing in the presence of the remaining four fractions (Fig. 1). The purification yielded PP2Cγ, a type-2C Ser/Thr phosphatase of previously unknown function. Previous work suggested that dephosphorylation of splicing factors may be important for catalysis after spliceosome assembly, although the identities of the specific phosphatases involved remained unclear. We showed that human PP2Cγ is physically associated with the spliceosome in vitro throughout the splicing reaction but is first required during the early stages of spliceosome assembly for efficient formation of the A complex. An active-site mutant of the enzyme did not support spliceosome assembly, demonstrating that phosphatase activity is required for the splicing function of PP2Cγ. The requirement for PP2Cγ is highly specific, as seen by the fact that the closely related phosphatase PP2Cα could not substitute for PP2Cγ. Consistent with a role in splicing, PP2Cγ localized to the nucleus in vivo. Therefore, at least one specific dephosphorylation event catalyzed by PP2Cγ is required for formation of the spliceosome. Current experiments are aimed at identifying the relevant substrates of PP2Cγ in splicing, determining the basis for its physical association with the spliceosome, and understanding the mechanistic consequences of the specific dephosphorylation events.

THE AT-AC INTRON SPLICING PATHWAY

We have continued to study the mechanisms of AT-AC pre-mRNA splicing, which is a minor pathway for processing approximately 0.1% of metazoan introns. We previously developed an in vitro splicing system using a sodium channel minigene transcript with an AT-AC intron. Q. Wu demonstrated that purine-rich exonic enhancers can stimulate AT-AC splicing in vitro, there-

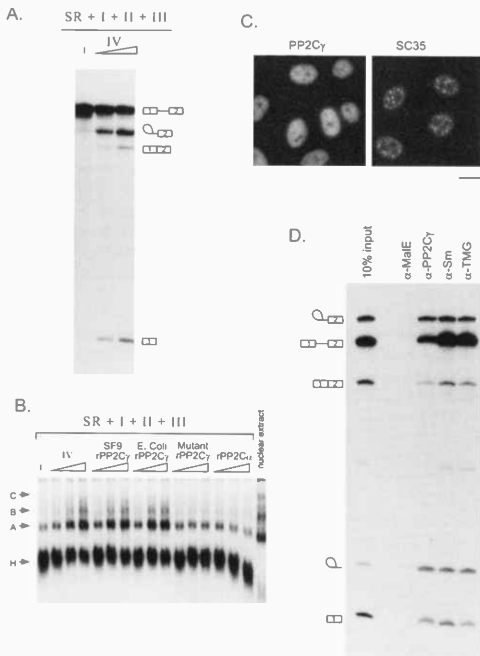


FIGURE 1 Human PP2Cγ, a Ser/Thr protein phosphatase, is a pre-mRNA splicing factor. (*A*) In vitro splicing complementation assay. Nuclear extract competent for in vitro splicing was separated into four crude chromatographic fractions (I through IV) plus purified SR proteins. The indicated combinations of fractions were incubated with labeled β-globin pre-mRNA under splicing conditions and the RNAs were analyzed by denaturing PAGE. None of the individual fractions can splice β-globin pre-mRNA, but activity is restored when all five fractions are added in combination. The assay shown demonstrates the dependence on fraction IV for the first catalytic step of splicing. Extensive purification of this fraction yielded human PP2Cγ. (*B*) Spliceosome assembly assay. Prespliceosomal and spliceosomal β-globin pre-mRNA complexes assembled in nuclear extract or in complementation reactions were assayed by native PAGE. In the absence of fraction IV or PP2Cγ, only a low level of the prespliceosomal complex A is formed. Addition of either partially purified fraction IV or recombinant PP2Cγ expressed in baculovirus or bacteria greatly stimulates A-complex formation. Mutant PP2Cγ containing an active-site point mutation does not stimulate A-complex formation in this assay nor does a related type-2C phosphatase, PP2Cα. As a control, the complexes formed in a 30-min incubation with crude nuclear extract are also shown. (C) Nuclear localization of PP2Cγ. The intracellular localization of PP2Cγ in HeLa cells was determined using anti-PP2Cγ monoclonal antibody and FITC-conjugated secondary antibody. SC35 localization is included as a positive control and as a marker for the speckle region of the nucleus. Bar, 10 μm. (*D*) Stable association of PP2Cγ with the spliceosome. Splicing reactions in nuclear extract with β-globin pre-mRNA were immunoprecipitated after a 1-hr incubation. Radioactively labeled RNA was recovered from the immunoprecipitates and analyzed by denaturing PAGE. The amount of input RNA shown is equivalent to 10% of the amount used for each immunoprecipitation. Anti-Sm and anti-trimethyl guanosine (TMG) immunoprecipitations were included as positive controls, and an anti-maltose-binding protein (MalE) antibody served as a negative control.

by extending the parallels between the major and minor pathways. Taking advantage of the unique snRNA requirements for this pathway, he showed that enhancer function does not require intact U1 or other major snRNAs. The lack of a U1 requirement distinguishes the mechanisms of splicing activation by an exonic enhancer and by a downstream 5′ splice site, both of which are thought to contribute to exon definition. This finding is probably also applicable to the major splicing pathway. The lack of a polypyrimidine tract for binding by U2AF and the close proximity between the branch site and 3′ splice site in these introns make it unlikely that U2AF is involved in AT-AC splicing stimulation by downstream 5′ splice site or enhancer elements, in contrast to current models for splicing enhancement of the major pathway. M. Hastings is now pursuing these findings by investigating the protein factors involved in basal AT-AC splicing and in activation of this pathway by downstream enhancers or conventional 5′ splice sites.

PUBLICATIONS

Cáceres J.F., Screaton G.R., and Krainer A.R. 1998. A specific subset of SR proteins shuttles continuously between the nucleus and the cytoplasm. *Genes Dev.* **12:** 55–66.

Hanamura A., Cáceres J.F., Mayeda A., Franza Jr., B.R., and Krainer A.R. 1998 Regulated tissue-specific expression of antagonistic pre-mRNA splicing factors. *RNA* **4:** 430–444.

Liu H.-X., Zhang M., and Krainer A.R. 1998. Identification of functional exonic splicing enhancer motifs recognized by individual SR proteins. *Genes Dev.* **12:** 1998–2012.

Mayeda A., Munroe S.H., Xu R.-M., and Krainer A.R. 1998. Distinct functions of the closely related tandem RNA-recognition motifs of hnRNP A1. *RNA* **4:** 1111–1123.

Misteli T., Cáceres J.F., Clement J.Q., Krainer A.R., Wilkinson M.F., and Spector D.L. 1998. Serine phosphorylation of SR proteins is required for their recruitment to sites of transcriptions in vivo. *J. Cell Biol.* **143:** 297–307.

Wu Q. and Krainer A.R. 1998. Purine-rich enhancers function in the AT-AC pre-mRNA splicing pathway and do so independently of intact U1 snRNP. *RNA* **4:** 1664–1673.

In Press

Ding J., Hayashi M.K., Krainer A.R., and Xu R.-M. 1999. Crystal structure of the two-RRM domain of hnRNP A1 (UP1) complexed with single-stranded telomeric DNA. *Genes Dev.* (in press).

Jiang J., Zhang Y., Krainer A.R., and Xu R.-M. 1999. Crystal structure of human p32, a doughnut-shaped acidic mitochondrial matrix protein. *Proc. Natl. Acad. Sci.* **96:** 3572–3577.

Koizumi J., Okamoto Y., Onogi H., Mayeda A., Krainer A.R., and Hagiwara M. 1999. The subcellular localization of SF2/ASF is regulated by direct interaction with SR protein kinases (SRPKs). *J. Biol. Chem.* **274:**11125–11131.

Lopato S., Kalyna M., Dorner S., Kobayashi R., Krainer A.R., and Barta A. 1999. atSRp30, one of two SF2/ASF-like proteins from *Arabidopsis thaliana*, regulates splicing of specific plant genes. *Genes Dev.* **13:** 987–1001.

Mayeda A. and Krainer A.R. 1999. Mammalian in vitro splicing assays. *Methods Mol. Biol.* **118:** (in press).

Mayeda A. and Krainer A.R. 1999. Preparation of HeLa cell nuclear and cytosolic S100 extracts for in vitro splicing. *Methods Mol. Biol.* **118:** (in press).

Mayeda A., Screaton G.R., Chandler S.D., Fu X.-D., and Krainer A.R. 1999. Substrate specificities of SR proteins in constitutive splicing are determined by their RNA-recognition motifs and composite pre-mRNA exonic elements. *Mol. Cell. Biol.* **19:** 1853–1863.

Murray M.V., Kobayashi R., and Krainer A.R. 1999. The type 2C Ser/Thr protein phosphatase PP2Cγ is a pre-mRNA splicing factor. *Genes Dev.* **13:** 87–97.

Wu Q. and Krainer A.R. 1999. AT-AC pre-mRNA splicing mechanisms and conservation of minor introns in voltage-gated ion channel genes. *Mol. Cell. Biol.* **19:** (in press).

REGULATION OF APOPTOSIS IN CANCER CELLS

Y. Lazebnik H. Fearnhead L. Faleiro
J. Rodriguez M. Elgersma

Apoptosis is a fundamental biological process critical for maintaining tissue homeostasis. Consequently, deregulation of apoptosis contributes to cancer. Our laboratory is investigating how the apoptotic machinery can be used to kill cancer cells. The main problem is to find out how to kill cancer cells selectively, and we are trying to understand how to achieve this goal.

An essential component of the apoptotic machinery is the 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. We study which caspases are involved in apoptosis, 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.

ONCOGENE-DEPENDENT APOPTOSIS IS MEDIATED BY CASPASE-9

The answer to selective killing of cancer cells may lie in the observation that the expression of oncogenes that deregulate the cell cycle can either induce apoptosis or sensitize cells to pro-apoptotic stimuli. An implication of this observation is that oncogene expression generates a pro-apoptotic signal that is present in transformed cells but absent in normal cells. When this signal is uncoupled from the apoptotic machinery transformed cells can survive and become resistant to chemotherapeutic drugs. In principle, restoring the link between the signal and the apoptotic machinery should selectively kill transformed cells because although untransformed cells have the machinery, they lack the signal. Understanding how the apoptotic machinery is regulated by oncogenic transformation is the first step in testing this hypothesis.

A central component of the apoptotic machinery is a family of cysteine proteases called caspases that are expressed as inactive precursors which are activated by proteolytic processing. According to the current model, two classes of caspases, initiators and effectors, are involved in apoptosis. Pro-apoptotic signals activate initiator caspases. This activation is autocatalytic and requires the binding of specific cofactors. Activated initiator caspases process effector caspases, which in turn cause cell collapse by cleaving a specific set of substrates. It appears that 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 linking this signal to a particular initiator caspase. The initiator caspase that mediates oncogene-dependent apoptosis is elusive.

To investigate how the apoptotic machinery is regulated by oncogene expression, we previously developed a cell-free system that mimics oncogene-dependent apoptosis. This system is based on the observation that extracts from cells transformed with the adenoviral oncogene *E1A* ("transformed extracts") spontaneously activated caspases, whereas extracts from untransformed cells ("untransformed extracts") did not, an observation consistent with the pro-apoptotic effect of this oncogene in cells. We suggested that the activity that triggered caspase activation is induced by expression of *E1A*, and we called this activity OGA (oncogene-generated activity). OGA partially purified from 293 cells (a transformed human epithelial kidney cell line that expresses the *E1A* and *E1B* oncogenes) activated caspases when added to untransformed extracts, thus mimicking the effects of *E1A* expression in cells. Therefore, we suggested that OGA is a link between oncogene expression and the apoptotic machinery.

We have purified OGA to apparent homogeneity and have identified it as APAF-1 (apoptotic protease activating factor 1), a cofactor required for caspase-9 activation. We found that caspase-9 was indeed required for caspase activation in our cell-free system, as was cytochrome *c*, a second caspase-9 cofactor. These observations predicted that caspase-9 is the initiator caspase that mediates oncogene-dependent apoptosis in cells and that expression of oncogenes sensitizes the cells to apoptosis by facilitating activation of this caspase. To test these predictions, we used human primary fibroblasts (IMR90) that were sensitized to drug-induced apoptosis by expression of adenoviral oncogene *E1A*. Consistent with the results from the cell-free system, a caspase-9 dominant-nega-

tive mutant blocked drug-induced apoptosis in cells expressing *E1A*. This finding led us to investigate how *E1A* expression controlled caspase-9 activation. We conclude that this control is achieved through several mechanisms, one of which is regulating cytochrome *c* release from mitochondria.

Because our results indicated that caspase-9 is involved in *E1A*-dependent apoptosis and because cytochrome *c* is required for caspase-9 activation, we asked whether *E1A* sensitizes cells to drug-induced apoptosis by facilitating cytochrome *c* release. We found that although etoposide induced cytochrome *c* release in cells expressing *E1A* irrespective of other manifestations of apoptosis, it did not have this effect in normal IMR90 cells, except in the small fraction that underwent apoptosis following etoposide treatment (<5% of the whole population). Thus, it appears that *E1A* controls drug-induced apoptosis, at least in part, by regulating cytochrome *c* release and thereby facilitating caspase-9 activation. This facilitation may be a biochemical mechanism underlying oncogene-induced sensitization of cells to apoptosis.

How *E1A* regulates the release of cytochrome *c* remains to be elucidated. One way can be through induction of proteins that release cytochrome *c* such as Bax. Considering that *E1A*-dependent Bax expression is controlled by p53, a tumor suppressor, it is a reasonable speculation that the function of p53 in oncogene-dependent apoptosis is to induce proteins required for cytochrome *c* release.

Although *E1A* can control caspase-9 activation by facilitating cytochrome *c* release, our findings in vitro suggest that additional mechanisms are involved. In the cell-free system, transformed and untransformed extracts have similar concentrations of cytochrome *c*, because breaking mitochondria during extract preparation bypasses any mechanism that regulates cytochrome *c* release in cells. However, caspases become active only in transformed extracts, which led us to propose that an activity, OGA, accounts for the difference. Yet, OGA, which was identified here as APAF-I, is also present in both extracts, as is caspase-9. Our hypothesis is that oncogenic transformation not only affects cytochrome *c* release, but also somehow "activates" other components required for caspase-9 activation.

LOCALIZATION OF CASPASES INVOLVED IN APOPTOSIS

Where are the components of the apoptotic machinery in a cell is one of the many mysteries of apoptosis. Not knowing where these components are makes it difficult to devise a viable model of cell death. Indeed, a significant part of our knowledge, as well as many assumptions, about the mechanisms of apoptosis comes from studies on cell-free systems, which are made of a homogeneous cell extract and isolated nuclei. Therefore, during the last year, we continued to analyze systematically the intracellular localization of caspases.

PUBLICATIONS

Fearnhead H.O., Rodriguez J., Govek E., Guo W., Kobayashi R., Hannon G., and Lazebnik Y.A. 1998. Oncogene-dependent apoptosis is mediated by caspase-9. *Proc. Natl. Acad. Sci.* **95:** 13664–13669.

Review

Thornberry N.A and Lazebnik Y. 1998. Caspases: Enemies within. *Science* **281:** 1312–1316.

REGULATION OF APOPTOSIS AND SENESCENCE

S. Lowe G. Ferbeyre A. Samuelson
A. Lin C. Schmitt
M. McCurrach E. de Stanchina
J. Polyakova M. Soengas

Apoptosis is a genetically controlled form of cell death that is important in normal development and in maintaining tissue homeostasis. Senescence produces "genetic death" in that senescent cells permanently arrest and are incapable of further genome propagation. Both processes are frequently disrupted in cancer cells, implying that each can limit tumor development. Moreover, radiation and many chemotherapeutic agents can induce 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 is genetic, and we typically exploit simple cellular systems to study cancer gene function. We often use viral oncogenes to probe cellular processes involved in normal growth control but have more recently extended our studies to cellular oncogenes. Finally, we are currently developing animal models to confirm the relevance of our simple systems for multistep carcinogenesis and cancer therapy.

Activation of p53 by E1A

E. de Stanchina, M. McCurrach, A. Samuelson
[in collaboration with C. Prives, Columbia University, and C. Sherr and M. Roussel, St. Jude Children's Research Hospital]

Much of our previous work focused on the p53 tumor suppressor, the most frequently mutated gene in human cancer. p53 promotes cell cycle arrest or apoptosis in response to DNA damage, hypoxia, and mitogenic oncogenes. Following DNA damage, signaling to p53 is mediated, at least in part, by a kinase that phosphorylates p53 on Ser-15, thereby stabilizing the protein by preventing its association with Mdm2. Mitogenic oncogenes can also activate p53, leading to increased apoptosis or premature senescence. This year, we demonstrated that the adenovirus *E1A* oncogene activates p53 through a mechanism independent of DNA damage, which involves inactivation of retinoblastoma protein (RB) and activation of the *ARF* tumor suppressor (de Stanchina et al. 1998). Hence, the stabilized p53 in E1A-expressing cells lacks detectable Ser-15 phosphate, and E1A is unable to induce p53 or its transcriptional targets in *ARF*-null mouse embryonic fibroblasts (MEFs). Moreover, *ARF*-null MEFs expressing E1A are resistant to apoptosis following serum depletion, irradiation, or adriamycin treatment. Reintroduction of *ARF* restores both p53 accumulation and apoptosis. Of note, the functions of E1A that induce $p19^{ARF}$ and p53 and promote apoptosis are precisely those required for E1A's oncogenic potential, implying that these processes are tightly coupled. These effects are not unique to E1A, as other mitogenic oncogenes that activate p53 also induce $p19^{ARF}$ and require *ARF* to elicit their antiproliferative effects (see Sherr, *Genes Dev. 12:* 2984 [1998]). These data imply that $p19^{ARF}$ functions as part of a p53-dependent fail-safe mechanism designed to counter uncontrolled proliferation and that this pathway is distinct from that induced by DNA damage. The involvement of bona fide tumor suppressors (RB and ARF) in oncogene-mediated signaling to p53 underscores the role of the ARF-p53 pathway in tumor surveillance (see Lowe 1999).

E1A and Chemosensitivity

M. McCurrach, E. de Stanchina, J. Polyakova, A. Samuelson

E1A has the remarkable property of making cells extremely sensitive to the cytotoxic effects of radiation and many chemotherapeutic drugs, and we continue to use E1A as a tool to understand factors that modulate

cellular radio- and chemosensitivity. This year, we demonstrated that a major component of this effect results from the ability of E1A to induce p19^{ARF}. *ARF* can stabilize p53 in response to oncogenes, but it does not activate p53 in response to DNA damage (see above). However, in the presence of an oncogenic stimulus, we demonstrated that p19^{ARF} can synergize with DNA damage to activate p53 and promote apoptosis (de Stanchina et al. 1998). Hence, *ARF*-null cells expressing E1A display attenuated apoptosis in response to radiation or adriamycin treatment compared to their normal counterparts, and reintroduction of *ARF* into *ARF*-null cells expressing E1A produces massive increases in p53 protein and radio- and chemosensitivity. Of note, since p19^{ARF} targets p53, reintroduction of *ARF* into *p53*-null cells has no effect on radio- or chemosensitivity. These data imply that activation of p53 by DNA-damaging agents can be markedly influenced by peripheral signals affecting p53 function. Moreover, if similar processes occur in human cancer, therapeutic strategies to exploit p19^{ARF} activation may enhance the radiosensitivity or chemosensitivity of p53-expressing tumors.

Other studies from our laboratory indicate that the ability of E1A to induce *ARF* cannot fully explain its ability to enhance anticancer agent-induced apoptosis. Therefore, we are performing extensive structure-function analysis on E1A to identify (ultimately) the cellular factors and processes underlying these alternative mechanisms. In addition, we continue to collaborate with Y. Lazebnik here at the Laboratory to explore how E1A can ultimately potentiate activation of the apoptotic machinery. We hope that a better understanding of this phenomenon may ultimately suggest cellular targets for novel therapies to enhance the radiosensitivity and/or chemosensitivity of human tumors.

p53-dependent and p53-independent Apoptotic Pathways

M. Soengas, M. McCurrach [in collaboration with T. Mak, Amgen Institute]

We continue to compare and contrast p53-dependent and p53-independent apoptotic programs in cells expressing E1A. E1A sensitizes cells to apoptosis induced by serum depletion, ionizing radiation, chemotherapeutic agents, hypoxia, and tumor necrosis factor (TNF). By introducing E1A into fibroblasts from normal and "knockout" mice, we can determine whether the deleted gene is involved in the above forms of apoptosis. For example, *p53*$^{-/-}$ cells expressing E1A are less sensitive to most anticancer agents than their *p53*$^{+/+}$ counterparts, yet display similar sensitivities to TNF. Using knockout MEFs expressing E1A, we assessed the requirement for p19^{ARF}, p53, Bax, caspase-3 (Casp3), caspase-9 (Casp9), Fas-associated death domain protein (FADD), and Apaf1 during apoptosis following adriamycin (p53-dependent) and TNF (p53-independent) treatment. To varying degrees, we see that inactivation of *ARF* (de Stanchina et al. 1998), *Bax*, *Casp9* (Hakem et al. 1998), and *Apaf1* (M. Soengas and S. Lowe, unpubl.) suppress cell death induced by ADR but not TNF. In contrast, *FADD* inactivation prevents TNF-induced death but not ADR (Yeh et al. 1998). Finally, inactivation of *Casp3* attenuates cell death leading to an aberrant form of apoptosis following treatment with either agent (Woo et al. 1998), implying that the p53-dependent and p53-independent pathways ultimately converge.

The studies defined from the simple genetic system clearly define at least two apoptotic pathways in oncogene-expressing cells. Since we hypothesize that the p53 pathway acts to suppress transformation, a clear prediction of our model is that disruption of the downstream components of the p53 apoptotic pathway should facilitate oncogenic transformation even in the presence of normal p53. Preliminary studies suggest this is the case (M. Soengas and S. Lowe, unpubl.).

Induction of Cellular Senescence by Oncogenic Ras

A. Lin, G. Ferbeyre [in collaboration with L. Van Aelst, Cold Spring Harbor Laboratory]

We also examined the Ras signaling pathway(s) responsible for p53 activation and premature senescence of nonimmortal human and mouse fibroblasts. Known Ras effectors include Raf-1 and components of the MAPK cascade, PI3-kinase (PI3K), Akt, c-Jun-N-terminal kinase (JNK), Rac the Rho proteins, and NF-κB. In normal cells, Ras induces premature senescence

through activation of the MAPK cascade, the same pathway important for Ras-induced mitogenesis in immortal cells (Lin et al. 1998). In human fibro-blasts, Ras "effector loop" mutants that retain their ability to bind Raf-1 promote premature senescence, and among a series of Ras downstream components examined, only activated Raf-1 and MEK induce p53, p16, and features of senescence. Moreover, a MEK inhibitor (PD98059) prevents Ras-induced cell cycle arrest and senescence. In primary murine fibroblasts, activated MEK arrests wild-type MEFs but forces uncontrolled mitogenesis and transformation when expressed at comparable levels in $p53^{-/-}$ or $INK4a^{ex2-/-}$ MEFs. The precisely opposite response of normal and functionally immortal cells to constitutive MAPK activation implies that premature senescence acts as a fail-safe mechanism to limit the transforming potential of excessive Ras mitogenic signaling. Thus, constitutive MAPK signaling activates p53 and p16 as tumor suppressors. More recently, we demonstrated that, like E1A, oncogenic *ras* activates p53 through $p19^{ARF}$ (G. Ferbeyre, A. Lin, D. de Stanchina, and S. Lowe, unpubl.).

In addition to their relevance for understanding multistep carcinogenesis, we believe our data provide new insights into the process of cellular senescence. Senescence was originally defined by the observation that primary cells have a genetically determined limit to their proliferative potential in cell culture, after which they permanently arrest with characteristic features. Because of the "end-replication problem," telomeres shorten during each cell division unless telomerase is expressed, and it has been proposed that some aspect of excessive telomere shortening activates cell cycle arrest and other characteristics of senescence. Our data demonstrate that senescence can be activated in response to deregulated MAPK activation (Lin et al. 1998). Although it remains possible that telomere shortening or some other cell-doubling-sensitive mechanism activates senescence through MAPK signaling, our hypothesis is that senescence can be activated by diverse stimuli leading to engagement of a common cell cycle arrest program. In this view, the process of senescence is conceptually related to apoptosis, a genetically controlled program carried out by a common cell-death machinery. Diverse stimuli and signaling pathways feed into this machinery, allowing regulation of cell death to be highly versatile. Consequently, the biological roles of cellular senescence may go well beyond the control of cellular or organismal aging, acting as a global antiproliferative response to a variety of cellular stresses.

In Vivo Models of Drug Sensitivity and Resistance

C. Schmitt, M. McCurrach

Our research is based on the premise that apoptosis provides a natural defense against tumor development and contributes to the cytotoxicity of most current anticancer agents. If true, then mutations that disrupt apoptosis should produce drug resistance. Although this appears to be true in at least some settings, the relationship between apoptosis and therapy is controversial. Thus, not all studies see an impact of specific anti-apoptotic changes on clonogenic survival of cultured cells nor correlate apoptotic mutations with drug resistance in patients. The picture is further clouded by the fact that cellular context can influence the results of clonogenic survival assays, and most commonly studied human tumor lines are derived from patients who received chemotherapy and have been further selected for growth in culture; hence, the response of these cells to anticancer agents may differ substantially from primary human tumor cells.

Since cancer occurs in whole animals, cell culture models may not recapitulate in vivo processes. Nevertheless, studies in human patients are complicated by extreme genetic heterogeneity and ethical issues. Mouse models provide an attractive alternative, since (1) large numbers of genetically identical individuals can be analyzed, (2) transgenic and knockout strains can be used to produce tumors with specific genetic alterations, and (3) samples can be isolated from animals undergoing therapy. To this end, our laboratory is studying the molecular genetics of drug sensitivity and resistance in the *Eμ-myc* transgenic mouse developed by Adams et al. (*Nature 318:* 533 [1985]). These animals constitutively express c-Myc in the B-cell lineage and typically succumb to pre-B or B-cell lymphoma at 4–6 months of age, many with associated leukemia. We hypothesized that the *Eμ-myc* transgenic mouse would be a tractable model for studying factors involved in therapy-induced cell death, since (1) tumor burden can be easily monitored by lymph node palpation or blood smears, (2) lymphomas are detectable long before the animal dies, so animals can be treated early, (3) large numbers of pure tumor cells can be isolated from mice undergoing therapy, (4) therapy is performed in immunocompetent mice, and (5) lymphoma cells readily adapt to culture and can be transplanted into syngeneic mice.

We have characterized the response of *E*μ-*myc* lymphomas to several anticancer drugs and have developed methods to facilitate the analysis of genetic factors involved in drug sensitivity and resistance. In addition, we have shown that *E*μ-*myc* lymphomas typically retain p53 function and are highly responsive to DNA damaging agents such as adriamycin and ionizing radiation, which produce massive p53 induction and apoptosis shortly after treatment. In contrast, *E*μ-*myc* lymphomas lacking functional p53 (generated by crossing to the p53 knockout mouse) display reduced apoptosis and are typically resistant to these same therapies. Although *p53* mutations are relatively rare in primary human leukemias or lymphomas, patients harboring these tumors rarely respond to cancer therapy. Therefore, the behavior of *E*μ-*myc* lymphomas recapitulates the behavior of human tumors and strongly suggests that this model will be useful for identifying additional factors relevant to drug sensitivity and resistance in human cancer.

PUBLICATIONS

de Stanchina E., McCurrach M.E., Zindy F., Shieh S.-Y., Ferbeyre G., Samuelson A.V., Prives C., Roussel M.F., Sherr C.J., and Lowe S.W. 1998. E1A signaling to p53 involves the p19^{ARF} tumor suppressor. *Genes Dev.* **12:** 2434–2442.

Hakem R., Hakem A., Duncan G.S., Henderson J.T., Woo M., Soengas M.S., Elia A., de la Pompa J.L., Kagi D., Khoo W., Potter J., Yoshida R., Kaufman S.A., Lowe S.W., Penninger J.M., and Mak T.M. 1998. Differential requirement of caspase 9 in apoptotic pathways in vivo. *Cell* **94:** 339–352.

Lin A.W., Barradas M., Stone J.C., van Aelst L., Serrano M., and Lowe S.W. 1998. Premature senescence involving p53 and p16 is activated in response to constitutive MEK/MAPK mitogenic signaling. *Genes Dev.* **12:** 3008–3019.

Lowe S.W. 1998. Tumor suppressor gene action in chemosensitivity and oncogenic transformation. In *Pharmaceutical intervention in apoptotic pathways* (ed. J.F. Nagelkerke et al.), pp. 143–150. Elsevier-North Holland, Amsterdam.

Woo M., Hakem R., Soengas M.S., Duncan G.S., Shahinian A., Kagi D., Hakem A., McCurrach M., Khoo W., Kaufman S.A., Senaldi G., Howard T., Lowe S.W., and Mak T.W. 1997. Essential contribution of caspase 3/CPP32 to apoptosis and its associated nuclear changes. *Genes Dev.* **12:** 806–819.

Yeh W.-C., de la Pompa J.L., McCurrach M.E., Shu H.-B., Elia A.J., Shahinian A., Ng M., Wakeham A., Khoo W., Mitchell K., El-Deiry W.S., Lowe S.W., Goeddel D.V., and Mak T.W. 1998. FADD: Essential for embryo development and signaling from some, but not all, inducers of apoptosis. *Science* **279:** 1954–1958.

In Press

Lowe S.W. 1999. Activation of p53 by oncogenes. *Endo. Related Can.* (in press).

Lowe S.W. 1999. p53, apoptosis, and chemosensitivity. In *Cancer chemotherapy and apoptosis* (ed. J. Hickman and C. Dive). Humana Press. (In press.)

Schmitt C.A. and Lowe S.W. 1999. Apoptosis and therapy. *J. Pathol.* **187:** 127–137.

Soengas M.S., Alarcón R.M., Yoshida H., Giaccia A.J., Hakem R., Mak T.W., and Lowe S.W. 1999. Apaf-1 and Caspase 9 in p53-dependent apoptosis and tumor inhibition. *Science* (in press).

Wallace-Brodeur R. and Lowe S.W. 1999. Clinical implications of p53 mutations. In *p53. Cellular and molecular life sciences* (ed. M. Oren and V. Rotter). Birlhauser, Basel. (In press.)

CELL SIGNALING IN HIV PATHOGENESIS

J. Skowronski Y.-N. Chang, M.E. Greenberg, B. Hill, A.J. Jafrate, A. Janardhan, M. Lock, 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 function of Nef, a regulatory protein of human and simian immunodeficency viruses (HIV and SIV), which is an important determinant of viral pathogenicity. Nef is likely an attractive potential target for the development of new drugs for AIDS treatment because viral replication is low and AIDS is attenuated in humans and animal models of AIDS. In addition, deleting the *nef* gene is a common promising strategy to attenuate immunodeficiency viruses for use as live attenuated vaccines.

Natural Nef proteins have several conserved effects on signal transduction and protein-sorting machineries that probably enhance viral virulence in vivo. One effect of Nef is to down-regulate expression of the CD4 molecule, an essential component of the cellular receptor for HIV and SIV virions and a component of the antigen receptor on class II major histocompatibility complex (MHC)-restricted T lymphocytes, from the cell surface. Nef also down-regulates expression of class I MHC from the cell surface, and, through this effect, Nef can probably help infected cells evade the immune response in the infected host. During the last year, we focused on the mechanism of down-regulation of class I MHC by Nef. We also continued our studies of the mechanism of Nef-induced down-regulation of CD4 expression and, in collaboration with Dr. Tom Kirchhausen (Harvard University), obtained evidence suggesting that Nef interacts directly with the AP-2 adaptor protein complex. Finally, we initiated the studies of the role of class I MHC down-regulation by Nef for the development of AIDS.

NEF INDUCES THE ACCUMULATION OF CLASS I MHC IN THE *TRANS*-GOLGI

Experiments were performed to address the fate of class I MHC in cells expressing HIV-1 Nef. Using fluorescence microscopy, we found a dramatic accumulation of assembled class I MHC in the Golgi of Nef-expressing cells. Colocalization experiments with proteins known to reside in various subcompartments of the Golgi revealed that class I MHC accumulates in the *trans*-Golgi compartment that also contains the AP-1 clathrin adaptor complex, found in the *trans*-Golgi and in transport vesicles linking the *trans*-Golgi with the endocytotic pathway. In contrast, very little if any class I MHC is found at this location in the absence of Nef.

Class I MHCs are normally transported to the plasma membrane by the default exocytotic pathway. Since the *trans*-Golgi is a late-sorting station for the protein traffic to and from the plasma membrane, class I MHC molecules that accumulate in the *trans*-Golgi could be sorted to this location directly from the exocytotic pathway, or following endocytosis from the plasma membrane, or by both of these mechanisms. We addressed these possibilities and found that class I MHC that are internalized from the plasma membrane accumulate in *trans*-Golgi in cells that express Nef. These and other observations indicate that Nef promotes class I MHC internalization from the cell surface to the *trans*-Golgi. Many viruses are known to interfere with the normal expression of class I MHC at the cell surface. They usually do so by disrupting the biogenesis and/or transport of the class I MHCs along the exocytotic pathway. Therefore, the mechanism whereby Nef promotes the internalization of class I MHC molecules to the *trans*-Golgi, likely followed by transport to the endosome-lysomal pathway and degradation, is a novel mechanism for down-regulating class I MHC expression by a viral protein. Our current experiments address the possibility that Nef alters additional events in the assembly and/or transport of class I MHC.

SEPARATE FUNCTIONS OF NEF DOWN-REGULATE CD4 AND CLASS I MHC EXPRESSION

Involvement of endocytosis with both the down-regulation of CD4 and class I MHC expression by Nef

suggested that these effects may involve similar mechanisms. Since we know that the ability of Nef to down-regulate CD4 involves its colocalization with the AP-2 clathrin adaptor, we were interested in whether this interaction is also required for down-regulation of the class I MHC. Interestingly, we found that the mutations in Nef disrupting the interaction with AP-2 on class I MHC down-regulation had no effect on down-regulation of class I MHC. These observations indicate that Nef down-regulates class I MHC and CD4 molecules *via* different interactions, with possibly different components of the protein-sorting machinery.

DOWN-REGULATION OF CLASS I MHC LIKELY INVOLVES NEF INTERACTION WITH AN SH-3 DOMAIN-CONTAINING PROTEIN

We asked whether the down-regulation of class I MHC maps to surfaces in Nef with other known functional roles. Using a panel of previously characterized mutant Nef proteins, we found that mutations that disrupt the SH3-domain-binding surface of Nef also disrupt class I MHC down-regulation. The mutations included substitutions disrupting the PPII helix in Nef and the canonical SH3 ligand-binding motif, as well as those disrupting other aspects of the SH3-domain-binding surface of Nef. These observations suggest that down-regulation of class I MHC expression probably involves an interaction of Nef with an SH3-domain-containing protein.

The ability of Nef to down-regulate class I MHC expression also requires a cluster of acidic amino acid residues conserved in HIV-1 Nef. This acidic element is not known to be a part of the surface of Nef that mediates interactions with SH3 domain ligands, suggesting that interactions of Nef with a non-SH3-domain-containing protein are also important for the down-regulation of class I MHC expression. Although we do not yet have a candidate protein for this interaction, the acidic element in Nef may function as a sorting signal because a similar cluster of acidic amino acids are part of *trans*-Golgi retrieval signals found in cytoplasmic domains of proteins that reside in the *trans*-Golgi. Interestingly, the acidic region and the PPII helix of Nef are also important for the ability of Nef to block CD3-initiated signaling in T cells, thus linking the sorting and signal transduction machineries in the regulation of class I MHC expression by Nef.

HIV-1 NEF BINDS AP-2 TO DOWN-REGULATE THE CD4 MOLECULES FROM THE CELL SURFACE

We previously found that HIV-1 Nef colocalizes in the cell with AP-2 clathrin adaptor complex and with CD4. The AP-2 adaptor mediates clathrin-dependent endocytosis by linking sorting signals in cytoplasmic domains of integral membrane proteins to clathrin in clathrin-coated pits at the plasma membrane. Furthermore, our observations that mutations which disrupt colocalization of Nef with AP-2 also disrupt the ability of Nef to down-regulate CD4 expression indicated that Nef down-regulates CD4 endocytosis by recruiting CD4 to clathrin-coated pits at the plasma membrane and accelerating CD4 endocytosis via this route; these observations also suggested that Nef may interact directly with the AP-2 adaptor complex.

During the last year, experiments were performed to further characterize the putative direct interaction between HIV-1 Nef and AP-2. First, in collaboration with Tom Kirchhausen (Harvard University), we identified a sequence in the carboxy-terminal disordered region in Nef that is essential for down-regulation of CD4. This sequence has the characteristics of the dileucine motif, one of two well-characterized sorting signals identified in other systems as being required for targeting membrane proteins to clathrin-coated membranes. The dileucine-containing Nef peptide binds directly and specifically to the β-subunit of the clathrin-adaptor complexes AP-1 and AP-2. In contrast to wild-type Nef, a Nef mutant that lacks this motif does not colocalize with AP-2 in vivo and does not down-regulate CD4 expression. These findings support the possibility that HIV-1 Nef binds directly to AP-2 via its acceptor site for dileucine-based sorting signals and that this interaction is likely critical for its ability to down-regulate CD4, possibly by promoting the recruitment of CD4 to AP-2. Current experiments are aimed toward understanding the interactions of Nef resulting in the recruitment of CD4.

ROLE OF CLASS I MHC RESTRICTED ANTIVIRAL RESPONSES IN THE DEVELOPMENT OF AIDS

Cytotoxic-T-cell responses directed toward viral antigens presented by class I MHC molecules by virally infected cells provide the fundamental mechanism for the antiviral responses of the host. Down-regulation of

class I MHC on the cell surface by Nef provides a possible mechanism through which immunodeficiency viruses could evade the defenses of the infected host and thereby permit the establishment of progressive infection. This effect of Nef could be very important for the development of AIDS, but little is known about its role for viral replication in vivo or about the role of the cytotoxic-T-cell response in HIV/SIV infection.

To address these questions, we initiated experiments in collaboration with R.C. Desrosiers (Harvard University) to assess the role of class I MHC down-regulation by Nef for SIV replication and development of AIDS in rhesus macaques, the best currently available animal model of AIDS. As a first step, we identified a number of point mutations in the carboxy-terminal region of SIV Nef that disrupt class I MHC down-regulation but have little effect on other known Nef functions. We are currently developing mutant Nef proteins with deletions in those regions that would selectively disrupt class I MHC down-regulation. We hope that it will be more difficult for such mutations to revert in vivo and that we will be able to measure the effect of this Nef function on SIV replication in the context of a functional immune system and for disease progression.

PUBLICATIONS

Greenberg M., DeTulleo L., Rapoport I., Skowronski J., and Kirchhausen T. 1998. A dileucine motif in HIV-1 Nef is essential for sorting into clathrin-coated pits and for down-regulation of CD4. Curr. Biol. **22:** 1239–1242.

Greenberg M.E., Iafrate A.J., and Skowronski J. 1998. The SH3 domain-binding surface and an acidic motif in HIV-1 Nef regulate trafficking of class I MHC complexes. *EMBO J.* **17:** 2777–2789.

In Press

Carl S., Iafrate A.J., Skowronski J., Stahl-Henning C., and Kirchhoff F. 1999. The effect of the attenuating deletion and of sequence alterations evolving in vivo on simian immunodeficiency virus C8-Nef function. *J. Virol.* (in press).

Carl S., Iafrate A.J., Lang S.M., Stahl-Henning C., Kuhn E.M., Fuchs D., Matz-Rensing K., Heeney J.L., Haft P., Skowronski J., and Kitchhoff F. 1999. The acidic region and conserved putative protein kinase C phosphorylation site in Nef are important for SIV replication in rhesus macaques. *Virology* (in press).

N. Shohdy

MOLECULAR BIOLOGY OF PAPILLOMAVIRUSES

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

The papillomaviruses infect and transform the basal epithelium of their hosts, inducing proliferation of the cells at the site of infection. The resulting tumors 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. In total, HPV DNA can now be found in biopsies from approximately 80% of all cervical carcinomas.

A key impediment to the study of papillomaviruses has been an inability to define simple in vitro cell culture systems for HPVs, largely because these viruses normally require specialized differentiating cells that only with great difficulty can be generated in cell culture. Therefore, a bovine papillomavirus (BPV-1) has become the prototype virus for the papillomavirus group largely because a convenient cell culture system exists for this virus. In this cell culture system, viral gene expression, oncogenic transformation, and viral DNA replication can be studied. The DNA replication properties of papillomaviruses show some unique and interesting characteristics. As a 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 the infected cells. The copy number of the viral DNA appears to be tightly controlled, and the viral DNA is stably inherited under these conditions. This system therefore provides a unique opportunity to study plasmid replication in mammalian cells.

In previous years, we have reported the characterization of the papillomavirus replicon and the identification of the viral components required for viral DNA replication. More recently, we have directed our attention toward detailed biochemical analysis of the replication process. We are studying the biochemical properties of the virus-encoded E1 and E2 proteins that are required for viral DNA replication. We are also studying how these two proteins interact with the ori and with each other to generate initiation complexes. From these studies, we now have a relatively clear picture of the roles of the E1 and E2 proteins in replication. E1 has all the characteristics of an initiator protein, including ori recognition, DNA-dependent ATPase activity, and DNA helicase activity. E1 can also function to unwind a supercoiled plasmid that contains the ori sequence. The E2 polypeptide, whose function has remained more elusive, appears to serve largely as a specificity factor for E1. Through physical interactions with both E1 and the ori, E2 can provide sequence specificity in the formation of the initiation complex.

Our attention has now turned toward elucidation of the precise biochemical events that precede initiation of replication at the origin of replication, i.e., binding of the initiator to the ori, the initial opening of the DNA helix, and the assembly and loading of the E1 replication helicase at the replication fork. Our studies indicate that initiation of DNA replication is an ordered multistep process involving the sequential assembly of E1 onto the ori to generate different complexes that in turn serve to recognize the ori, to destabilize the double helix at the ori, and to function as a replication helicase.

DNA Binding by the Initiator Protein E1

G. Chen

The initiator protein E1 has a poorly characterized DNA-binding activity that serves to recognize the papillomavirus origin of DNA replication and thus determines where DNA replication initiates. We have previously established that E1 can form several different complexes with ori DNA. To understand how these forms are generated and how they function in replication, we are interested in determining the stoichiometry of binding as well as defining the sequences that constitute specific recognition sequences for E1. We have identified and isolated a minimal domain from E1 that is capable of binding specifically to the ori. This domain, which consists of the sequences between amino acids 142 and 308 in E1, can form several different complexes with the ori as judged from gel-shift analysis and can also bind cooperatively with the E2 protein. To determine the stoichiometry of binding, we performed mixing experiments using two DNA-bind-

ing domain fragments of different sizes. The results from these studies demonstrated that the E1 DNA-binding domain, which is monomeric in solution, can bind to the ori as one to four monomers. Interference analysis in combination with mutational analysis demonstrated that each monomer of E1 recognizes a hexanucleotide sequence which exists in six copies in the ori sequence. In the presence of E2, E1 binds as two monomers, which is also the form of E1 that cooperates with the E2 protein for binding. Binding of E1 is primarily in the major groove where bases from both strands are recognized.

Interactions between the DNA-binding Domains of the E1 and E2 Proteins

G. Chen

In most eukaryotic replicons that have been studied, binding sites for transcription factors constitute a part of the *cis*-acting sequences required for replication activity. In the majority of cases, including, for example, SV40, polyomavirus, and ARS elements from *Saccharomyces cerevisiae*, this auxiliary activity can be supplied by various transcriptional *trans*-activators with little apparent specificity. A similar requirement exists also for papillomavirus replicons; however, only the virus-encoded transcription factor E2 can serve as an auxiliary factor for DNA replication. The involvement of E2 in replication of BPV extends beyond a mere requirement for E2 bound to the ori: A physical interaction with E1 is also required. This interaction can be detected as cooperative binding of the two proteins to the ori. The interaction between the E1 and E2 proteins appears to take place in a two-step process where an initial weak physical interaction between E1 and the E2 DNA-binding domain allows a productive interaction to occur between E1 and the activation domain of E2. We have used site-directed mutagenesis to identify the specific residues in the E2 DNA-binding domain that are required for the interaction with E1. These studies have mapped two small patches of hydrophobic residues in the DNA-binding domain of E2 that are important for the interaction with E1, verifying the specificity of this interaction and allowing us to address the importance of this protein-protein interaction in the viral life cycle. Mutations in these residues abolish the cooperative binding between E1 and E2 DNA-binding domains. Furthermore, these point mutations in the E2 DNA-binding domain result in a severe defect in the interactions between the full-length E1 and E2 proteins, indicating that, indeed, the interaction between the two DNA-binding domains is a prerequisite for the interaction beween the E2 activation domain and E1. Interestingly, these same mutations, when introduced into the viral genome, result in a severe defect in transformation. When replication of the viral genome is measured, the mutant viral DNA fails to accumulate, demonstrating a defect in viral DNA replication. Thus, most likely, the defect in transformation is caused by the defect in replication of the viral genome.

Cooperative Binding of E1 and E2 Induces Structural Changes in the ori

E. Gillitzer

As discussed above, cooperative binding of the E1 and E2 proteins is required for DNA replication in vivo. We have previously demonstrated that this interaction has two separate components. The DNA-binding domains of the two proteins interact, and the activation domain of E2 interacts with the helicase domain of the E1 protein. As mentioned above, the interaction between the activation domain of E2 and E1 is the productive interaction, whereas the interaction between the two DNA-binding domains serves to facilitate this interaction. To determine how the physical interaction between the two DNA-binding domains triggers the productive interaction, we have analyzed the structure of the ori in response to binding of the two DNA-binding domains. Both the E1 and E2 proteins individually generate modest bends 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 the E2 DNA-binding domain mutants described above that fail to interact with the E1 DNA-binding domain. Interestingly, although the intrinsic bend generated by the mutant E2 DNA-binding domains was of a magnitude similar to that generated by the wild-type E2 DNA-binding domain, the mutant E2 DNA-binding domains failed to generate a sharp bend in combination with the E1 DNA-binding

domain. These results suggest that when the E1 and E2 DNA-binding domains interact physically, the result is the generation of a bend of the sequences between the two sites. This bend, in turn, appears to facilitate the interaction between the *trans*-activation domain of E2 and the helicase domain in E1.

Assembly of Initiator Complexes

C. Sanders

An intriguing aspect of viral initiator proteins is their ability to perform several different, seemingly unrelated biochemical functions. The E1 protein is known to be a sequence-specific DNA-binding protein, but it can also distort the ori and serve as a DNA helicase. One model to explain how these activities can reside in one single polypeptide is that different oligomeric forms of the protein may have different activities. Our previous genetic and biochemical studies have indicated that both of the two specific E1-containing complexes that we can detect on the ori are important for initiation of DNA replication. These two complexes, which we have termed the E1E2-ori complex and the E1-ori complex, differ in that E2 is present in one of the complexes and also that a larger number of E1 molecules are present in the E1-ori complex. However, the complexes are clearly related, and the E1 molecules bound in the E1E2-ori complex constitute a subset of the E1 molecules bound in the E1-ori complex. To address whether these two complexes represent stages in an assembly pathway, we have analyzed their biochemical properties as well as their relation to each other. These studies have revealed a very interesting relationship, consistent with a role for both complexes in the ordered assembly of an initiator complex on the BPV ori. The E1E2-ori complex binds to the ori with very high affinity and specificity, whereas the E1-ori complex shows limited sequence specificity. By using an epitope tagging technique to follow the fate of the E1 molecules from one particular complex, we have been able to demonstrate that the E1E2-ori complex is a preferred substrate for the formation of the E1-ori complex. In a reaction that requires hydrolysis of ATP, the E1E2-ori complex can be converted into the E1-ori complex. As a consequence of this reaction, E2 is displaced from its binding site and additional E1 molecules are added to the complex. Because the E1-ori complex formed from the E1E2-ori complex appears to be identical to the E1-ori complex formed in the absence of E2, these results identify a role for the viral transcription factor E2 as a factor required transiently and catalytically for the assembly of the initiator complex. One clear implication of these results is that a crucial function for E2 in DNA replication is to form a cooperative complex with E1, increasing the specificity and affinity of E1 binding. Thus, this pathway identifies a strategy for depositing a complex with limited sequence specificity (the E1-ori complex) onto a specific site. This is accomplished through the initial formation of the sequence-specific E1E2-ori complex which subsequently is converted to the less sequence-specific E1-ori complex. This process shows interesting parallels to loading of both prokaryotic and eukaryotic replication factors such as, for example, proliferating cell nuclear antigen (PCNA) and Dna B.

PUBLICATIONS

Chen G. and Stenlund A. 1998. Characterization of the DNA-binding domain of the bovine papillomavirus replication initiator E1. *J. Virol.* **72:** 2567–2576.

Sanders C.M. and Stenlund A. 1998. Recruitment and loading of the E1 initiator protein: An ATP dependent process catalysed by a transcription factor. *EMBO J.* **17:** 7044–7055.

Sedman J. and Stenlund A. 1998. The papillomavirus E1 protein forms a DNA-dependent hexameric complex with ATPase and DNA helicase activities. *J. Virol.* **72:** 6893–6897.

DNA SYNTHESIS

B. Stillman	S. Waga	J. Mendez	M. Weinreich	L. Zou
	J. Chong	K. Shibahara	Z. Zhang	M. Waga
	V. Ellison	K. Simpson	Y.-C. (Nancy) Du	K. Cronin
	C. Liang	A. Verreault	X.H. Zou-Yang	P. Wendel

Our research continues to focus on the mechanism and regulation of DNA replication and the mechanisms that ensure inheritance of chromatin structures in proliferating eukaryotic cells. During the past year, considerable progress has been made toward these goals. Furthermore, we have become increasingly more interested in how epigenetically determined states of chromatin are inherited. In particular, we have uncovered interactions between the DNA replication protein machinery and the machinery that facilitates chromatin assembly. In addition to these studies, we have continued to investigate how initiation of DNA replication occurs and how this process is controlled by cell cycle events.

THE INITIATION OF DNA REPLICATION

Characterization of the DNA elements that constitute a functional origin of DNA replication and identification of the origin recognition complex (ORC) that binds to these origins have proved to be significant advances. The origin-bound ORC is the focus of a series of protein-protein interactions that determine how initiation of DNA replication occurs and how it is controlled. Our studies since these two discoveries have been directed toward understanding how ORC functions as an origin-specific landing pad for other proteins that eventually cause the initiation of DNA replication at each origin during the S phase of the cell cycle.

ORC was originally discovered in the yeast *Saccharomyces cerevisiae* as a six-subunit protein complex that bound to autonomously replicating sequences (ARS) in an ATP-dependent manner. We had previously identified the ORC1 and ORC2 subunits from human cells and other species, suggesting that ORC is conserved. This may have seemed rather obvious, but the DNA sequences within origins of DNA replication have not been conserved, even among the fungi. Thus, it was not obvious that the mechanism of initiation involving ORC would be conserved. In the last year we have, in collaboration with Tim Hunt's laboratory (Imperial Cancer Research Laboratories, United Kingdom), purified and sequenced subunits of ORC from *Xenopus*, identified cDNAs encoding the *Xenopus* ORC3 and ORC5 subunits, and cloned cDNAs encoding the human ORC3, ORC4, and ORC5 subunits. Combined with the sequences of the yeast orthologs, we realized that the ORC1, ORC4, and ORC5 subunits are structurally related to each other, belonging to a class of predicted ATP-binding proteins. Andy Neuwald's group here at the Laboratory independently identified these three ORC subunits as belonging to a large superfamily of known and putative ATPases called the AAA$^+$ protein family. Also belonging to this family of proteins are the Cdc6 and the six minichromosome maintenance (MCM) proteins, both of which cooperate with ORC to initiate DNA replication. Thus, one challenge has been to determine how ATP controls the functions of these proteins. Steve Bell's laboratory (Massachusetts Institute of Technology) has characterized the ATP dependence of ORC interaction with the origins of DNA replication.

To further characterize the function of the initiation proteins, we have performed structure-function analysis of yeast Cdc6p from *S. cerevisiae*. Mutagenesis studies suggest that ATP binding, but not ATP hydrolysis, is required for Cdc6p-dependent loading of the Mcm proteins on to chromatin to form the prereplication complex (pre-RC). Furthermore, we and others have demonstrated that Cdc6p in yeast is loaded on to chromatin as cells exit mitosis and is then removed from chromatin as cells enter S phase, after the activation of the S-phase-specific cyclin-dependent protein kinases (CDKs). In contrast, the Mcm proteins are loaded on to chromatin soon after the Cdc6p, but in contrast to Cdc6p, they are removed from chromatin gradually during S phase. Similar findings on Cdc6p and Mcm protein binding to chromatin have emerged from the laboratories of Steve Bell, Kim Nasmyth, and John Diffley.

In recent years, the genomes of several species of Archaea have been completely sequenced, and open reading frames with high sequence similarity to the

Orc1/Cdc6p sequences and the Mcm protein sequences have been identified. The Archaea are a newly recognized group of organisms that are believed to bridge the differences between bacteria and eukaryotic species. The Mcm proteins are essential for initiation of DNA replication in yeast and form part of the pre-RC at origins of DNA replication prior to S phase. Like Orc1p and Cdc6p, sequences related to the Mcm protein have been identified in Archaea. To study the structure and function of these putative initiation proteins, we have cloned the Mcm and Orc1/Cdc6-related proteins from a number of Archaea species and, in collaboration with the Rui-Ming Xu laboratory, are biochemically and structurally characterizing these proteins. We anticipate that these studies will complement similar studies with the yeast proteins.

In the previous year, we reported that Cdc45p is loaded on to chromatin just prior to S phase in a manner that was dependent on the CLB-CDKs. These CDKs are essential for initiation of DNA replication and act to trigger formation of a preinitiation complex (pre-IC). In the past year, we have characterized another protein kinase that is essential for initiation of DNA replication, the Cdc7-Dbf4p kinase. Recombinant versions of this kinase have been purified to homogeneity, and using this enzyme, we have demonstrated that the kinase autophosphorylates the Dbf4p subunit, Mcm2p, Mcm3p, Mcm4p, Mcm6p, and Mcm7p, and the largest subunit of DNA polymerase α primase. Interestingly, Mcm5p appears not to be a substrate, yet it is known that mutations in the gene encoding this protein bypass the need for Cdc7-Dbf4p kinase in mitotically dividing cells. We have also shown that the Dbf4p subunit is cell-cycle-regulated in its abundance and is loaded onto chromatin at the G_1 to S phase transition, about the same time as Ccd45p, and is removed as cells enter into mitosis. At this time, Dbf4p is degraded in a manner dependent on functional anaphase-promoting complex (APC), a protein complex that facilitates ubiquitin-mediated protein degradation by the 26S proteosome. How both protein kinases act to trigger entry into S phase is under further investigation.

DNA-REPLICATION-DEPENDENT CHROMATIN ASSEMBLY

Previous studies using the SV40 DNA replication system have uncovered a DNA replication-dependent chromatin assembly mechanism. A central component of this mechanism is the chromatin assembly factor-1 (CAF-1), a three-subunit protein that can mediate assembly of nucleosomes during DNA replication and DNA repair. We have shown that elimination of CAF-1 from yeast leads to sensitivity of the yeast strains to UV light and to reduced silencing of the epigenetically determined transcriptional state of reporter genes that are placed adjacent to telomeric heterochromatin. In a population of CAF-1-defective yeast cells, the silenced state is inefficiently maintained and the gene expression is variegated.

We have long wondered why the CAF-1-dependent nucleosome assembly occurred preferentially on DNA that was replicated or had undergone DNA repair. In the last year, we have shown that the replicated DNA becomes marked with a noncovalent imprint during DNA replication and that the marked, completely replicated DNA can be isolated and used for chromatin assembly. Removal of this mark on the replicated DNA caused the DNA to become incompetent for chromatin assembly. Analysis of the nature of the noncovalent mark on the DNA resulted in the demonstration that the proliferating cell nuclear antigen (PCNA), a protein we have shown previously to be a DNA polymerase clamp protein, remained linked to the DNA after DNA replication. Removal of PCNA from the DNA by the clamp unloader protein, replication factor C (RFC), removed the imprint and prevented chromatin assembly on the replicated DNA. We further showed that PCNA directly bound to PCNA via the p150 subunit of CAF-1. These studies show that PCNA is necessary for a DNA-replication (and DNA-repair)-dependent mark on the DNA that facilitates CAF-1-dependent chromatin assembly (Fig. 1). Because of the asymmetric nature of the DNA replication process and the consequent asymmetry of PCNA on leading and lagging strands at the DNA replication fork, we suggest that PCNA may be part of a mechanism that allows asymmetric inheritance of chromatin structures to daughter cells during development (Fig. 1).

It is known that PCNA also binds to the [cytosine-5] methyltransferase, suggesting that PCNA left on DNA after DNA replication and repair may mediate DNA methylation. Thus, PCNA may well be a mark on replicated DNA for the stable inheritance of epigenetically determined chromosomal states, be they noncovalent protein or covalent modifications such as cytosine methylation. Consistent with this observation, it is known that mutations in the gene encoding *Drosophila* PCNA cause suppression of position effect variegation and sensitivity to DNA-damaging agents, phenotypes remarkably similar to those displayed by yeast strains that lack CAF-1.

We suggest that CAF-1 and PCNA are components of a system to ensure the stable propagation of chro-

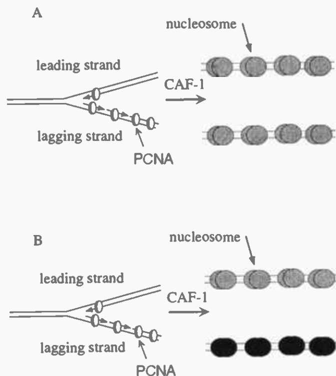


FIGURE 1 Model for PCNA-mediated inheritance of chromatin on replicated DNA. PCNA binds to CAF-1 and mediates DNA-replication-coupled chromatin assembly. In *A*, the nucleosomes are assembled concomitant with passage of the DNA replication fork, and a similar chromatin structure is formed on both leading and lagging strands. In *B*, the asymmetric distribution of PCNA on the leading strand versus the lagging strand could promote asymmetric chromatin states on the two sister chromatids that then could be distributed to the two daughter cells, creating phenotypic differences between them.

mosomal complexes in eukaryotic cells. Current efforts are to test this hypothesis further and to characterize other proteins that are essential for this process.

PUBLICATIONS

Ellison V. and Stillman B. 1998. Reconstitution of recombinant human replication factor C (RFC) and identification of an RFC subcomplex possessing DNA-dependent ATPase activity. *J. Biol. Chem.* **273:** 5979–5987.

Li R., Yu D.S., Tanaka M., Zheng L., Berger S.L., and Stillman B. 1998. Activation of chromosomal DNA replication in *Saccharomyces cerevisiae* by acidic transcriptional activation domains. *Mol. Cell. Biol.* **18:** 1296–1302.

Martini E., Roche D.M.J., Marheineke K., Verreault A., and Almouzni G. 1998. Recruitment of phosphorylated chromatin assembly factor 1 to chromatin following UV irradiation of human cells. *J. Cell Biol.* **143:** 563–575.

Ni T.-H., McDonald W.F., Zolotukhin R., Melendy T., Waga S., Stillman B., and Muzyczka N. 1998. Cellular proteins required for adeno-associated virus DNA replication in the absence of adenovirus coinfection. *J. Virol.* **72:** 2777–2787.

Tugal T., Zou-Yang X.H., Gavin K., Pappin D., Canas B., Kobayashi R., Hunt T., and Stillman B. 1998. The Orc4p and Orc5p subunits of the *Xenopus* and human origin recognition complex are related to Orc1p and Cdc6p. *J. Biol. Chem.* **273:** 32421–32429.

Verreault A., Kaufman P.D., Kobayashi R., and Stillman B. 1998. Nucleosomal DNA regulates the core histone-binding subunit of the human Hat1 acetyltransferase. *Curr. Biol.* **8:** 96–108.

Waga S. and Stillman B. 1998. The DNA replication fork in eukaryotic cells. *Ann. Rev. Biochem.* **67:** 721–751.

Waga S. and Stillman B. 1998. Cyclin-dependent kinase inhibitor p21 modulates the DNA template-primer recognition complex. *Mol. Cell. Biol.* **18:** 4177–4187.

Yan Z., DeGregori J., Shohet R., Leone G., Stillman B., Nevins J.R., and Williams R.S. 1998. Cdc6 is regulated by E2F and is essential for DNA replication in mammalian cells. *Proc. Natl. Acad. Sci.* **95:** 3603–3608.

Zou L. and Stillman B. 1998. Formation of a pre-initiation complex by S-phase cyclin CDK-dependent loading of Cdc45p onto chromatin. *Science* **280:** 593–596.

IN PRESS

Duncker B.P., Pasero P., Braguglia D., Heun P., Weinreich M., and Gasser S.M. 1999. CyclinB/Cdk1 kinase stimulates ORC- and Cdc6-independent steps of semi-conservative plasmid replication in yeast nuclear extracts. *Mol. Cell. Biol.* **19:** 1226–1241.

Shibahara K. and Stillman B. 1999. Replication-dependent marking of DNA by PCNA facilitates CAF-1 coupled inheritance of chromatin. *Cell* **96:** 576–585.

Weinreich M., Liang C., and Stillman B. 1999. The Cdc6p nucleotide binding motif is required for loading Mcm protein onto chromatin. *Proc. Natl. Acad. Sci.* **96:** 441–446.

ONCOPROTEIN DESTRUCTION

W. Tansey A. Herbst S. Salghetti
S.Y. Kim K. Tworkowski
M. Muratani

The maintenance of normal cellular growth and differentiation ultimately depends on mechanisms which 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 oncogene transcription factors are regulated at the level of their own 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. Consistent with its potent growth-promoting properties, the expression and activity of Myc are tightly regulated at many levels, including transcription, mRNA stability, translation, and protein stability. Indeed, Myc—like many of the transcription factors that regulate cell growth—is a highly unstable protein, with a typical half-life of about 30 minutes. The extreme instability of Myc and these other regulators helps keep their intracellular levels low and maintains tight control over cell proliferation, a role which is underscored by the fact that some viral oncogenic transcription factors escape degradation and accumulate to higher levels than their cellular counterparts.

Work in our laboratory has demonstrated that Myc is unstable because it is destroyed by a process known as ubiquitin-mediated proteolysis. In this process, the covalent attachment of Myc to the protein ubiquitin signals Myc destruction by the 26S proteasome, a large complex with several proteolytic activities. Protein ubiquitylation is a highly specific multistep process, which begins when an element within the target protein, termed a degron, is recognized by the cellular ubiquitylation machinery. After the degron has been recognized and bound, ubiquitin 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 proteasomal 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 ubiquitin-mediated proteolysis.

In the last year, we have made considerable progress in understanding how Myc is targeted for destruction and how loss of control of this process relates to human cancer. First, using a reverse genetic approach, we have defined regions in the Myc protein that determine its stability in human cells. We have found that the Myc degron resides within the amino-terminal 128 residues of the protein, a region that overlaps closely with the transcriptional activation domain (TAD) of Myc. Indeed, despite our best efforts, we have been unable to separate genetically the transcriptional activation function of this region from its degron function, implying that it is the TAD per se that signals Myc destruction. In contrast to the amino-terminal degron, we have also identified an element in the carboxyl terminus of Myc that promotes Myc stability. This element appears to function by association with the POZ domain transcription factor Miz-1, a protein that binds to Myc and converts it from a transcriptional activator to a transcriptional repressor. The significance of Miz-induced stabilization of Myc is unclear at this stage, but it may allow the formation of stable Miz-Myc repressor complexes that can efficiently repress gene transcription. We are currently exploring this possibility.

Second, we have explored the ubiquitin-mediated destruction of Myc in yeast. We reasoned that as the Myc TAD activates transcription in the yeast *Saccharomyces cerevisiae*, it may also function as a degron in this species. This is indeed the case. We have found that Myc is a highly unstable protein in yeast, with a typical half-life of approximately 5 minutes. Removal of the Myc TAD or genetic disruption of the proteasome stabilizes Myc, demonstrating that the Myc TAD signals ubiquitin-mediated Myc turnover in yeast, as it does in mammalian cells. This result not only demonstrates an extraordinary conservation of proteolytic targeting mechanisms, but also provides an opportunity to dissect genetically Myc destruction in yeast, and we have devised a number of screening strategies to identify genes required for Myc destruction.

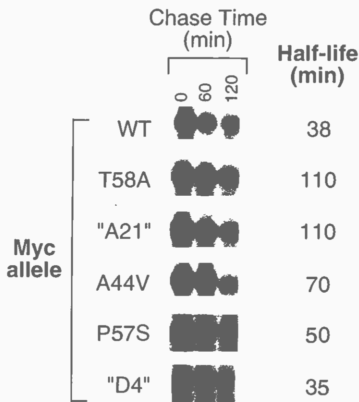


FIGURE 1 Lymphoma-associated and transforming mutations stabilize Myc. Human U2OS cells were transiently transfected with expression constructs encoding wild-type human Myc (WT) or the indicated mutant Myc alleles. Protein stability was determined by pulse-chase analysis. The alleles shown represent a cross section of different mutations. The T58A mutation is found in several viral Myc proteins and has been shown to enhance the transforming potential of Myc in vitro; residue 58 is also one of the major mutational hot spots within the Myc-coding sequence. Allele A21 is a complex mutation (S71Y, L82H, Δ115-120) identified in a tumor sample from a patient suffering from an aggressive, AIDS-associated, lymphoma. The A44V mutation has been found in a patient with Burkitt's lymphoma and in a separately derived Burkitt's lymphoma cell line. The P57S mutation was identified in a Burkitt's lymphoma patient, whereas allele D4 (mutations: N127D and I129L) was identified in a tumor sample from a patient suffering from a non-AIDS-associated lymphoma.

Finally, we have asked whether Myc destruction is changed in human malignancy. Missense mutations within the first 130 amino acid residues of Myc are commonly found in a variety of lymphomas. Because these mutations lie within the Myc degron, we asked whether they alter Myc stability. As shown in Figure 1, four out of five mutant forms of Myc that we tested were more stable than wild-type Myc and accumulated to higher steady-state levels (not shown). In one case, a single point mutation, Thr-58 to Ala-58, increased the stability of Myc from 35 minutes to approximately 2 hours: This mutation occurs at a major site of growth-regulated Myc phosphorylation and has been shown to increase the transforming potential of Myc in vitro. Thus, cancer-associated and transforming mutations disrupt Myc degron function, demonstrating that the rapid destruction of Myc is subverted in cancer. These data provide one of the first examples of how a defect in ubiquitin-mediated proteolysis can be a mechanism of cellular oncogene activation. Given the frequent occurrence of mutations within the Myc degron in cancer, we speculate that enhanced protein stability is a common mechanism contributing to oncogenic transformation by Myc.

In Press

Salghetti S.E., Kim S.Y., and Tansey W.P. 1999. Destruction of Myc by ubiquitin-mediated proteolysis: Cancer-associated and transforming mutations stabilize Myc. *EMBO J.* **18:** 717–726.

MOLECULAR GENETICS

The section on molecular genetics covers many diverse problems, from organisms from three different kingdoms of life, and from biological phenomena as diverse as cancer, cell cycle, programmed cell death, growth and differentiation, and intercellular communication. The scientific groups are united only in their adoption of genetic approaches to the study of their problems.

- Dr. Wigler's group studies tumor suppressors and is developing new genetic tools for the study of human disease. These tools, based on representational difference analysis, identify the somatic mutations that underlie human cancer and the germ-line mutations that underlie sporadic hereditary diseases. Part of this effort is directed at developing new applications of micro-arrayed DNA probes for the analysis of genomic changes.
- Dr. Hamaguchi's group is isolating tumor suppressor genes by applying newly developed technologies in positional cloning.
- Dr. Beach and Dr. Hannon's group have developed new technology for the genetic manipulation of mammalian cells, using highly modified retroviral tools. This technology is expected to increase both speed and accuracy of the elucidation of genes involved in disease, their functions, and the associated genetic pathways.
- Dr. Hengartner's group uses the nematode *C. elegans* to study conserved biological processes, with an emphasis on apoptosis (programmed cell death) and developmental neurobiology.
- Dr. Futcher's laboratory studies the control of cell division, with special emphasis on cyclin-dependent kinases and their substrates. This year, microarray technology allowed all the cell cycle regulated genes of yeast to be identified and placed in the cell cycle transcriptional loop. The laboratory also works on telomeres and their relationship to aging.
- Dr. Grossniklaus' group studies the genetic and molecular basis of sexual plant reproduction and apomixis, focusing on the development and function of the haploid female gametophyte. They also explore how the female gametophyte controls subsequent events during seed formation and found that epigenetic mechanisms such as genomic imprinting play a crucial role.
- Dr. Jackson's group studies a novel mechanism of cell-to-cell communication in plants involving the intercellular trafficking of regulatory proteins. They are also characterizing novel mutants of maize that affect fundamental aspects of plant growth such as the pattern of leaf initiation and the control of stem cell proliferation.
- Dr. Grotewold's group has significantly contributed to understanding the evolution of plant transcription factors containing the conserved Myb DNA-binding domain and to elucidating the mechanisms by which they control gene expression. They also explored successfully the possibility of manipulating plant metabolism by ectopically expressing transcription factors in cultured maize cells.
- Dr. Ma's laboratory continues to identify new mutants using the gene trap transposon mutagenesis system developed at Cold Spring Harbor Laboratory. Several new mutants with defects in floral organ development and male meiosis have been identified, and their characterization should uncover new genes regulating reproductive development in plants.
- Dr. Martienssen's laboratory continues to do research on other aspects of plant genetics as well, and he recently made an important discovery about the movement of intracellular proteins across cell membranes. They have shown that a gene, *hcf106*, codes for a membrane protein that resembles certain bacterial proteins and is vital to protein translocation in maize and bacteria.

EUKARYOTIC CELL CYCLE CONTROL

D. Beach J. Chen P. Sun
D. Conklin J. Wang

During the course of the year, Susan Allan accepted a position at Mitotix in Cambridge, Massachusetts, and Pedro de Campos Lima took up a fellowship at the Institute of Child Health in London.

MDM2 Overexpression May Contribute to TGF-β Resistance in Tumors

P. Sun

Transforming growth factor-β (TGF-β) is a multifunctional cytokine that inhibits the growth of cells of epithelial, lymphoid, and neuroectodermal origin. TGF-β sensitivity often declines during the progression of tumors derived from these cells. In several types of tumors, the extent of TGF-β resistance correlates with malignancy. These observations implicate the involvement of TGF-β in tumorigenesis. Currently, the mechanisms of TGF-β resistance in tumor cells are not well understood. Some tumors may become TGF-β-resistant following c-Myc overexpression or inactivation of essential components of the TGF-β signaling pathway (e.g., loss of TGF-β receptor function and mutations in *Smad2* and *Smad4* genes), or through deletion of the $p15^{INK4B}$ locus. However, such alterations cannot account for the majority of cases in which TGF-β responsiveness is lost. Therefore, TGF-β resistance must also be achieved by mechanisms that are as yet unknown.

To search for additional genetic alterations leading to TGF-β resistance in tumors, we performed a screen to identify genes that overcome the TGF-β sensitivity of mink lung epithelial (Mv1Lu) cells. We found that overexpression of an oncoprotein, MDM2, rescued the TGF-β-sensitive phenotype of Mv1Lu cells. MDM2 was previously shown to associate with and inactivate the p53 tumor suppressor protein. However, the capacity of MDM2 to bypass TGF-β-induced growth arrest was independent of its ability to inactivate p53, because two dominant negative p53 alleles (p53Val135 and p53-175H) did not confer TGF-β resistance, although they could interfere with the function of endogenous, wild-type p53 in Mv1Lu cells. Moreover, an MDM2 mutant which could no longer bind to p53 also conferred TGF-β resistance in Mv1Lu cells. We further demonstrated that MDM2 overcomes TGF-β through interference with the retinoblastoma (RB)/E2F pathway. More specifically, MDM2 prevented the dephosphorylation of RB and down-regulation of E2F-1 expression induced by TGF-β.

TGF-β induces growth arrest in normal human lymphocytes, melanocytes, and breast epithelial cells. However, cells from human leukemia, lymphomas, melanomas, and breast carcinomas are often TGF-β-resistant. Coincidentally, MDM2 is commonly overexpressed in these tumors (e.g., 73% in human breast carcinomas). We found that enforced expression of MDM2 alone in primary human mammary epithelial cells (HMEC) was sufficient to convert these TGF-β-sensitive cells to a resistant phenotype. These observations raised the possibility that increased MDM2 expression might contribute to TGF-β resistance in tumors.

We therefore examined the correlation between MDM2 expression levels and TGF-β responsiveness in seven human breast tumor cell lines. T-47D, ZR-75-1, and HTB20 cells expressed MDM2 at levels comparable to those observed in cells (HMEC and Mv1Lu) that had been made TGF-β-resistant by infection with MDM2 retroviral vectors. These three cell lines were completely resistant to TGF-β-induced growth arrest. The two cell lines (MCF-7 and BT549) that were most sensitive to TGF-β treatment had very low MDM2 levels, similar to those seen in TGF-β-sensitive, normal HMECs. Thus, in several tumor cell lines, increased MDM2 expression strictly correlated with the ability to escape TGF-β-induced growth inhibition. Two other breast carcinoma cell lines (HBL100 and MDA-MB-468) exhibited partial resistance to TGF-β despite low levels of MDM2 expression, confirming that other mechanisms (e.g., c-*myc* overexpression and receptor mutation) must also contribute to TGF-β-resistance.

Our data therefore suggest that overexpression of MDM2 is a potential mechanism leading to TGF-β

insensitivity in tumors and that MDM2 can contribute to tumorigensis by antagonizing both p53 and RB tumor suppressor pathways, functioning in many respects as a cellular version of SV40 large T antigen.

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. Calcium ion is necessary and sufficient for cell cycle progression during the maturation of oocytes and marine eggs. Calcium has been identified in genetic studies as an important regulator of cell cycle progression at G_1/S and at mitosis in lower eukaryotes. Direct observation of mammalian cells with calcium indicators and imaging techniques has determined that Ca^{++} transients occur at several points in the cell cycle including G_0/G_1, G_1/S, S, and M. 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 channel antagonists to inhibit both the transients and the subsequent cell cycle progression in a number of cell types.

In an effort to identify gene products involved in the calcium regulation of cell proliferation, retroviral cDNA libraries have been screened for genes that, when overexpressed, allow cells to escape calcium channel antagonist-induced growth arrest. Several known genes involved in signal transduction have been isolated in these screens, including *ras*, *jun*, a G-protein-coupled serpentine receptor, and a calcium-binding protein.

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. Since this gene confers *r*esistance to two structurally unrelated compounds that both function as *c*alcium *c*hannel *b*lockers, it was named *CCBR1*. Overexpression of *CCBR1* does not confer increased resistance to several other drugs that inhibit proliferation. Overexpression of the human *p*-glycoprotein (MDR1), which is responsible for the detoxification of a number of toxic substances, does not confer resistance to nifedipine. Collectively, these results indicate that the resistance conferred by *CCBR1* does not result from increased general stress response or drug detoxification and that *CCBR1* is likely to be related to some aspect of calcium biology.

Sequence analysis indicates that the *CCBR1* gene encodes a previously unknown member of a family of proteins involved in both cell proliferation and transport, recently identified as light chains of the activation antigen CD98. CD98 is a heterodimeric surface glycoprotein initially isolated on activated T lymphocytes; however, it has subsequently been found on a number of rapidly proliferating cells including most tumor cells. The heavy chain has been implicated in a variety of cell functions including integrin action, human immunodeficiency virus (HIV) gp160-induced cell fusion, and amino acid transport.

CD98 has also been implicated in Ca^{++} homeostasis in cardiac smooth muscle cells and T cells. Current studies are focused on the effects of overexpression of *CCBR1* and the other calcium channel blocker resistance genes on intracellular calcium levels.

Immortalization of Human Primary Cells

J. Wang

The c-*myc* gene was identified as the cellular homolog of the v-*myc* oncogene found in several transforming retroviruses. It is expressed at elevated levels in a variety of human tumors, indicating that its deregulation may be an important component in tumorigenicity. c-*myc* has been shown to have a dual function in mammalian cells. On the one hand, it acts as a central regulator of cellular function, involved in the regulation of proliferation, mitogenesis, and differentiation. On the other hand, c-*myc* appears to be involved in the regulation of apoptotic cell death.

The *ras* genes are also critical regulators of numerous physiological functions, including cell growth and differentiation. Activated or oncogenic *ras* genes have been implicated in many types of human cancers. Although increased expression of normal *ras* proto-oncogenes can induce certain manifestations of the malignant phenotype, *ras* oncogenes activated by sin-

gle point mutations displayed more efficient transforming ability.

Immortalization is a critical step during tumorigenesis. Oncogenic *ras* efficiently transforms most immortal rodent cell lines but not primary cells. However, *ras* cooperates with certain other oncogenic alterations to transform primary rodent cells. Similarly, deregulated *myc* expression alone is insufficient to elicit a transformed phenotype. It is well established that *myc* can cooperate with an activated *ras* oncogene to transform primary rat fibroblasts, rat embryo cells, and pre-B cells.

Although *myc* and *ras* behave very similarly in many aspects, they act in a totally opposite way in senescence control. Oncogenic *ras* has been shown to provoke premature cell senescence in primary human or rodent cells. We have recently reported that *myc* can bypass replicative senescence through activating telomerase in human primary epithelial cells and fibroblasts. Furthermore, we found that *myc* or *hEST2* can immortalize human primary cells through a telomere maintenance mechanism. The *myc* or *hEST2*-infected HMEC and IMR-90 cells have bypassed the normal senescence point (>150 population doublings) and still grow well. Their telomere length remains stable even after hundreds of population doublings. On the other hand, activated *ras*V12 can overcome *myc* or *hEST2* to induce cellular senescence in HMECs. It is not through inactivating telomerase or shortening telomere in those cells. However, the mechanism is unknown. We suggest that *ras*V12 induces senescence through a mechanism different from natural replicative senescence.

In addition, we have characterized the *myc* and *hEST2* cells by comparing the expression levels of several cell cycle regulatory proteins including p16, p21, and p53.

Western blots showed that p21 decreased significantly in HMEC/*myc* and/or *hEST2* cells compared with vector cells, whereas p53 stayed unchanged, and that both p16 and p21 decreased dramatically in IMR-90/*myc* or *hEST2* cells compared with vector control cells, whereas p53 did not. These results indicate that loss of p16 and/or p21 but not p53 contributes to immortalization of HMEC and IMR-90 cells.

Human primary cells HMEC and IMR-90 can be immortalized by a single step: overexpression of *myc* or *hEST2*. This is through maintaining or elongating telomere length by activation of telomerase. However, oncogenic *ras*V12 can overcome the effects of *myc* and *hEST2* and prevent immortalization by arresting *myc* or *hEST2* cell growth. This growth arrest has been characterized to be senescence by several criteria. RasV12-induced senescence is telomere-length-independent. The mechanism of this senescence is unknown.

PUBLICATIONS

Sun P., Dong P., Dai K., Hannon G.J., and Beach D. 1998. p53-independent role of MDM2 in TGF-β1 resistance. *Science* **282:** 2270–2272.

Wang J., Xie L.-Y., Allan S., Beach D., and Hannon G.J. 1998. Myc activates telomerase. *Genes Dev.* **12:** 1769–1774.

CELL CYCLE CONTROL IN *SACCHAROMYCES CEREVISIAE*

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

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 basic cell cycle oscillation. Many key cell cycle events are regulated by protein kinase complexes formed between Cdc28p and one of nine cyclins. These fall into two broad groups: the G_1 cyclins including Cln1p, Cln2p, and Cln3p that regulate Start and the mitotic B-type cyclins Clb1p, Clb2p, Clb3p, and Clb4p. Two other cyclins, Clb5p and Clb6p, 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 Cdc28p kinase activity is required for mitosis. At the end of anaphase, the Cdc28p 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 the Cdc28p kinase is inactivated by destruction of cyclins, production of the Cdk inhibitor Sic1p, and possibly by other mechanisms. A network of genes 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.

One gene involved in exit from mitosis is *SDB24*. Sdb24p has no close homologs, but it has six potential sites for phosphorylation by Cdc28p, and it has a clear coiled-coil domain. The transcript is cell-cycle-regulated, with a peak in S phase. The *sdb24* null mutant is viable but is synthetically lethal with *dbf2* and with *cdc23*. The synthetic lethality of *dbf2 sdb24* can be rescued by overexpression of the mitotic exit genes *CDC5, SIC1*, and *SPO12*, but not *CDC15* or *TEM1*. *GAL-CLB2* and *GAL-CLB5* are lethal in an *sdb24* background.

Sdb24p also has connections to early spindle events. The protein is found in early spindles and is apparently at kinetochores. The *sdb24* mutation is synthetically lethal with mutations in the kinesin-like motor proteins Cin8p and Kip1p, both of which are important for forming the mitotic spindle.

All of these results are consistent with the general idea that Sdb24p is important for spindle formation and function. We speculate that because of a slightly aberrant spindle, *sbd24* mutants have difficulty signaling the end of anaphase and so have difficulty inactivating Cdc28p at the end of mitosis. In short, Sdb24p is one of the proteins comprising a checkpoint that allows Cdc28p inactivation and exit from mitosis.

Dissection of Nuclear and Cytoplasmic Roles of Cyclins

N. Edgington

Nine different yeast cyclins bind to and activate the Cdc28p kinase. Although the enzymatic properties of the nine different cyclin-Cdc28p complexes are similar, these nine complexes have very different biological roles. We asked how many of these differences could be accounted for by differences in location. We have created "forced localization" tags, which, when appended to a protein, force it to be either cytoplasmic or nuclear. Using such tagged proteins, we have found that nuclear Cln3p, but not cytoplasmic Cln3p, can complement all Cln3p functions, suggesting the Cln3p is solely nuclear. On the other hand, most functions of Cln2p are complemented only by cytoplasmic Cln2p. Most functions of Clb2p are complemented

only by nuclear Clb2p, and yet nuclear Clb2p is not entirely wild type, suggesting that it may have a cytoplasmic role or may need to transit through the cytoplasm.

Affinity Purification Tags

S. Honey, B. Schneider

We have been interested in identifying the components of various protein complexes, such as cyclin-Cdk complexes. To aid in this, we have developed new methods for rapid purification. We have made a *Not*I cassette carrying six histidines (allowing binding to nickel columns and elution with imidizole), a calmodulin-binding peptide (allowing binding to calmodulin and elution with EGTA), and a triple hemagglutinin epitope (allowing immunoprecipitation with monoclonal antibody 12CA5). This tag has allowed us to purify Clb2p and associated proteins to homogeneity in 1 day. Tandem mass spectrometry identified Cdc28p, Sic1p, and Cks1p as Clb2-associated proteins. We are currently expanding these methods to other complexes.

Gene Arrays for Analysis of Cell Cycle Gene Expression

G. Sherlock [in collaboratoin with P. Spellman, D. Botstein, P. Brown, M. Zhang, and others]

The laboratories of Pat Brown and David Botstein have arrayed all 6000 yeast genes on glass slides, such that the relative level of expression of each gene can be assayed by hybridization. We have used these microarrays to analyze the pattern of gene expression in yeast through synchronous cell cycles. We synchronized yeast in G_1 phase with α factor, or in late mitosis with cdc15-2, or in G_1 phase by elutriation. These three cultures were then allowed to progress through several synchronous cycles. Samples were taken frequently, and the level of mRNA for all 6000 genes was assayed by hybridization to a microarray; about 400,000 pieces of data were generated in this way. By doing extensive statistical analysis, including fitting results for each gene to a sine wave, we were able to identify 800 genes (~13% of the total genes) whose transcripts oscillated in abundance in a cell-cycle-dependent way.

The 800 genes were further analyzed using a clustering algorithm that grouped genes which behaved similarly. This established ten groups containing about 400 genes. For each group, we identified a common promoter element, and for most of these promoter elements, we were able to identify a transcription factor that bound. Thus, we are able to explain at least 400 cell-cycle-regulated genes in terms of upstream elements and transcription factors.

For one important group of genes, the "*CLB2*" cluster, we defined an upstream element, but no transcription factor was known for this element. We guessed that the responsible transcription factor(s) might itself be cell-cycle-regulated. Thus, we looked on the list of cell-cycle-regulated genes for putative transcription factors of unknown function. There were only a few. Furthermore, other workers have previously used genetic screens to try to find the regulator of *CLB2* and other genes in the *CLB2* cluster without success. This failure suggested to us that two or more genes might redundantly encode the transcription factor. Our list of cell-cycle-regulated putative transcription factors contained one pair of homologs, *FKH1* and *FKH2*. We therefore examined mutants in these genes (with the help of T. Davis, Seattle). We found that the *fkh1 fkh2* double mutant was defective in cell-cycle-regulated expression of *CLB2*, *SWI5*, and other genes in the *CLB2* cluster. Instead, these genes were now expressed at a constitutively low level. Thus, we believe we have identified a new cell cycle transcription factor. Interestingly, Fkh2p contains many consensus Cdc28p phosphorylation sites, suggesting it may be under the direct control of Cdc28p. In addition, the *fkh1 fkh2* double mutant has a constitutive pseudohyphal growth phenotype, suggesting that we may have identified the pathway, and the effector genes, for pseudohyphal growth.

Analysis of *WHI3*, a New Size Control Gene

T. Volpe

The *whi3* mutation was isolated some years ago by Rob Nash, and we are continuing its characteriza-

tion. It contains an RNA-binding motif, and we have recently shown that this RNA-binding motif is essential for Whi3p function. Like other *whi* mutants, it gives a small cell phenotype, which seems to be the reason *whi3* mutants overexpress *CLN1* and *CLN2* G_1 cyclins. Overexpression of *WHI3* turns off *CLN1* and *CLN2* and is lethal. Presumably, then, the normal role of *WHI3* is to somehow repress *CLN1,2* expression.

The level at which *WHI3* interferes with *CLN* expression, however, has been unclear. *WHI3* does not interfere with *CLN1* or *CLN2* expression from a *GAL* promoter, so *WHI3* probably does not act directly on the *CLN1,2* mRNAs. *CLN1* and *CLN2* transcription depends on the transcription factors Swi4p and Swi6p, and these are somehow activated by *CLN3*. We asked whether *WHI3* might interfere with the activation of Swi4p/Swi6p by *CLN3*. The answer may be yes, because (1) a *whi3* mutation cannot reduce the size of a *cln3* mutant (i.e., *cln3* is epistatic to *whi3*), and (2) a carboxy-terminally truncated Swi4p, which does not depend on activation by *CLN3*, can promote *CLN2* transcription even in the presence of excess Whi3p.

Presumably, Whi3p binds some RNA or RNAs. We immunoprecipitated Whi3p, and then carried out Northern analysis on the immunoprecipiates to determine whether *CLN3* or any other relevant mRNA was coprecipitated. So far, no such RNA has been found. Recently, RNAs coimmunoprecipitated with Whi3p have been sent for microarray analysis to determine if any RNAs can be identified.

Mechanisms of Transcriptional Activation by Cyclin-Cdc28p Complexes

H. Wijnen

One effect of the Cln3-Cdc28p complex—perhaps the only effect—is to induce transcription of a large family of genes involved in the G_1/S transition. We are trying to discover the mechanism of induction. The promoters of the *CLN3*-inducible genes all include binding sites for the Swi4p transcription factor or for its close relative Mbp1p. The Swi4p and Mbp1p DNA-binding proteins each forms a complex with another protein called Swi6p; these two complexes are called the SBF or MBF transcription factors, respectively. We have found that *swi6* mutants are completely defective for *CLN3*-induced transcription, and this argues that Swi6p is the direct or indirect target for Cln3p.

One model for activation of SBF/MBF by Cln3p is that Cln3p/Cdc28p phosphorylates SBF/MBF. This does not seem to be the case, because mutants lacking all consensus Cdc28p phosphorylation sites in SBF are wild type for activation by Cln3p. Another model is that Cln3p joins the SBF/MBF complex and acts relatively directly on transcription. This also does not seem to be correct, because (1) *cln3* mutants with an appended VP16 transcriptional activation domain do not hyperinduce transcription and (2) *cln3* mutants with an appended *TUP1* repression domain still induce transcription normally. Finally, it does not appear that *CLN3* activates an activator of SBF/MBF, because no such activator could be identified in a saturating high-copy screen.

A remaining model 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 Cln3p/Cdc28p) inhibits retinoblastoma (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*. Several such mutants have been found. One is in a gene encoding a component of a histone deacetylase, and the human homolog of this gene is a known RB-binding protein.

Finally, we have looked for mutants of Swi6p that can activate transcription even in the absence of *CLN3*. Alleles of Swi6p lacking the leucine zipper behave in this way, suggesting that the leucine zipper might be the binding site of the putative inhibitor of SBF.

New Telomere Maintenance Mutants

F. Ferrezuelo

We have done a screen to identify new yeast telomere maintenance mutants. Several mutants have been obtained, and these include two new alleles of *est2*, showing that the screen works. Another mutant was recently identified as *mtr10*, a karyopherin β involved in nuclear import. It may be important for import of

Est2p, Tlc1p (the telomerase RNA), or the Est2p/Tlc1p complex. We still have several unidentified mutants, which produce short telomeres.

Analysis of an RNA-binding Motif in the Telomerase Component Est1p

J. Zhou

The telomerase component Est1p has a homolog we call Est10p. Whereas *est1* mutants have a senescent phenotype ("ever shorter telomeres," leading to senescence and death), the *est10* mutant has only a short telomere phenotype. We noted a clear RNA-binding motif in Est10p, and a less clear RNA-binding motif in Est1p and found that these motifs were at the center of the most highly conserved region of the two proteins. This suggested that the role of Est1p/Est10p might be to bind the Tlc1p telomerase RNA and help form the complex with the catalytic subunit, Est2p. We destroyed the RNA-binding motif in Est1p by site-directed mutagenesis and found that this gave a dominant-lethal allele, consistent with the model. Furthermore, when the RNA-binding site was reconstructed, but with different amino acids, *EST1* function was restored. Immunoprecipitation of Est1p and *est1* mutants, followed by Northern analysis, showed that Tlc1p coprecipitated with Est1p, and with Est1p with a reconstructed RNA-binding site, but did not coprecipitate with the *est1* mutant lacking the RNA-binding site. This coprecipitation did not require the presence of Est2p (the catalytic reverse transcriptase), suggesting that the interaction of Est1p with Tlc1p is direct. Finally, it has been proposed that Est1p acts as a single-stranded DNA-binding protein. We could detect very little single-stranded DNA-binding activity, but such activity as was present was not affected by the RNA-binding site mutations.

In summary, we conclude that Est1p is likely an RNA-binding protein that binds Tlc1p, and in this way helps form the Est2p-Tlc1p complex. Other telomerases also contain RNA-binding proteins, although they are not homologous to Est1p.

PUBLICATIONS

Schneider B.L., Patton, E.E., Lanker S., Mendenhall M.D., Wittenberg C., Futcher B., and Tyers M. 1998. Yeast G_1 cyclins are unstable in G_1 phase. *Nature* **395:** 86–89.

Spellman P.T., Sherlock G., Zhang M.Q., Iyer V.R., Anders K., Eisen M.B., Brown P.O., Botstein D., and Futcher B. 1998. Comprehensive identification of cell cycle regulated genes of the yeast *Saccharomyces cerevisiae* by microarray hybridization. *Mol. Biol. Cell* **9:** 3273–3297.

In Press

Futcher B. 1999. Cell cycle synchronization in yeast cells by elutriation and block release methods. *Methods Cell Sci.* (in press).

DEVELOPMENTAL GENETICS OF PLANT REPRODUCTION

U. Grossniklaus R. Baskar, P. Hallock, P. Pultorak, I. Siddiqi, A. Coluccio, J. Moore, Y. Redler, J. Thomas, K. Gremski, J. Parkey, C. Spillane, J.-P. Vielle-Calzada

We use genetic and molecular approaches to characterize the developmental control of plant reproduction. We focus on the events that lead to the formation of the female gametes, the molecular and genetic basis of which is largely unknown. Unlike in animals, where the meiotic products differentiate directly into gametes, the spores of plants give rise to multicellular haploid organisms that produce the gametes later in their development. The key events in female gametogenesis occur within ovules, which are specialized reproductive structures of sporophytic origin that harbor the female gametophyte. The highly polar nature and simple organization of ovule and megagametophyte make it an ideal system to study fundamental aspects of plant development. The long-term goal of our research is to elucidate the role of cell lineage, positional information, and cell-cell communication in plant morphogenesis and cellular differentiation.

In addition to yielding important insights into fundamental concepts of plant development, a better understanding of the mechanisms controlling plant reproduction will also provide tools to manipulate the reproductive system. We are particularly interested in engineering components of apomixis, a natural form of asexual reproduction through seeds. The introduction of apomixis into crops promises economic and social benefits exceeding those of the green revolution. We are using the tools we generate in our basic research for a biotechnological manipulation of the sexual system toward apomixis in *Arabidopsis*. In addition, we also use genetic strategies to isolate mutants displaying apomictic traits in maize and, in collaboration with R. Pruitt (Harvard University), in *Arabidopsis*.

A collection of over 4000 transposants forms the basis for our analysis of female gametogenesis in *Arabidopsis*. Enhancer detection allows the identification of genes based on their pattern of expression. We are using the enhancer/gene trap system developed here at the Laboratory by V. Sundaresan, R. Martienssen, and coworkers (Sundaresan et al., *Genes Dev. 9:* 1810 [1995]). Our transposant collection was subjected to four independent screens to identify genes involved in ovule morphogenesis, megasporogenesis, female gametophyte development, and fertilization.

Alterations of Megasporogenesis and Induction of Apomixis in *Arabidopsis*

J.-P. Vielle-Calzada, U. Grossniklaus

Megasporogenesis is initiated as a single hypodermal cell in the ovule primordium differentiates into the megaspore mother cell (MMC). The MMC undergoes meiosis to produce four haploid megaspores. Only one of the megaspores survives, divides three times, and ultimately gives rise to a functional megagametophyte. Whereas most flowering plants including *Arabidopsis* reproduce sexually, some species can form embryos that are genetically identical to the maternal parent through apomixis. Apomictic embryos arise from a cell lineage lacking both meiosis and fertilization, leading to the formation of clonal seeds. Apomixis is genetically controlled, but the molecular mechanisms and genetic basis of its regulation are largely unknown. The determination of apomictic initial cells occurs during megasporogenesis. It is likely that some of the genes regulating sexual development are also responsible for the induction of apomixis.

To test this hypothesis, we are isolating genes that act during early ovule development in *Arabidopsis*. By screening reporter gene expression in over 1100 transposants, we identified 27 lines that show expression in the developing ovule. In most, expression can be traced back to the onset of MMC differentiation. Some of the genomic sequences flanking the insertions show similarity to genes with important roles in plant and animal development (e.g., cell cycle regulators, receptor kinases, MAP kinase kinases, and chromatin remodeling factors). Three lines show expression patterns that are of particular interest to megasporogenesis and the induction of apomixis: In the first one, a few cells in the nucellus that might correspond to a group of cells with the potential to differentiate into a MMC express the reporter gene. In the second line, expression is restricted to the MMC itself, whereas in the third line, only the surviving megaspore stains. Regulatory sequences controlling nucellar and MMC-specific expression will serve as important tools to alter determination and cellular interactions during

megasporogenesis and to manipulate the reproductive potential of sexual plants toward apomixis.

Genetic Analysis of *dyad*, a Mutation Affecting Female Meiosis in *Arabidopsis*

I. Siddiqi, U. Grossniklaus

The *dyad* mutant of *Arabidopsis* causes female sterility by affecting the division of the MMC. The mutant was isolated at the Centre for Cellular and Molecular Biology, Hyderabad, India, and the experiments described below were conducted during a sabbatical visit here at the Laboratory. In the *dyad* mutant, normal meiosis is altered such that the MMC undergoes a single division giving rise to a dyad instead of a tetrad, which then arrests. Understanding the function of the *dyad* gene is of particular interest with respect to processes that are unique to female meiosis and megasporogenesis. What type of division does the MMC undergo in the *dyad* mutant? The mutant phenotype could be due to an arrest at the end of meiosis I, or, alternatively, it could be that the MMC undergoes a mitotic division instead of a meiotic one. We had prior evidence in support of the first explanation based on the appearance of a cytological marker (callose) that is expressed during meiosis in the MMC.

At Cold Spring Harbor, we further addressed the issue of whether the MMC in *dyad* divides meiotically or mitotically by examining the expression of the meiosis-specific gene *AtDMC1* by RNA in situ hybridization using protocols developed by J.-P. Vielle-Calzada. We also examined chromosome segregation during the division using confocal microscopy. In addition, ultrastructural studies were carried out by transmission electron microscopy. The results from all three approaches indicated that the MMC in *dyad* undergoes meiosis I but arrests before meiosis II. This suggests that the *dyad* gene is specifically required during female meiosis for entry into meiosis II. We are continuing this collaboration by examining the expression of ovule- and megagametophyte-specific markers in the *dyad* mutant.

Characterization of *tlazolteotl*, a Mutant Affecting Ovule Morphogenesis

J.-P. Vielle-Calzada, K. Gremski (URP Program), P. Hallock (Partner for the Future), U. Grossniklaus

We have made substantial progress in the characterization of one of the female sterile mutants we identified. Ovules of this mutant have shorter integuments, a dense region of proliferating cells at the chalaza, and abnormal embryo sacs often protruding through the micropyle (Fig. 1). After fertilization, a few viable seeds are formed. The *GUS* expression pattern in this transposant correlates with the tissues affected in the mutant. We named it *tlazolteotl* (*tlz*), after the Aztec goddess of fertility who is often represented as giving birth through the mouth. Complementation analysis has shown that *tlz* is not allelic to previously characterized mutants (*ant*, *bel*, *ino*, *sin*, and *exc*). Reversion analysis showed that the single *Ds* element present in this line is responsible for the mutant phenotype. The sequence flanking the insertion site shows strong homology with a hypothetical protein with domains often found in transcription factors. We are currently focusing on the isolation of a *TLZ* cDNA, the determination of the pattern of *TLZ* mRNA localization, and the investigation of genetic interactions of *tlz* with other genes controlling ovule development.

Characterization of Mutants Disrupting Megagametogenesis

J. Moore, J. Parkey, P. Pultorak, U. Grossniklaus

During megagametogenesis, the mature female gametophyte consisting of seven cells (1 egg cell, 2 synergids, 3

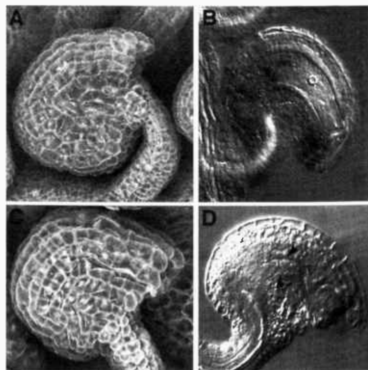


FIGURE 1 Morphological comparison of wild-type (*A,B*) and *tlazolteotl* (*tlz*) ovules (*C,D*). Fixed specimens were either gold-coated and observed with a scanning electron microscope (*A,C*) or cleared in Herr's solution and observed with Nomarski optics (*B,D*). In contrast to wild-type, *tlz* ovules have short integuments, a dense region of proliferating cells at the proximal pole (arrowheads in *D*), and abnormal embryo sacs often protruding through the micropyle (*D*).

antipodals, and 1 binucleate central cell) is formed. After double fertilization of both the egg and the central cell, the ovule develops into a seed. Despite the importance of this process for plant reproduction, only a small number of mutants affecting megagametogenesis have been described. We identified about 50 mutants affecting the gametophytic phase of the plant life cycle and characterized 14 of these at the genetic and morphological level. Of the 14 mutants, 12 have been mapped and were shown to represent independent loci distributed over all chromosomes. The transmission efficiency of the *Ds* elements associated with these mutations through male and female gametophytes was determined. All but two mutants were found to affect the female, but most also affected the male to various degrees. A morphological characterization of male gametophyte development in mutants with reduced male transmission has been performed in collaboration with D. Twell (University of Leicester). We also thank Nikhil Laud and Sean Kass (both from Roslyn High School), who helped with reversion analyses and the characterization of pollen tube guidance in these mutants, respectively.

Based on morphological defects, the mutants can be grouped into four classes: (1) mitotic division cycles are abnormal, (2) nuclear fusion of polar and/or sperm nuclei is affected, (3) abnormal degeneration processes occur, and (4) the megagametophyte develops to maturity, but fertilization is not properly effected. The phenotype of one member of each class is described as an example. In *haumea* (*hma*), named after a Polynesian-Hawaiian goddess of fertility, the regulation of the mitotic division cycles is disturbed, resulting in mitotic arrests, asynchronous nuclear divisions, or sometimes in additional division cycles such that megagametophytes with up to 22 nuclei have been observed. In *jumis* (*jum*), named after a Latvian god of fertility, karyogamy is disrupted, and the polar nuclei in the central cell do not fuse nor do the sperm nuclei fuse with their targets. In *anat* (*ana*), which honors a Canaanite fertility goddess, the antipodals and synergids, which usually degenerate, often persist. The megagametophytes of *feronia* (*fer*), named in honor of the Etruscan goddess of fertility and fire, develop normally and attract pollen tubes, but seed development is not initiated, suggesting that double fertilization or the activation of seed development is disrupted. To date, we have obtained sequences flanking the insertion site for 12 of these mutants. As expected, some of these have similarities with proteins involved in basic cellular processes, whereas others appear to have regulatory functions.

Cell Specification in the Female Gametophyte

R. Baskar, J. Moore, J.-P. Vielle-Calzada, U. Grossniklaus

Although the seven cells of the megagametophyte are clonally derived, they develop along four alternative developmental pathways, making it an ideal system to study cell specification. We have screened over 2300 transposants for reporter gene expression in mature ovules. Among the enhancer detector transposants, about 5% show expression in the female gametophyte. Whereas some of these show expression in all cells of the megagametophyte, others are specific to a subset of cells or to individual cell types. We are particularly interested in the specification and function of the egg cell. The egg-cell-specific expression of the two transcription units we identified was verified by in situ hybridization, and genomic phages spanning the insertion sites were isolated. Reporter gene constructs containing up to 4 kb of 5′ sequences of one of these genes are being analyzed by *Agrobacterium*-mediated transformation. Once identified, egg-cell-specific promoters will be used for genetic cell ablation and misexpression of putative regulatory genes involved in egg activation. This will allow us to investigate cellular interactions between the cells of the megagametophyte and to probe the potential of the egg cell for autonomous activation, an important component of apomictic reproduction.

The *medea* Locus Is Regulated by Genomic Imprinting

J.-P. Vielle-Calzada, J. Thomas, U. Grossniklaus

We have previously shown that the *Arabidopsis* mutant *medea* (*mea*) displays a gametophytic maternal effect. Seeds derived from megagametophytes carrying a mutant *mea* allele abort after delayed morphogenesis with excessive cell proliferation in the embryo and reduced free nuclear divisions in the endosperm. *MEA* encodes a SET domain protein of the *Polycomb* and *trithorax* group, which are believed to maintain repressed or active states of gene expression during development by modulating higher-order chromatin structure. During the last year, we elucidated the nature of the *mea* maternal effect. Because the endosperm inherits two maternal copies but only one paternal copy of the genome, *mea* could affect a dosage-sensitive gene required for endosperm development. Alternatively, the mutation could disrupt a maternally produced gene product stored in the egg and/or central cell, which is required for seed development. As a third possibility, the mutation could affect an imprinted gene that is

only transcribed from the maternally inherited allele.

Using duplex tetraploid plants (*mea/mea/MEA/MEA*) that produce endosperm carrying from 0 to 6 mutant *mea* alleles, we could exclude gene dosage as the cause for the *mea* maternal effect. The latter two possibilities were distinguished using in situ hybridization and reverse transcription (RT)-PCR. *MEA* is expressed prior to fertilization in the megagametophyte, but also after fertilization in the embryo and endosperm. The high level of *MEA* transcript detected several days after fertilization suggests that *MEA* is transcribed not only maternally, but also zygotically. In the endosperm, we could directly visualize zygotic transcription of the *mea* locus by a sensitive in situ hybridization protocol that allows the detection of nuclear dots representing nascent transcripts. In triploid endosperm nuclei, only two nuclear dots are visible, suggesting that only two of the three *mea* loci are actively transcribed after fertilization. Using PCR conditions that allow a distinction between various *mea* alleles, we could independently confirm that paternally inherited *MEA* alleles are not transcribed in either fertilization product. Thus, the *mea* locus is regulated by genomic imprinting in both the embryo and endosperm.

Regulation of Genomic Imprinting in Plants

C. Spillane, A. Coluccio, J. Thomas, U. Grossniklaus

Genomic imprinting, where individual genes are expressed depending on their parental origin, has only been described in mammals and plants. This may have its evolutionary origin in a shared strategy to produce offspring (placental habit) that led to a parental conflict over the allocation of nutrients from the mother to her offspring (Haig and Westoby, *Am. Nat. 143*: 147 [1989]). Only a few imprinted genes have been described in plants. *MEA* is the first one that shares many features with imprinted genes in mammals: *MEA* is the only known imprinted plant gene that affects morphogenesis and where imprinting is not allele-specific. Despite its importance in development and disease, the regulation of imprinting is still poorly understood. Using *mea* as a tool, we try to elucidate the regulation of imprinting using molecular and genetic approaches. Several constructs have been made to identify the *cis*-regulating sequences that control parent-of-origin-specific expression of *MEA*. In addition, we use the powerful genetics of *Arabidopsis* to identify *trans*-acting factors involved in imprinting. To this aim, we use chemical mutagenesis to isolate mutants that modify the *mea* seed abortion phenotype. To date, we have identified several mutations that alter the frequency of seed abortion in a heterozygous *mea* plant, some of which may be regulators of genomic imprinting. As an alternative to isolating new mutations that modify the *mea* phenotype, we are screening the natural variation in different *Arabidopsis* ecotypes for modifier loci. We are also analyzing genetic interactions with mutants that have a known effect on transgene silencing or DNA methylation which were isolated in other laboratories. A better understanding of imprinting in plants will be essential for the engineering of apomixis in sexual crops. These are usually highly sensitive to imbalances in the ratio of maternal to paternal genomes in the endosperm as occurs if a reduced sperm fertilizes a nonreduced central cell. Imprinting is also thought to be an obstacle to wide crosses that have a great potential for plant breeding.

PUBLICATIONS

Grossniklaus U. and Vielle-Calzada J.-P. 1998. Response: Parental conflict and infanticide during embryogenesis. *Trends Plant Sci.* **3:** 328.

Grossniklaus U. and Schneitz K. 1998. The molecular and genetic basis of ovule and megagametophyte development. *Sem. Cell Dev. Biol.* **9:** 227–238.

Grossniklaus U., Moore J.M., and Gagliano W. 1998. Molecular and genetic approaches to understanding and engineering apomixis: *Arabidopsis* as a powerful tool. In *Advances in hybrid rice technology*. Proceedings of the 3rd International Symposium in hybrid rice, 1996 (ed. S.S. Virmani et al.), pp. 187–211. International Rice Research Institute, Manila, Philippines.

Grossniklaus U., Koltunow A., and van Lookeren Campagne M. 1998. A bright future for apomixis. *Trends Plant Sci.* **3:** 415–416.

Grossniklaus U., Vielle-Calzada J.-P., Hoeppner M.A., and Gagliano W.B. 1998. Maternal control of embryogenesis by *MEDEA*, a *Polycomb*-group gene in *Arabidopsis*. *Science* **280:** 446–450.

Howden R., Park S.-K., Moore J.M., Orme J., Grossniklaus U., and Twell D. 1998. Selection of T-DNA-tagged male and female gametophytic mutants by segregation distortion in *Arabidopsis*. *Genetics* **149:** 621–631.

Moore J.M., Grossniklaus U., Vielle-Calzada J.-P., and Gagliano W.B. 1998. Altering sexual development in *Arabidopsis*. *J. Plant Biol.* **41:** 73–81.

In Press

Grossniklaus U. 1999. From sexuality to apomixis: Molecular and genetic approaches. In *Advances in apomixis research* (ed. Y. Savidan and J. Carman). (In press.)

Lemieux B. and Grossniklaus U. 1999. Paper alert in genomics and molecular genetics. *Curr. Opin. Plant Biol.* (in press).

Messing J. and Grossniklaus U. 1999. Genomic imprinting in plants. In *Results and problems in cell differentiation* (ed. R. Ohlsson). Springer Verlag, Berlin, Germany. (in press.)

Naumova T.N. and Vielle-Calzada J.-P. 1999. Ultrastructural analysis of apomictic development. In *Advances in apomixis research* (ed. Y. Savidan and J. Carman). (In press.)

PLANT GENETICS

E. Grotewold E.L. Braun P.D. Rabinowicz
M.J. Hernandez G.M. Sosa
M. Pizzirusso

Evolution of Plant Myb Domain Proteins

P.D. Rabinowicz, E.L. Braun (Ohio State University), E. Grotewold

Transcription factors containing the Myb homologous DNA-binding domain are widely found in eukaryotes. In plants, R2R3 Myb domain proteins are involved in the control of form and metabolism. The *Arabidopsis* genome harbors more than 100 *R2R3 myb* genes, but few have been found in monocots, animals, and fungi. Using reverse transcriptase–polymerase chain reaction (RT-PCR) from different maize organs, we cloned 480 fragments corresponding to a 42–44-residue-long sequence spanning the region between the conserved DNA-recognition helices (Myb^{BRH}) of the *R2R3 myb* domains. We determined that maize expresses more than 80 different *R2R3 myb* genes. Evolutionary distance analyses among maize Myb^{BRH} sequences indicate that most of the amplification of the *R2R3 myb* gene family occurred within the past 550 million years, possibly after the origin of land plants, but prior to the separation of the two main groups of flowering plants. Interestingly, particular classes of *R2R3 myb* genes have suffered a dramatic amplification within the past 30 million years within the grasses, adding support to the idea that expansion of this gene gamily has occurred in conjunction with the development of novel cellular functions.

Cloning and Characterization of a Maize WD40 Protein That Has High Homology with Regulators of Anthocyanin Accumulation and Trichome Formation

M. Pizzirusso (Partners for the future student 1997), M.J. Hernandez (Ohio State University), G.M. Sosa (Ohio State University), E. Grotewold

The An11 protein of Petunia controls anthocyanin accumulation, probably by modulating the function of the Myb domain protein An2. Using degenerate primers derived from conserved regions of the *An11* gene, a PCR fragment was generated from cDNA obtained from maize seedling poly(A)$^+$ RNA. After cloning and sequencing, the PCR fragment was used as a probe to screen maize cDNA and genomic libraries. Sequence analysis of the corresponding genomic and cDNA clones revealed a gene without introns with an open reading frame (ORF) about 410 amino acids long encoding an MP1 protein of 45 kD. The MP1 protein contains conserved WD40 repeats and has a very high identity to An11 and TTG. Northern analysis indicated that *MP1* is expressed in all maize organs investigated including silks, seedlings, roots, tassels, and immature ears. This is similar to what has been previously observed for An11. Mapping experiments positioned *MP1* to the short arm of chromosome 5, close to the recently identified *pale aleurone color1* (*pac1*) maize mutant.

The MP1 protein was expressed in *Escherichia coli* as a histidine-tagged fusion (N6His-MP1), affinity-purified, and injected into rabbits. Polyclonal antibodies will be used to determine whether the subcellular localization of MP1 is cytoplasmic, as observed for An11. Purified N6His-MP1 protein was utilized in in vitro kinase assays together with total protein extracts from maize aleurones and pericarps in the presence of [γ-^{32}P]ATP. A phosphorylated band of the correct molecular weight in PAGE-SDS gels was identified in reactions containing MP1, but absent in control reactions. These preliminary findings suggest that MP1 can be phosphorylated by a protein kinase in maize extracts and provide additional support to the idea that MP1, TTG, and An11 are part of a signal transduction cascade.

PUBLICATIONS

Mol J., Grotewold E., and Koes R. 1998. How genes paint flowers and seeds. *Trends Plant Sci.* **3:** 212–217.

Grotewold E., Chamberlain M., Snook M., Siame B.A., Butler L.G., Swenson J., Maddock S., St. Claire G., and Bowen B. 1998. Engineering secondary metabolism in maize cells by ectopic expression of transcription factors. *Plant Cell.* **10:** 721–740.

van Aalten D.M.F., Grotewold E., and Joshua-Tor L. 1998. Essential dynamics from NMR clusters: Dynamic properties of the Myb DNA-binding domain and a hinge-bending enhancing variant. *Methods—A companion to Methods in Enzymology* **14:** 318–328.

CANCER GENETICS

M. Hamaguchi B. O'Connor
J. Troge

We are interested in the genes responsible for carcinogenesis and metastasis. The analysis of cancer genes is the first step in elucidating the molecular mechanisms of the development of cancer. For this purpose, we have applied and developed genetic tools, including representational difference analysis (RDA), rapid isolation of cDNAs by hybridization (RICH), and exon trapping. Genetic lesions were first discovered by RDA. A transcript map was constructed by utilization of RICH, a new technique, as well as other available methods including exon trapping, database searches, and sequencing. We are also trying to develop a mutation detection system based on RDA.

New Technology for Positional Cloning

M. Hamaguchi, B. O'Connor [in collaboration with R. McCombie and M. Wigler, Cold Spring Harbor Laboratory]

Powerful methods facilitate the localization of disease genes to chromosomal regions. We have applied positional cloning to discover these genes. The regions covered by contigs of large genomic clones (yeast artificial chromosomes or YACs, bacterial artificial chromosomes or BACs, and P1s) must be analyzed to identify transcribed sequences. Candidate genes are winnowed out from the transcripts in the region by mutation analyses.

The rate-limiting step in positional cloning is the search for transcribed sequences in a given genomic region. This problem has been approached in a number of ways: exon trapping, computational analysis after DNA sequencing, and direct hybridization selection. Exon trapping is effective when the splice sites of the genes in question are recognized accurately by the host cells, but cryptic splice sites cause a background of false positives. DNA sequencing is still costly and time consuming. Direct hybridization selection is useful in some cases, but it diminishes in usefulness when the target genes are rare messages. This method also suffers from background problems created by repetitive sequences.

We have developed RICH, a new method of isolating cDNAs that have sequences in common with large genomic clones. RICH is meant to complement the isolation of transcribed sequences by exon trapping and DNA sequencing. The ends of cDNA fragments are modified so that they can be selected only when they hybridize to fragments derived from large genomic clones covering the regions of interest. We have demonstrated that RICH is a powerful tool when applied to amplified loci such as the c-*myc* locus. We have also demonstrated that RICH works on the suppressor locus PTEN (*p*hosphatase and *ten*sin homolog). The yield of PTEN fragments was less satisfactory than that of c-*myc*, we believe, due to a reduced level of expression of PTEN. An additional prescreening of the products, however, is helpful for subsequent cloning.

We have also introduced a variation in our method: selectively amplifying genomic DNA fragments that have homology with cDNAs, i.e., RICH in reverse. This method is called rapid isolation of genes by hybridization to transcripts (RIGHT). The RIGHT products are all genomic fragments and therefore are ready to be used for polymerase chain reaction (PCR)-based analysis such as radiation hybrid mapping and deletion mapping by quantitative PCR (Q-PCR). Utilization of both methods may facilitate the confirmation of cDNAs and aid in the determination of intron-exon boundaries.

Loci Deleted in Breast Cancer

M. Hamaguchi, J. Troge, B. O'Connor [in collaboration with R. McCombie and M. Wigler, Cold Spring Harbor Laboratory]

Loss of heterozygosity (LOH) has reportedly occurred in more than half of breast cancers. RDA has identi-

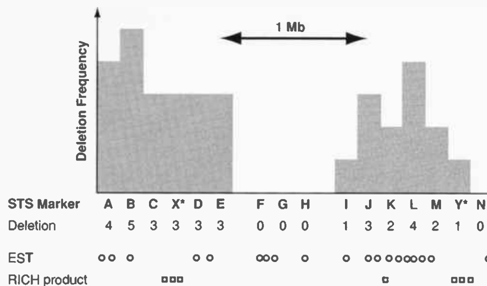


FIGURE 1 Deletion map of the short arm of chromosome 8. Double arrowheads indicate approximately 1 Mb. The letters represent STSs used for deletion analysis. The deletion numbers are listed under the corresponding STS markers and represented by the height of the columns. (*Gray circles*) Localization of ESTs found in the databases; (*gray squares*) RICH products. Two deletion peaks are demonstrated in the figure. One epicenter is mapped to 8p23 (B) and the other to 8p22 (L).

fied two homozygous deletions of 8p22 and 8p23. These facts imply the presence of tumor suppressor genes at 8p22 and 8p23. Our efforts have been directed at cloning these suppressor genes.

First, RDA probes were localized by radiation hybrid mapping. The radiation hybrids are rodent cells containing fragments of human genomic DNA generated by irradiation. The relative distance between known sequence tagged site (STS) markers and the probe in question is obtained by comparison of the presence of the marker and the probe in each radiation hybrid. One RDA probe was mapped to 8p23 and the other to 8p22. Second, physical maps of these regions were constructed. Library screening and database searches generated 20 YACs, 9 BACs, and 2 P1s. STSs around the original RDA probes were isolated by database searches, subcloning of genomic clones, and sequencing of P1s. These STSs were further mapped by radiation hybrids and analyzed by PCR against genomic clones to determine their physical order. Subsequently, deletion mapping was carried out by Q-PCR on 87 breast tumors using 16 STSs.

Figure 1 demonstrates two distinctive deletion epicenters that are mapped to 8p22 and 8p23. The size of each deletion is estimated at 1 Mb. A transcript map was then constructed by database searches, RICH, exon trapping, and sequencing. This procedure has provided 32 candidate sequences including 19 expressed sequence tags (ESTs), 7 RICH products, 2 trapped exons, and 4 exons predicted by computational analysis. Reverse transcriptase PCR analysis showed that 7 out of 32 fragments are expressed in normal mammary glands. Among them, three sequences have detected positive clones in cDNA libraries from normal breast tissues. These candidate genes are being analyzed further.

Identification of Mutations in Tumors

M. Hamaguchi, J. Troge [in collaboration with
S. Lowe and M. Wigler, Cold Spring Harbor Laboratory]

We recently commenced a project utilizing a modified RDA technique to detect mutations with LOH. Homozygous deletions identified by conventional RDA are generally large, and laborious deletion analysis is unavoidable in narrowing down the commonly deleted region. Moreover, tumor suppressor genes are frequently inactivated by mutations accompanied by LOH. We believe that isolation of mutant genes in the LOH region is directly linked to the discovery of tumor suppressor genes. We are now exploring the possibility of improving the protocols of cDNA-RDA for this purpose. When genomic alterations are accompanied by functional LOH, i.e., any suppression of transcription such as physical LOH, genomic imprinting, and repression, only the mutants exist in the cDNA representation. If the mutations alter recognition sites of frequent cutters, modified RDA with restriction enzyme digestion will detect these mutations as well as basic differences in the transcripts of two tumors. In contrast, conventional cDNA-RDA identifies only basic differences. Mutations of the genes in the LOH region will be identified by comparison of the products from both modified and conventional RDA procedures. We are testing this idea by examining mouse tumors.

PUBLICATIONS

Hamaguchi M., O'Connor E.A., Chen T., Parnell L., McCombie R.W., and Wigler M.H. 1998. Rapid isolation of cDNA by hybridization. *Proc. Natl. Acad. Sci.* **95:** 3764–3769.

GROWTH CONTROL IN ANIMAL CELLS

G. Hannon P. Dong
C. Frangou

The long-standing goal of our laboratory remains an understanding of the mechanisms that contribute to tumorigenesis. Normal cells are protected from oncogenic transformation by homeostatic responses that prevent cell proliferation in the face of potentially oncogenic alterations. These oversight mechanisms require well-known tumor suppressor pathways such as p53 and p16. During the past year, we have focused our efforts on two processes that have a clear relationship to neoplastic transformation:cellular mortality control and oncogene-dependent apoptosis.

PROTECTION FROM ONCOGENE-DEPENDENT APOPTOSIS

During the past several years, it has become clear that normal cells respond to oncogene activation in a surprising way. For example, normal cells that are forced to express activated *ras* senesce. Upon expression of oncogenes such as *myc* or *E1A*, normal cells are sensitized to programmed cell death. These responses are likely to be key tumor suppressive mechanisms.

Since oncogenes such as *myc* predispose normal cells to apoptosis, additional genetic alterations must occur during tumorigenesis that counteract the pro-apoptotic effects of oncogenes. During her stint as a Visiting Scientist here at the Laboratory, Roberta Maestro (now at the CRO, Aviano, Italy) devised a genetic screen to identify genes that could protect normal cells from oncogene-dependent cell death. One of the genes isolated through this approach was a developmentally regulated transcription factor, Twist.

Twist is a basic helix-loop-helix protein that has been implicated in the control of diverse developmental processes. Mice specifically lacking the *twist* gene die at day 9.5 p.c. and show massive apoptosis in the developing somites, a site of embryonic *twist* expression. We found that in cell culture, ectopic *twist* expression protected from the predisposition to apoptosis that occurs upon expression of both cellular (c-*myc*) and viral (*E1A*) oncogenes.

Oncogene-dependent apoptosis proceeds largely through a mechanism that requires an intact p53 tumor suppressor protein. Twist could interfere with p53-dependent apoptosis in MEF (mouse embryonic fibroblast) cells that had been transformed with *E1A* plus *ras*, a system originally characterized by Scott Lowe here at the Laboratory. Twist could also interfere with other p53 activities. MEF cells that have been engineered to express a temperature-sensitive version of p53 arrest upon shift to the permissive temperature. However, in the presence of Twist, this arrest is suppressed.

Since Twist interfered with oncogene-dependent apoptosis and with the activity of a known tumor suppressor, we sought to determine whether inappropriate Twist expression might contribute to tumorigenesis. We surveyed a number of tumor types and found frequent Twist expression in rhabdomyosarcomas.

Rhabdomyosarcoma is the most common sarcoma in children. This tumor is thought to derive from skeletal muscle precursor cells that fail to complete terminal differentiation. Twist is generally excluded from differentiating muscle cells, but in 50% of rhabdomyosarcomas, Twist expression is maintained.

Inappropriate expression of Twist in rhabdomyosarcomas is interesting from several standpoints. First, enforced Twist expression had previously been shown to block myogenic differentiation, although the precise mechanism was unclear. We have shown that Twist can protect from programmed cell death and antagonize a tumor suppressor. Thus, *twist* may contribute to the formation of rhabdomyosarcomas through multiple routes.

The diverse effects of *twist* may have a single underlying biochemical basis. In collaboration with Larry Kedes' group (University of California, Los Angeles), we have shown that Twist can interact physically with a promiscuous coactivator, p300. Recently, p300 has been shown to promote transcriptional activation by p53. The p300 protein is an acetyltransferase that can modify histones and thus affect chromatin structure. p53 itself is also acetylated by p300, and this modification may affect both DNA binding and transcriptional activation. It has recently been shown that Twist can inhibit p300 acetyltransferase activity. Since p300 serves as a coactivator for diverse transcription factors (including myogenic proteins), the interaction between Twist and p300 provides a

potential biochemical mechanism for the effects that we see on p53 function and for additional effects of *twist* on myogenic differentiation and other diverse developmental processes.

CELLULAR MORTALITY CONTROL

Normal human cells have a limited replicative life span. When placed in culture, these cells can execute only a predetermined number of cell divisions before entering an irreversible state of growth arrest termed cellular senescence. Tumor cells often escape from these life-span limitations, thus becoming capable of indefinite proliferation. It is likely that inactivation of mortality controls and release from proliferative limits are essential for tumorigenesis, particularly for the formation of metastases.

The consumption of proliferative potential by a normal cell in culture is measured by loss of telomeres. Telomeres are simple, repetitive sequences that cap the ends of chromosomes. At each cell division, these repeats are progressively lost due to the inability of the DNA replication machinery to completely reproduce a linear DNA molecule. As these repeat arrays become critically short, an as yet mysterious signaling mechanism triggers senescence.

In immortal cells, such as germ-line and tumor cells, telomeres are maintained by the action of telomerase, an enzyme that adds repeats to chomosome ends. At the end of last year, we showed, in collaboration with Jenny Wang and David Beach (CSHL, The Institute of Child Health, London), that the life span of a normal cell could be extended by artificially activating telomerase in an otherwise mortal cell. We have now demonstrated that telomerase activation is sufficient to immortalize these cells, which have now been passed continuously in culture for more than 1 year.

Although telomerase is activated in a high percentage of human cancers, the underlying mechanism of this activation was unknown. We found that expression of the limiting subunit of telomerase, hTERT, was regulated by a human oncogene, c-*myc*, that shows increased activity in a broad spectrum of human tumors. This provided an explanation for telomerase activation in transformed cells and added at least one piece to the puzzle of how *myc* activation contributes to tumorigenesis.

Despite the fact that we could immortalize some cultured human cells through a single genetic alteration, it is unlikely that telomerase activation is sufficient to allow continuous propagation of any primary human cell. In fact, one of the "normal" cell lines used in our studies had already silenced the $p16^{INK4a}$ tumor suppressor prior to our introduction of telomerase. In the other cell type, normal human fibroblasts, we find that extended life-span populations are under continuous pressure to extinguish p16 expression. This is consistent with recent work that has defined a second mortality control point that uses p16 as an effector but that is telomere-independent. Current efforts are aimed at defining the spectrum of molecular events that are required to allow unlimited propagation of a truly normal human cell in culture. Through a definition of these events, we may devise methods for the expansion of normal human cells in a way that does not select for populations that contain potentially oncogenic alterations.

PUBLICATIONS

Carnero A. and Hannon G.J. 1998. The INK4 family of CDK inhibitors. *Curr. Top. Microbiol. Immunol.* **227:** 43–55.

Fearnhead H.O., Rodriguez J., Govek E.E., Guo W., Kobayashi R., Hannon G., and Lazebnik Y.A. 1998. Oncogene-dependent apoptosis is mediated by caspase-9. *Proc. Natl. Acad. Sci.* **95:** 13664–13669.

LaMontagne K.R., Hannon G., and Tonks N.K. 1998. Protein tyrosine phosphatase PTP1B suppresses p210 bcr-abl-induced transformation of rat-1 fibroblasts and promotes differentiation of K562 cells. *Proc. Natl. Acad. Sci.* **95:** 14094–14099.

Sun P., Dong P., Dai K., Hannon G.J., and Beach D. 1998. p53-independent role of MDM2 in TGF-β1 resistance. *Science* **282:** 2270–2272.

Wang J., Xie L.Y., Allan S., Beach D., and Hannon G.J. 1998. Myc activates telomerase. *Genes Dev.* **12:** 1769–1774.

In Press

Hannon G.J., Sun P., Carnero A., Xie L.Y., Maestro R., Conklin D.S., and Beach D. 1999. MaRX: An approach to genetics in mammalian cells. *Science* **283:** 1129–1130.

CAENORHABDITIS ELEGANS DEVELOPMENTAL GENETICS

M. Hengartner S. Desnoyers, R. Dodes (URP), A. Fraser, A. Gartner, T. Gumienny, D. Hoeppner, J. Keller, Q. Liu, S. Milstein, M. Spector, S. Tharin

Ever since its introduction to the research community by Sydney Brenner, the small nematode *Caenorhabditis elegans* has been used extensively as a model organism for the study of complex biological phenomena, including developmental and signal transduction pathways, neurobiology, and aging. The value of *C. elegans* for such studies derives in part from its great simplicity at the anatomical and developmental levels, from its powerful genetics and ease of maintenance, and from the availability of powerful reverse genetic tools (gene knockout, RNA-mediated interference). The completion of the worm genome at the end of this year has made *C. elegans* ready for the next generation of molecular and developmental biologists.

Despite its great simplicity, 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 used as a useful model system. Our laboratory uses *C. elegans* to study two basic biological problems: programmed cell death (apoptosis) and nervous system development and function.

PROGRAMMED CELL DEATH

Programmed cell death (PCD), or apoptosis, is a highly regulated program of cell suicide. Apoptosis is employed in many key biological processes, from the removal of unnecessary cells during development to the maintenance of cell populations and the deletion of potentially dangerous (e.g., cancer-prone) cells in adults. Defects in cell death also have a central role in the pathogenesis of many human diseases including AIDS, various neurodegenerative diseases, and cancer. An understanding of the underlying molecular mechanisms of both the regulation and execution of cell death may thus allow significant improvement in therapeutic design to treat these diseases.

More than 12 genes have been identified that function in programmed cell death in *C. elegans*. These genes divide the process into three distinct steps: execution of the death sentence, engulfment of dying cells, and degradation of dead engulfed cells. 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 antagonized by the Bcl-2 family member CED-9. CED-9 has been proposed to prevent death by sequestering CED-4 and proCED-3 in an inactive ternary complex, the apoptosome. In cells fated to die, CED-9 is in turn inactivated by the pro-apoptotic BH3 domain-containing protein EGL-1. The structural and functional conservation of cell death genes between nematodes and mammals strongly suggests that the apoptotic program is ancient in origin and that all metazoans share a common mechanism of apoptotic cell killing.

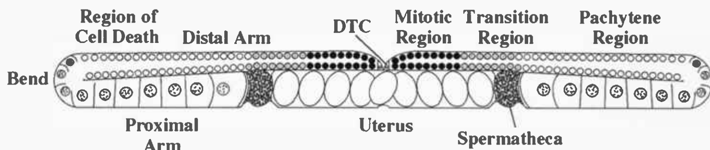
Physiological Cell Death in the *C. elegans* Germ Line

T. Gumienny, S. Milstein

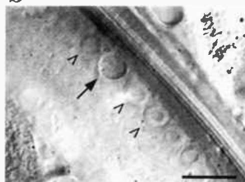
How is the apoptosome regulated? The answer to this question is likely to be complex, as even a single cell might need to integrate multiple signals to determine whether it should activate the death pathway or keep on living. To address this question in *C. elegans*, we have set out to determine how specific cell types decide between life and death.

One of the cell types that we chose to focus on is the germ cell. PCD plays a major part in the adult hermaphrodite germ line. Our previous studies have shown that a majority of the germ cells that differentiate along the oogenic pathway undergo PCD (Fig. 1). We suspect that these deaths serve a homeostatic function and are needed to regulate the number of cells that are allowed to differentiate into oocytes. Indeed, loss of germ cell death results in germ line hyperplasia, which likely accounts for the lower fertility of *ced-3* and *ced-4* mutants.

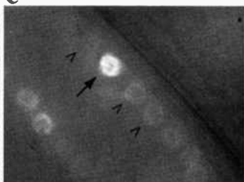
a



b



c



d

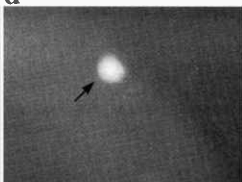


FIGURE 1 Germ cell apoptosis. (*a*) Schematic diagram of an adult hermaphrodite *C. elegans* gonad. (*b–d*) Apoptotic germ cells (arrows) can readily be distinguished from their surviving sisters (arrowheads) by DIC light microscopy (*b*), Hoechst nuclear staining (*c*), and SYTO12 vital dye staining (*d*). Bar, 10 μm.

The CED-9/CED-4/CED-3 apoptosome appears to be regulated differently in the germ line than in the soma. Loss of *egl-1* function or gain of *ced-9* function prevents most, if not all, developmental deaths. In contrast, these mutations have no effect on germ cell death, suggesting that perhaps another BH3 domain protein inhibits CED-9 in the germ line.

To further decipher how germ cells make the decision between life and death, we have screened for mutations that specifically affect PCD in the germ line. We have thus far isolated more than two dozen mutations that result in excessive germ cell death. We are now in the process of genetically characterizing and classifying these mutations, as a prelude to positional cloning.

DNA-damage-induced Apoptosis in *C. elegans*

A. Gartner, S. Milstein

During our studies of the regulation of germ cell death, we noticed that ionizing radiation, as well as chemical mutagens, can induce germ cell death and germ cell proliferation arrest. Both responses require the function of the *rad-5* gene. Interestingly, although *egl-1* is not involved in physiological germ cell death, loss of *egl-1* function significantly reduces (but does not abrogate) radiation-induced germ cell death. Thus, at least three distinct pathways can lead to apoptosome activation in *C. elegans*.

Genes Involved in the Engulfment of Dying Cells

T. Gumienny, Q. Liu

Once a cell activates 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.

Six genes have previously been shown to function in this process in *C. elegans*. In the past year, we have reported our cloning and molecular characterization of

one of these six genes, *ced-6*. CED-6 contains a phosphotyrosine-binding (PTB) domain and a number of potential SH3-binding sites, suggesting that CED-6 might act in a signal transduction cascade activated once a cell recognizes that one of its neighbors is undergoing PCD. Interestingly, overexpression of CED-6 can partially rescue the engulfment defect of *ced-1* and *ced-7* mutants, suggesting that *ced-6* might act downstream from these genes to promote cell engulfment.

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 allow us to readily address complex questions at the genetic and molecular levels.

Axonal Guidance and Outgrowth

S. Tharin [in collaboration with B. Wightman, Muhlenberg College, Pennsylvania; G. Garriga, University of California, Berkeley; E. Hartwieg, and R. Horvitz, HHMI/Massachusetts Institute of Technology]

C. elegans has proven to be a useful system to identify conserved genes that function in nervous system development. We have recently found that the *unc-69* gene is required for axonal guidance, outgrowth, and fasciculation in *C. elegans*. We have cloned the *unc-69* gene and found that it encodes a small novel protein with a predicted coiled-coil motif near its carboxyl terminus. Rescuing *unc-69::gfp* fusion constructs are expressed throughout development in the nervous system, predominantly in axons, suggesting that the *unc-69* gene acts cell autonomously to promote axonal guidance and outgrowth. Our current data are consistent with a model where *unc-69* participates in a signal transduction pathway that relays external guidance cues to the axonal cytoskeleton.

PUBLICATIONS

Gartner A. and Hengartner M.O. 1998. Genetic approaches to programmed cell death in *C. elegans*. In *When cells die* (ed. R.A. Lockshin et al.), pp. 131–146. John Wiley & Sons, New York.

Gutch M.J., Flint A.J., Keller J., Tonks N.K., and Hengartner M.O. 1998. The *Caenorhabditis elegans* SH2 domain-containing protein tyrosine phosphatase PTP-2 participates in signal transduction during oogenesis and vulval development. *Genes Dev.* **12:** 571–585.

Hengartner M.O. 1998. Apoptosis: Death cycle and Swiss army knives. *Nature* **391:** 441–442.

Hengartner M.O. 1998. The apoptosome: Molecular control of apoptosis in the nematode *C. elegans*. In *Pharmaceutical intervention in apoptotic pathways* (ed. J.F. Nagelkerke et al.), pp. 45–51. Elsevier-North Holland, Amsterdam.

Hengartner M.O. 1998. Apoptosis: Death by crowd control. *Science* **281:** 1298–1299.

Lin X., Hengartner M.O., and Kolesnick R. 1998. *Caenorhabditis elegans* contains two distinct acid sphingomyelinases. *J. Biol. Chem.* **273:** 14374–14379.

Liu Q.A. and Hengartner M.O. 1998. Candidate adaptor protein CED-6 promotes the engulfment of apoptotic cells in *C. elegans*. *Cell* **93:** 961–972.

In Press

Gumienny T.L., Lambie E., Hartwieg E., Horvitz H.R., and Hengartner M.O. 1999. Genetic control of programmed cell death in the *Caenorhabditis elegans* hermaphrodite germ line. *Development* **126:** 1011–1022.

Liu Y., Hengartner M.O., and Herr W. 1999. Selected elements of herpes simplex virus accessory factor HCF are highly conserved in *Caenorhabditis elegans*. *Mol. Cell. Biol.* **19:** 909–915.

PLANT DEVELOPMENT

D. Jackson J.M. Arroyo G. Sabino
G. Birkenmeier D. St. Fleu
Z. Khalfan Z. Yuan

Our goal is to understand the molecular and genetic mechanisms controlling plant development. Plants have unique developmental capabilities because they possess pools of stem cells, called meristems, that enable them to initiate new organs throughout their life cycle. For example, the shoot apical meristem gives rise to the shoot organs such as leaves and flowers. The fate of any given cell during plant development depends mainly on its position with respect to surrounding cells, rather than its developmental history or lineage. The cytoplasmic connections between neighboring cells, called plasmodesmata, are likely to be one route through which cells exchange positional information. This is supported by a growing body of evidence which indicates that macromolecules of viral and plant origin traffic through plasmodesmata.

Perhaps the best known example of a plant developmental gene that acts nonautonomously is *knotted1* (*kn1*) from maize. The *kn1* gene was first identified by dominant mutations that cause ectopic growths, called knots, on the leaves. When *Kn1* is expressed only in the middle mesophyll layers of the leaf, this is sufficient to induce the extra cell divisions in all cell layers, leading to knot formation. The discovery that KN1 protein can be detected outside the domain of detectable transcript accumulation in the meristem as well as in knotted leaves suggested that intercellular movement of KN1 protein may account for the non-cell-autonomous behavior of *Kn1*. Previously, we showed that like viral movement proteins, fluorescently labeled KN1 protein rapidly moves from cell to cell when microinjected into maize or tobacco leaf mesophyll cells. *kn1* is a member of a small family of homeobox genes, and therefore our data supporting the intercellular trafficking of a putative transcription factor suggest a novel mechanism by which plant cells communicate during development.

The data supporting intercellular movement of the KN1 protein are compelling, but there is still no direct evidence that KN1 protein moves in the shoot meristem, where it is normally required, nor whether movement of KN1 is important for *kn1* gene function. Also, little is known about the mechanism by which macromolecules are targeted to and traffic through plasmodesmata.

HIGH-RESOLUTION IMAGING OF KN1

Cell-to-cell trafficking of viral and plant gene products has been evidenced, in part, by performing high-resolution localization experiments to demonstrate that these molecules are associated with plasmodesmata *in planta*. Recently, we were able to show that KN1 protein is detected in the plasmodesmata (Fig. 1) and nuclei (data not shown) in the maize inflorescence meristem. The level of labeling is weak compared to viral movement proteins, suggesting that the level of expression or the mechanism of trafficking may differ. Our results support the idea that the route for intercellular trafficking of

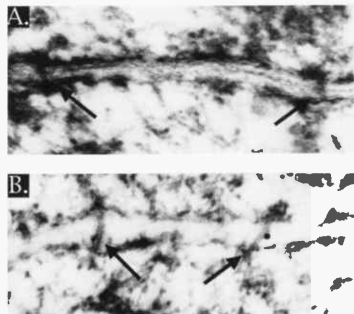


FIGURE 1 Sections of maize inflorescence meristem were incubated with a control preimmune serum (*A*) or anti-KN1 polyclonal antibodies (*B*) followed by incubation with protein A gold. Gold particles (*black dots*) are visible in plasmodesmata (*arrowed*) in *B*, but not in *A*.

KN1 is through the plasmodesmata. We have improved the specimen preparation procedure to look at the surface layers of the meristem, which were not well preserved in the procedures that we used initially.

IN VIVO IMAGING OF KN1 CELL-TO-CELL TRAFFICKING

Our current understanding of cell-to-cell trafficking of plant proteins is limited by the accessibility of tissues to microinjection. We are therefore developing new systems to visualize cell-to-cell trafficking of KN1 and other proteins in different tissues of intact plants. Several fusion proteins between KN1 and the green fluorescent protein (GFP) have been created. The GFP gene was isolated from jellyfish and encodes a protein that is naturally fluorescent. Using microprojectile bombardment to introduce the KN1-GFP fusion constructs into single epidermal cells, we have succeeded in demonstrating cell-to-cell trafficking of the fusion protein. The KN1-GFP fusion clone has also been expressed in stable transgenic *Arabidopsis* plants to follow trafficking in different tissues of the plant, using several approaches. First, we have grafted nontransgenic stems (the scion) onto plants that express the KN1-GFP fusion and have observed trafficking of the fusion protein into the stem of the nontransgenic scion. This experiment demonstrates the cell-to-cell trafficking of the KN1-GFP fusion but does not allow us to determine the extent of trafficking in a wide range of cell types. Second, we are using the Cre-*loxP* site-specific recombination system of bacteriophage P1 to create mosaic sectors of GFP-KN1 in *Arabidopsis*. The sector boundaries are marked with a different form of GFP that is targeted to a specific subcellular compartment, such as the mitochondria or chloroplasts. Transgenic plants harboring the *lox*P/GFP-KN1 constructs have been made and will be crossed to plants carrying the *cre* recombinase gene under the control of a heat shock promoter. Upon the application of a heat shock, *cre* expression will be induced and lead to the production of sectors that express KN1-GFP and lack the subcellular marker GFP. Imaging of the boundary between cells that express KN1-GFP and cells that do not will allow us to determine in which tissues and how far the KN1 protein can move in vivo. As a third approach, we are using a Gal4-based enhancer trap–*trans*-activation system developed by Dr. Jim Haseloff to express KN1-GFP in specific tissue and cell types. We have generated more than 100 enhancer trap lines that express GFP in specific cell or tissue types during development. These lines will be crossed to plants that carry the KN1-GFP fusion under the control of the Gal4UAS, so that the transgene will be activated in the specific cell or tissue types revealed by the enhancer trap expression pattern. These approaches will allow us to determine when and where during development the KN1 protein is able to traffic between plant cells.

MAIZE MUTANTS AFFECTING MERISTEM DEVELOPMENT AND PHYLLOTAXY

Plant architecture has a major impact on agricultural yield; for example, the introduction of dwarfing genes into wheat helped increase yields from 0.75 tons/hectare to up to 8 tons/hectare. Architecture is the end-product of morphogenesis, which in plants, as in animals, commences during embryogenesis with the development of distinct organ and tissue types. In plants, however, new organs are initiated throughout the life cycle from populations of stem cells in the meristems. The shoot organs such as leaves, axillary meristems, and flowers are initiated by the shoot apical meristem (SAM), usually in regular and defined patterns. SAMs show remarkable abilities to self-regulate their size during development, by balancing their own growth with the incorporation of cells into new primordia. We are investigating the mechanism of SAM size regulation through the molecular and phenotypic analyses of mutants in maize that cause enlargement or fasciation of the ear inflorescence SAM.

We have identified several spontaneous and transposon-induced mutants that have a fasciated phenotype, leading to enlarged and fasciated ears. Allelism tests indicate that the mutations correspond to several different genes and also suggest that certain of the gene products may interact directly. One of the mutants, *fasciated ear2* (*fae2*), was isolated in a nondirected *Mutator* (*Mu*) transposon screen conducted at Dekalb. We identified a *Mu* transposon that cosegregates with the *fae2* mutation in approximately 80 mutant individuals and have cloned a genomic DNA fragment containing the *Mu* element as well as flanking DNA that may contain the *fae2* gene. By analysis of RNA expression, we identified a region of the genomic clone that encodes a transcript which is preferentially expressed in ear primordia and is absent in *fae2* mutant ears, making this a good candidate for the *fae2* gene.

Experiments to definitively show whether the cloned sequence corresponds to *fae2* are in progress. We are also trying to determine whether this mutation directly affects the proliferation of stem cells in the meristem or whether it acts by an alternative mechanism.

An aspect of meristem function that has been difficult to address is how regular patterns of leaf initiation are generated. These patterns, known as phyllotaxies, have been studied for decades, but the molecular mechanisms involved are largely unknown. We have been characterizing a new recessive mutant of maize, *abphyl1* (*abph1*), that initiates leaves in opposite pairs rather than singly, as is typical for the grasses. The *abph1* mutation leads to the initiation of a larger shoot meristem domain in the embryo and an altered phyllotactic pattern. There are two possible models to explain the mechanism of action for the *abph1* mutation: (1) The mutation might affect the axial growth of the embryo, causing the initiation of a broader shoot meristem that initiates more primordia due to an increased morphogenetic field within which the primordia are specified or (2) the mutation could function by directly specifying extra primordia. In this case, the larger meristem domain would be a consequence of having extra primordia. Isolation of the *abph1* gene should allow testing of these models by determining when and where the gene is expressed. We therefore initiated a directed tagging experiment by crossing *abph1* mutant plants onto *Mu* active plants and screening in the F_1 generation for rare putative new alleles. Last winter in the greenhouse, we found several candidates, and since then, we have been able to show that at least two are heritable. We have now generated the families to start to look for cosegregating *Mu* transposons that would allow us to isolate the *abph1* gene and determine how it acts to control the phyllotaxy of the maize plant.

Understanding these fundamental aspects of plant development could provide a means to control the number, pattern, and size of organs produced during development and could therefore enhance crop plant productivity.

PUBLICATIONS

Zhang S., Williams-Carrier R., Jackson D., and Lemaux P.G. 1998. Expression of CDC2Zm and KNOTTED1 during axillary shoot meristem in-vitro proliferation and adventitious shoot meristem formation in maize (*Zea mays* L.) and barley (*Hordeum vulgare* L.). *Planta* **204:** 542–549.

In Press

Jackson D. and Hake S. 1999. Control of phyllotaxy by the *abphyl1* gene. *Development* **126:** 315–323.

Ori N., Juarez M.T., Jackson D., Yamaguchi Y., Banowetz G.M., and Hake S. 1999. Delay in leaf senescence caused by expression of the maize homeobox gene, *knotted1*, under control of the senescence activated promoter, SAG12. *Plant Cell* (in press).

ARABIDOPSIS FLOWER AND POLLEN DEVELOPMENT

H. Ma Y. Azumi E. Katz H. Onaka
A. Debrowski B. Laulicht P. Rubinelli
J. Ellstein D. Liu S. Virani
B. Horwitz S. Luo Y. Wang
Y. Hu S. Moran M. Yang

Our research continues to focus on the functional analysis of genes important for plant reproductive development in *Arabidopsis*. Our recent endeavor has been to isolate mutants defective in flower development and pollen development and to clone the affected genes, using the gene/enhancer trap system developed here at CSHL by Drs. V. Sundaresan and R. Martienssen.

In 1998, Peter Rubinelli, a graduate student of the SUNY, Stony Brook MCB program, completed his research project here at the Laboratory, successfully defended his thesis, and received his Ph.D. degree. For his postdoctoral training, he has chosen to work at Ohio State University. In addition, we welcomed several new members. Drs. Yixing Wang from Jinan University, China, and Hiroyasu Onaka from the University of Tokyo, Japan, joined our laboratory as postdoctoral associates. They have started experiments designed to gain further insights into the function of the floral regulatory gene *AGAMOUS*. Dr. Yoshitaka Azumi came from Kanagawa University, Japan, for his sabbatical leave. He has been learning the gene-trap system step by step and has participated in the analysis of a newly identified male meiosis gene. Dr. Benjamin Horwitz came for a short stay from The Technion Institute in Israel, working on constructing cDNA libraries as a part of a 3-year collaboration between our two laboratories. Shujin Luo, an URP from Peking University, performed studies on enhancer-trap lines with floral expression patterns. Elisa Katz, our Partner for the Future, completed her project with Ming Yang, a postdoctoral fellow in the laboratory, on the characterization of an *Arabidopsis* gene required for male meiosis. Finally, high school students Brian Laulicht, Sienna Moran, Amy Debrowski, and Joshua Ellstein continued their projects in the gene-trap effort. As always, we greatly appreciate the assistance by the Uplands Farm manager Tim Mulligan and his assistant Stan Schwarz.

In August, my laboratory concluded more than 8 years of research at CSHL, and we moved to the Pennsylvania State University Department of Biology, where I have started a position as an associate professor. I am fortunate that most of the full-time members in my laboratory have moved with me, including postdoctoral fellows Ming Yang, Yixing Wang, and Hiroyasu Onaka, visiting scientist Yoshitaka Azumi, and technician Yi Hu. Because of family reasons, postdoc Dehua Liu stayed at CSHL, working with Dr. Martienssen.

Characterization of a Ds-tagged Locus, *SKL1*

M. Yang, Y. Hu, H. Ma [in collaboration with M. Lodhi and R. McCombie, Cold Spring Harbor Laboratory]

We previously reported the isolation of a putative Ds-tagged mutant defective in male meiosis from a Ds insertional line called ET5223. Subsequent genetic studies have recovered several revertant sectors in mutant plants carrying an Ac element, presumably as the result of Ac-dependent transposition of the Ds element. Sequence analysis indicates that the Ds-disrupted locus is completely restored to the wild-type sequence in 12 revertants. Furthermore, in two other revertants at the site of Ds insertion, the Ds element is no longer present, but six nucleotide insertions remain and are capable of encoding two additional amino acid residues. These results provide direct evidence for the idea that the mutant phenotype is caused by the Ds insertion and suggest that the corresponding protein product can still function with these extra residues.

Portions of the sequences flanking the Ds element match perfectly cDNA sequences of an expressed sequence tag (EST) clone that is highly similar to a yeast gene and a human cDNA encoding the SKP1 proteins. These results indicate that the gene disrupted by the Ds element is a likely homolog of the yeast and human *SKP1* genes; thus, we have named the gene *SKL1*, for *SK*P1-*L*IKE*1*, and the Ds insertional mutant allele *skl1-1*. The Ds insertion in the mutant and the extra amino acid residues are in a nonconserved linker

region between two conserved domains, consistent with the fact that these additional amino acids do not disrupt the *SKL1* function. The *SKL1* gene is expressed throughout plant development, in all organs tested, including both vegetative and reproductive organs. In particular, RNA in situ hybridization revealed that *SKI1* is expressed in male meiotic cells. Analysis of RNA samples from the *skl1-1* mutant plants indicates that the *SKL1* message is not detectable.

Characterization of the Male Meiotic Defect in the *skl1-1* Mutant

M. Yang, E. Katz, B.B. Laulicht, Y. Hu, H. Ma

Normal *Arabidopsis* male meiosis in a microspore mother cell produces a tetrad containing four equal-sized spores. Our previous studies indicate that the *skl1-1* mutant exhibits defects during male meiosis, resulting in abnormal "tetrads" with a variable number of spores that are uneven in size. Our further analysis indicates that the mutant fails to separate homologous chromosomes during meiosis I in the microspore mother cell. Proper chromosomal association and subsequent segregation are essential for normal passage of genetic information through cell divisions. However, little is known about the association and separation of homologous chromosomes during meiosis. Using fluorescence microscopy, we found that chromosomal condensation at metaphase I appeared to be similar between wild-type and *skl1-1* mutant male meiosis, but the mutant chromosomal materials were abnormally extended during anaphase I. Furthermore, fluorescence in situ hybridization analysis of chromosome 2 using rDNA as a probe revealed that the rDNA region is localized to the central portion of the extended DNA materials. These results strongly suggest that homologous chromosomes in the mutant fail to separate following metaphase I, and the region of association is small. In particular, the region of attachment on chromosome 2 may be near or overlap with the rDNA region. As reported above, the *SKL1* gene product is a member of a highly conserved family that includes the human and yeast SKP1 proteins. The yeast SKP1 protein targets specific proteins for degradation by ubiquitin-mediated proteolysis or for phosphorylation in the kinetochore. We propose that the *Arabidopsis* SKP1 protein controls homolog separation during male meiosis I by degrading or otherwise removing a protein complex that holds homologs together prior to anaphase I.

Genetic Studies of a Possible Interaction between *SKL1* and *UFO*

M. Yang, B. Laulicht, H. Ma

Molecular genetic studies have identified a number of genes whose functions are specifically required for controlling floral organ identity. At the same time, it is likely that other genes with more pleiotropic functions also contribute to the regulation of floral organ identity. While studying the male-sterile mutant *skl1-1*, we noticed that in addition to its defects in male meiosis, it also has abnormalities during vegetative and flower development. We have begun to characterize these abnormalities. In the *skl1-1* mutant, rosette leaf growth is reduced, resulting in slightly smaller than normal rosette leaves throughout vegetative development. After flowering, the mutant has less internode elongation than wild type. Although some mutant flowers have four normal-sized petals, many flowers have one or more petals that are reduced in size. The filaments are more uniformly shortened, and there are slightly fewer petals and stamens. In addition, petal/stamen chimeric organs are found in many flowers. These results indicate that the *SKL1* gene affects the size of vegetative and floral organs.

The *skl1* floral phenotype resembles, but is not as severe as, that of the *Arabidopsis ufo* mutants; we therefore have initiated experiments to test for possible interactions by examining the *ufo-2 skl1-1* double mutant. Crosses were carried out between *skl1-1* and *ufo-2* mutant plants. In addition, other crosses were performed to generate double mutants to investigate potential interactions between *SKL1* and other floral regulatory genes. Analysis of these double mutants should uncover genetic interactions and provide insight about how *SKL1* functions in the regulation of flower development.

Preliminary Characterization of Putative Mutants

D. Liu, Y. Azumi, Y. Hu, S. Moran, H. Ma

We have screened our Ds insertional lines for visible developmental phenotypes in the flower. In addition to several mutants similar to known mutants, we also found a number of mutants that have novel floral phe-

notypes. These include mutants that have abnormal floral organ sizes and/or shape. In particular, several mutants have short filaments; some of these produce a large number of functional pollen grains but have reduced fertility because the pollen grains are not efficiently delivered to the stigma.

From among several new male sterile mutants, we have characterized in some detail one mutant from transposant ET2292 that has a defect in male meiosis. This mutant produced tetrads with four to six spores, and thus the mutant phenotype is different from that of the meiotic mutant from ET5223 or from those of several published mutants. Our results from pollination using normal pollen indicate that the ET2292 mutant is female fertile. Previously, we reported that we had obtained several revertant sectors among mutant plants carrying an Ac element. Further analysis indicated that the ET2292 mutant carries a single Ds insertion, and the revertant alleles have lost this Ds element. In particular, DNA sequences of the regions flanking the Ds element in the mutant and the corresponding regions from the wild-type and several revertants support the hypothesis that the Ds insertion is responsible for the mutant phenotype. We are in the process of isolating the cDNA and genomic clones.

Preliminary analysis of mutant meiosis using DAPI staining suggests that the homologous chromosomes fail to pair properly during meiosis I. Consequently, at metaphase I, ten univalents (single unpaired chromosomes) were observed in the mutant cells, as opposed to the five bivalents (pairs of homologous chromosomes) seen in the wild-type cells. Further investigation is in progress.

Screening Enhancer and Gene-trap Transposants for Addition Mutants

A. Debrowski, J. Ellstein, Y. Hu, H. Ma [in collaboration with Jean-Philippe Vielle Calzada and Ueli Grossniklaus, Cold Spring Harbor Laboratory]

To identify new genes regulating *Arabidopsis* flower development, we have continued to generate enhancer trap/gene trap transposon insertional lines. At the same time, we have screened for new mutants among Ds insertional lines. A number of new putative mutants were identified, and will be characterized in the near future. In particular, in collaboratoin with Jean-Philippe Vielle Calzada and Ueli Grossniklaus, we have screened for sterile or low-fertility mutants. Initial microscopic examinations suggest that three sterile mutants may have defects in male meiosis, whereas others have abnormal pollen development at later stages. We expect that further analysis should reveal the abnormalities more precisely.

PUBLICATIONS

Ma H. 1998. To be, or not to be, a flower: Control of floral meristem identity. *Trends Genet.* **14:** 26–32.

Ma H. 1998. A serpentine receptor surfaces in *Arabidopsis*. *Trends Plant Sci.* **3:** 248–250.

Ma H. 1998. Floral induction: From photoperiodism to florigen. *Current Biol.* **8:** R690–R692.

Rubinelli P., Hu Y., and Ma H. 1998. Identification, sequence analysis and expression studies of novel anther-specific genes of *Arabidopsis thaliana*. *Plant Mol. Biol.* **37:** 607–619.

PLANT DEVELOPMENTAL GENETICS AND FUNCTIONAL GENOMICS

R. Martienssen M. Byrne, M. Curtis, C. Kidner, A. Groover, B. May, P. Rabinowicz, E. Vollbrecht, M. Dunham [URP], A.M. Settles, J.-M. Arroyo, R. Shen, J. Simorowski, C. Yordan

We are using plant developmental genetics to study fundamental questions of patterning and differentiation during development. Currently, these studies are focused on the *Arabidopsis* leaf and in the maize inflorescence. Our primary tools in this work are functional genomics and gene trap transposon mutagenesis. In collaboration with Dick McCombie's laboratory at Cold Spring Harbor, we have also embarked on a sequence analysis of the *Arabidopsis* genome and have developed methods for sequencing maize and rice. The ability to examine complete sets of genes at the structural and functional levels across species barriers is leading to a revolution in our understanding of plant form and function and of how this relates to similar processes in animals and microbes.

Dorsoventral Axis Specification in the Arabidopsis Leaf

C. Kidner, R. Martienssen

The leaf is the ground-state organ of plants, and asymmetry in the dorsoventral axis is vital for producing the flattened blade that characterizes most leaves and leaf-like organs such as petals. We found that an *Arabidopsis* gene (*DANDELION* [*DND*]), which is required for proper formation of the dorsoventral axis, is a member of a large and evolutionary well-conserved gene family, with representatives in humans, insects, nematodes, and yeast as well as other plants.

Analysis of the function of *DND* in *Arabidopsis* is aided by an allelic series. The original dandelion mutation (*dnd1-1*) was isolated by A. Bhatt and C. Dean, and a similar mutant, *dnd1-2*, was recovered from our enhancer trap collection. These plants produce radial leaves and no flowers, and one out of five dies in the first week of growth. This phenotype is similar to another mutant, *argonaute* (*ago1*), that has recently been cloned molecularly (Bohmert et al., *EMBO 17:* 170 [1998]) and proved to be allelic. The gene trap lines also yielded a much weaker allele, *dnd1-3*. These plants have radial first leaves and floral organs, but subsequent leaves have some dorsoventral asymmetry. Scott Poethig (University of Pennsylvania) provided us with a similar mutant that proved to be an additional allele, *dnd1-4*, that is similar to *dnd1-3* but has trumpet-shaped leaves and a more severe floral phenotype. None of the alleles recovered from the enhancer trap lines were associated with a transposon insertion, but reverse transcriptase–polymerase chain reaction (RT-PCR) shows that *dnd1-3* and *dnd1-4* produce aberrant *DND* mRNAs of various sizes, the predominant size being about 50 bp smaller than wild type. This points to a partial splicing defect.

Using the allelic series and gene trap and enhancer trap expression patterns, we have found a number of genes in the same developmental pathway as *DND*. Enhancer traps have identified several genes that are misexpressed in a *dnd* background, including a gene with similarity to *teosinte branched*, a member of a large gene family controlling lateral organ growth in maize and its relatives.

Proximodistal Axis Specification in the Arabidopsis Leaf

M. Byrne, M. Dunham (URP), M. Curtis, R. Martienssen

Assymetric leaf1 (*as1*) is a classical mutant of *Arabidopsis* first isolated in the 1940s. Leaf shape is progressively altered in this mutant, with round, buckled rosette leaves being gradually replaced with lobed leaves and then dissected cauline leaves later in development. By examining a number of spatially restricted enhancer and gene trap reporter gene expression patterns, we have established that the mutant phenotype reflects the presence of multiple proximodistal axes and may have a partial alteration in dorsoventral polar-

ity. The *as1* gene maps to chromosome 2, and we have been isolating recombinants with flanking markers in a "chromosome landing" experiment designed to positionally clone the *as1* gene. We have narrowed the *as1* region to less than 50 kb and have identified a number of candidate genes in the region. These genes are being fine-mapped and tested for their ability to complement the mutant phenotype in transgenic plants.

Plant Vascular Differentiation

A. Groover, R. Martienssen

Vascular plants represent the most important group of terrestial plants in evolution, and the highly ordered and polar arrangement of veins within leaves is a striking example of spacing differentiation in plant development. Cells must assess their position relative to other cells within the tissue and to differentiate at the appropriate stage. This process underlies secondary growth in plants (the formation of wood).

We are using *Arabidopsis* gene traps to study signaling mechanisms controlling vascular development by (1) identifying secreted signaling proteins using a functional genomics approach and (2) identifying regulatory genes expressed in a specific vascular cell type, the tracheary element. Tracheary elements synthesize an elaborate secondary cell wall, and then undergo a process of programmed cell death. We are systematically identifying genes expressed within this cell type using gene traps. We have also developed a "secretion trap" screen that identifies secreted signaling proteins based on functional properties of the gene trap fusion protein. Preliminary results from this screen include the tagging of several membrane-bound and -secreted receptor-like molecules, as well as a number of novel genes.

Characterization of the *iojap* Gene in Maize and Bacteria

M. Byrne, R. Martienssen

The *iojap* mutant of maize was first characterized in the 1920s and 1940s and has a pattern of green and white stripes on its leaves. Green sectors have cells with normal chloroplasts, whereas white sectors have cells where plastids fail to differentiate. These mutant plastids, when transmitted through the female gametophyte, do not recover even in the presence of the wild-type *Iojap* (*Ij*) gene.

Biochemical evidence suggests that the Ij protein interacts with the 50S ribosomal subunit, and sequences homologous to *Ij* have been found in all eubacteria for which the complete genome sequence is available (except for mycoplasmas). The *Escherichia coli* homolog, *ybeB*, is the first gene in an operon of four genes, two of which (*pbpA* and *rodA*) encode gene products required for cell wall formation. Disruption of *pbp2* is conditionally lethal, but an *E. coli* strain carrying a chromosomal deletion in *ybe*B has normal growth rate compared with wild type. The morphology of mutant cells also appears to be unaltered, except for occasional colonies that exhibit filamentous growth. We are currently comparing the growth of the wild type and mutant under specific growth conditions such as limiting nutrients, and we are making double mutants with ribosome assembly and other relevant mutants.

We have used a His-tagged Ij fusion protein to generate both polyclonal antibodies and a collection of 20 monoclonal antibodies specific for Ij. These are being used in immunoprecipitation experiments to isolate proteins interacting with Ij.

Redundancy and Suppression in the Control of Chloroplast Protein Translocation by the *hcf106* Gene in Maize

M. Settles, R. Martienssen [in collaboration with A. Barkan, University of Oregon]

The *hcf106* gene on maize chromsome 2S is required for targeting and localization of a subset of thylakoid proteins in the chloroplast (Settles et al., *Science 278(5342):* 1467 [1998]). A homologous gene family in prokaryotes and another conserved membrane protein are required for similar protein targeting in *E. coli*. The *hcf106* secretion pathway is posttranslational and may tolerate tertiary structure, allowing tightly associated proteins to translocate as a single unit.

In maize, a second gene 88% identical to *hcf106* encodes a protein closely related to HCF106 (HCF106C) that is not expressed in *hcf106* mutant seedlings and maps close to a genetic factor required for the *hcf106* phenotype on chromosome 10L. We sequenced the *hcf106C* locus of both wild-type and mutant plants and found a 7-bp direct repeat in the

mutant *hcf106C* locus that causes a nonfunctional protein to be produced. Using this polymorphism, we have shown that the mutated *hcf106C* gene cosegregates with the *hcf106* phenotype.

The unlinked *hcf106C* mutation complicates genetic studies of double mutants. The *tha1* mutation of maize disrupts chloroplast SecA of the Sec post-translational targeting pathway. We have made triple mutants with *hcf106, hcf106C*, and *tha1* which demonstrate that the HCF106 and SecA pathways are independent of each other, implying that the *hcf106* pathway does not share essential components with the Sec pathway. In the process of making this triple mutant, we found that the *tha1* mutation is epigenetically regulated by the activity state of Robertson's *Mutator* transposons. The *tha1* mutation contains a *Mu1* insertion in an intron. Intron Mu insertions are not generally thought to be epigenetically regulated, but we have confirmed that the *hcf106-mum4* allele, which also has an insertion in an intron, is also epigenetically regulated.

Exploring Lethality and Redundancy in the Arabidopsis Genome with Systematic Gene Trap Sequencing

J. Simorowski, C. Yordan, R. Shen, Q. Gu (University of Tennessee), P. Springer (University of California at Riverside), C. Kidner, M. Curtis, A. Groover, R. Martienssen [in collaboration with J. Healy, A. Reiner, and W.R. McCombie, Cold Spring Harbor Laboratory]

The genome of *Arabidopsis thaliana* has approximately 20,000 genes, of which only 10% encode essential functions. Reverse genetic studies have revealed that only a small proportion of the remaining genes have obvious roles in growth and development, even when they are known to be expressed. One explanation is that most genes have closely related homologs elsewhere in the genome that encode redundant functions.

To address redundancy and lethality in functional genomics, we are using a gene trap approach. The transposon system that we have devised is uniquely suited to this as it generates single, random insertions in the *Arabidopsis* genome via positive-negative selection for unlinked transpositions. We are generating thousands of gene trap and enhancer trap insertions and amplifying, sequencing, and staining each one to simultaneously determine allelic structure, gene expression, and genetic function. So far, 800 lines have been successfully sequenced.

A relational database of gene expression patterns and insertion site sequences has been established that allows gene expression, mutant phenotype, map position, and gene disruption data to be searched using sophisticated Web-based applications. In this way, disruptions in gene family members that are expressed in the same cells can be genetically combined to reveal redundancy.

The expression patterns of lethal genes can also be examined in viable heterozygotes, revealing clues to function. *prolifera* was the first lethal insertion to be recovered, and it proved to disrupt an essential cellular function, namely, DNA replication. We have now recovered many more lethals by systematically screening transposants for similar phenotypes as they are isolated. Lethal mutations in translation, transcription, and cell cycle function have been recovered. Most of these mutants have variable and occasionally maternally inherited phenotypes, including semisterility and embryonic lethality. These phenotypes are highly reminiscent of similar mutants in yeast, *Drosophila*, and *Caenorhabditis elegans* which cause early developmental arrest and maternal effects.

Genetically Filtered Shotgun Sequencing of the Maize Genome

P. Rabinowicz, C. Yordan, R. Martienssen [in collaboration with K. Schultz, N. Dedhia, L. Parnell, L. Stein, and R. McCombie, Cold Spring Harbor Laboratory]

The genomes of higher plants and animals are highly differentiated, comprising a relatively small number of genes and a large fraction of repetitive DNA. Recently, it has been shown that the bulk of this repetitive DNA constitutes transposable, and especially retrotransposable, elements. It has been hypothesized that most of these elements are heavily methylated relative to genes and that this methylation might serve to sequester transposons and other repeats from transcriptional complexes in the nucleus. However, the evidence for this is anecdotal and controversial.

We analyzed a random sample of a few hundred clones from maize genomic libraries using two kinds of hosts: *mcr*$^-$ (defective restriction-modification systems), which should include any sequence present in the maize genome, and *mcr*$^+$ (called genetically filtered libraries), which should restrict methylated

DNA. By judicious use of insert sizes less than the size of genes, we reasoned that genes should be preferentially represented in these libraries if methylation were restricted to repeats. 90% of the *mcr* clones contained repetitive DNA (mainly retrotransposons), as previous studies predicted. In contrast, almost 80% of the genetically filtered clones were low-copy DNA, and only 10% had high-copy repeats. By comparison with databases, most of the low-copy DNA in the genetically filtered libraries could be accounted for by genes and genic regions.

EST (expressed sequence tag) sequencing largely avoids intergenic and noncoding DNA, but many genes are also absent or underrepresented even in normalized cDNA libraries. Genomic sequencing approaches do not suffer these limitations, and relatively low-quality sequence from a genome of interest and its comparison to the complete and highly accurate sequence of a related genome would yield most of the information sought. The difficulty with such an approach for plant and mammalian genomes has always been the high density of repetitive sequences that consume sequencing capacity and prohibit assembly. The use of genetically filtered libraries could overcome this difficulty.

Mutator-targeted Mutagenesis in Maize

B. May, E. Vollbrecht, P. Rabinowicz, J.-M. Arroyo, R. Martienssen [in collaboration with M. Freeling, University of California at Berkeley; L. Senior, D. Alexander, Novartis Seeds; and L. Stein, Cold Spring Harbor Laboratory]

The availability of large amounts of DNA sequence information in plants allows a systematic approach to be taken to determine gene function. This approach, pioneered in animal genomes such as *Drosophila* and *C. elegans*, involves first constructing a library of thousands of organisms, each of which has a different spectrum of insertions or deletions. This library is then screened with a target sequence to identify plants that carry mutations in a given gene. Robertson's *Mutator* transposable elements are uniquely suited for this purpose, and we have previously developed a strategy allowing large populations of plants to be screened for mutations in pools (Das and Martienssen, *Plant Cell 7:* 287 [1995]). This strategy involves crossing active lines with a strain that inhibits *Mutator* activity, so that F_1 plants carry germinal but no somatic mutations (Martienssen and Baron, *Genetics 136:* 1157 [1994]). A background of somatic mutations has proven to be problematic in a similar system, now known as "TUSC," which was developed by Pioneer Hi-bred at around the same time (Bensen et al., *Plant Cell 7:* 75 [1995]).

As part of the new Plant Genome Initiative, we are establishing a library of 40,000 F_1 plants. So far, more than half of these plants have been self-pollinated and the seeds collected. DNA samples are being extracted from tissue samples and pooled using bar codes. A preliminary screen for kernel phenotypes has been used to estimate the mutation frequency. PCR can be performed on DNA pools derived from this population so that seeds corresponding to mutations in a given gene can be readily identified. Individual geneticists from the maize community will be able to send in sequence information for targeted disruption of genes of interest to them. The information will be entered into a database, and the seed corresponding to the mutation will be distributed to interested researchers to allow them to determine the function of each gene chosen in this way. The *Mutator*-targeted mutagenesis (MTM) system will thus be used to build up a database of gene function in maize.

PUBLICATIONS

Bevan M., Bennetzen J.L., and Martienssen R. 1998. Genome studies and molecular evolution commonalities, contrasts, continuity and change in plant genomes. *Curr. Opin. Plant Biol.* **1(2):** 101–102.

Gu Q., Ferrandiz C., Yanofsky M.F., and R. Martienssen. 1998. *FRUITFULL* encodes a MADS-box gene and mediates cell differentiation during *Arabidopsis* fruit development. *Development* **125:** 1509–1517.

Martienssen R. 1998. Chromosomal imprinting in plants. *Curr. Opin. Genet. Dev.* **8:** 240–244.

Martienssen R.A. 1998. Functional genomics: Probing plant gene function and expression with transposons. *Proc. Natl. Acad. Sci.* **95:** 2021–2026.

Martienssen R. 1998. Transposons, DNA methylation and gene control. *Trends Genet.* **14(7):** 263–264.

Martienssen R. and Dolan L. 1998. Patterns in vegetative development. In Arabidopsis (ed. M. Anderson and J.A. Roberts), vol. 1, pp. 262–298. Annual Plant Reviews, Sheffield Academic Press.

Settles A.M. and Martienssen R. 1998. Old and new pathways of protein export in chloroplasts and bacteria. *Trends Cell Biol.* **8(12):** 494–501.

In Press

Martienssen R. and Springer P. 1999. Enhancer and gene trap transposons in *Arabidopsis*. In *Insertional mutagenesis: A practical approach* (ed. G. Coupland). Academic Press. (In press.)

MAMMALIAN CELL GENETICS

M. Wigler C. Yen, M. Hamaguchi, M. Nakamura, R. Lucito, L. Serina, D. Esposito, E. Hatchwell, D. Dong, J. Stolarov, H. Tu, J. West, L. Rodgers, M. Riggs, J. Troge, B. O'Conner, V. Tu, A. Buglino, M. Katari, D. Almanzar, J. Brodsky, J. Douglas

Our overall research program is only modestly modified from last year's. Our general aims remain the same: to define the mutations that result in cancer and other human diseases; to understand the mechanisms by which these mutations cause disease; and to develop strategies to modify the course of disease. Our effort is specifically focused in three areas: *combinatorial chemistry*, in which we are trying to develop a general methodology that will enable us to manipulate the biochemical pathways involved in cancer; *signal transduction*, in which we study the pathways of intracellular communication that become deranged in cancer cells; and *genomics*, in which we apply and develop tools for the genetic analysis of human disease.

The combinatorial program (Dennis Dong), conducted in collaboration with Peter Nestler and colleagues here at the Laboratory, has demonstrated the feasibility of finding small branched peptidic molecules that recognize the peptide epitopes of proteins and alter their biochemical interactions.

Our signal transduction studies are focused in two areas: cellular pathways that relate to the oncogenic Ras proteins (Hua Tu and Dennis Dong) and the analysis of the PTEN tumor suppressor (Javor Stolarov, Ken Chang, and Dennis Dong, in collaboration with Mike Myers and Nick Tonks at CSHL).

Our genomic studies are now divided into three parts. First, we are searching for cancer genes at the many loci that we have discovered by the application of representational difference analysis, or RDA. We are seeking tumor suppressor genes at six of the loci that are commonly deleted in cancer (Clifford Yen, Masaaki Yamaguchi, Robert Lucito, and Diane Esposito, in collaboration with Dick McCombie and Larry Parnell at CSHL). The PTEN tumor suppressor was found this way. In collaboration with Scott Powers and colleagues at Tularik, we are also seeking oncogenes in the regions that are commonly amplified in cancer (Clifford Yen and Mariko Nakamura). In the second part, we are expanding the use of genomic representations and microarrays to develop "genomic chips" (Robert Lucito and Joe West, in collaboration with Andy Reiner at CSHL). We expect to develop thereby a vastly more powerful engine for the discovery of genes mutated in cancer. Additionally, we hope that genomic chips will provide a method for rapidly genotyping clinical human cancers, leading to improvements in the diagnosis and the selection of therapies. The cancer genomics studies are conducted in collaboration with Larry Norton of the Sloan Kettering Memorial Cancer Institute. In the third part, we are adapting the techniques of representational analysis to find spontaneous mutations that cause germ-line transmission of sporadic hereditary diseases (Eli Hatchwell and Lidia Serina).

Combinatorial Chemistry

D. Dong

In collaboration with Peter Nestler's lab here at the Laboratory, we are investigating the binding capacity of a class of branched peptidic molecules, or molecular "forceps." These "forceps" have been generated as members of encoded combinatorial libraries, a method developed in collaboration with C. Still's laboratory at Columbia University (Ohlmeyer et al., *Proc. Natl. Acad. Sci. 90:* 10922 [1993]). As targets, we have chosen the carboxyl end of H-RAS, because it is the substrate of farnesyl transferase, the first step in the essential processing of RAS proteins (Gibbs, *Cell 65:* 1 [1991]), and because this end is predicted by crystallographic studies to be an exposed peptide. Several forceps libraries were screened against both H-RAS

protein and H-RAS terminal peptides using "on-bead" assays. Several members of this forceps library thus found were shown to bind specifically to the carboxy-terminal sequences of H-RAS and to recognize these sequences when they are fused to other proteins. At high concentrations, these forceps block the enzymatic farnesylation of H-RAS, in a manner strongly suggesting competition with enzyme for substrate binding. These results (Dong et al. 1999) suggest that branched peptidic molecules have the general capacity to recognize peptides and could, in principle, be used to modulate biological systems. However, improvements in the chemical backbone and binding affinities are clearly necessary before this end can be achieved. Our current efforts are now focused on screening more selective libraries and validating the strategy of specifically blocking H-RAS maturation as a way to modulate tumor growth.

Studies of MAP Kinase Cascades

H. Tu, D. Dong

The MAP protein kinase cascade, consisting of components from the MEKK, MEK, and MAPK protein kinase families, is a conserved triad found in all eukaryotic organisms and is under the control of Ras proteins in multiple species from diverse phyla. Our first encounter with the triad was in the sexual differentiation pathway in the yeast *Schizosaccharomyces pombe*. In that organism, Ras1 acts directly on Byr2, an MEKK, by binding to its regulatory domain. We have conducted many studies of the integration and control of this pathway during the past years, and in previous years demonstrated that Shk1, a conserved protein kinase, activates Byr2 by "opening" its conformation. Shk1 is a member of the PAK family of protein kinases. Like the MEKK kinases, these kinases also have a carboxy-terminal catalytic domain and an amino-terminal regulatory domain that contains the binding site for certain RHO proteins, members of the Ras superfamily of GTPases. We have demonstrated that the tools for analyzing the regulatory components of Byr2 can be used for the study of the PAK family of kinases (Tu and Wigler 1999). In particular, the RHO proteins bind the regulatory domain of Shk1, and this domain of Shjk1 binds the catalytic domain. Moreover, mutations in the Shk1 regulatory region that abolish binding to the catalytic region open the conformation of Shk1 and activate it.

The PTEN Tumor Suppressor

J. Stolarov, K. Chang, C. Yen, D. Dong

The use of RDA led to the identification of a region on chromosome 10 deleted in a variety of human cancers, including breast, prostate, and brain cancers. We therefore suspected that this region harbored a tumor suppressor gene. In a collaboration with Ramon Parsons of Columbia University, a gene from this region, PTEN, was identified and found to be mutated in many cancers (Li et al., *Science 275:* 1943 [1997]). Parsons and collaborators found that the PTEN gene is mutated in the germ line of patients with Cowdens syndrome, an inherited predisposition to multiple neoplasms (Liaw et al., *Nat. Genet. 16:* 64 [1997]; Marsh et al., *Nat. Genet. 16:* 333 [1997]). More recently, other investigators have shown that transgenic mice hemizygous for PTEN are cancer prone, completing the evidence that PTEN is a tumor suppressor.

In a collaboration with Mike Myers and Nick Tonks here at the Laboratory, we showed that PTEN is a protein tyrosine phosphatase with a somewhat atypical substrate preference (Myers et al., *Proc. Natl. Acad. Sci. 90:* 10922 [1997]). More recently, we have shown that PTEN has a lipid phosphatase activity, specific for phosphatidylinositol-3,4,5 phosphate, PIP_3, (Myers et al. 1998). In fact, one of the mutations in Cowdens syndrome destroys lipid phosphatase activity, leaving the protein phosphatase activity apparently untouched, suggesting that the lipid phosphatase activity is essential for PTEN function. This hypothesis is made more plausible because PIP_3 is a second messenger that has been previously implicated in the growth and survival of cancer cells.

We are pursuing two lines of investigation into the biology of PTEN. First, we have expressed the PTEN protein in cultured human tumor cells that lack a functional PTEN gene. Constitutive expression of PTEN appears to slow the growth and flatten these cells, and significantly suppresses tumorigenicity of these cells in experimental animals. Our current efforts are directed at constructing an inducible expression system for

PTEN in cultured cells, so that these effects can be examined more accurately. Second, we are studying the PTEN homologs in the yeasts *Saccharomyces cerevisiae* and *S. pombe*. As yet, no phenotype is apparent in *S. cerevisiae* when the PTEN homolog, Tep1, is deleted or overexpressed. We are just initiating similar experiments in *S. pombe*.

Loci Deleted or Amplified in Breast Cancer

C. Yen, M. Hamaguchi, R. Lucito, Y. Han, D. Esposito, M. Nakamura

Many loci that are homozygously deleted or amplified in breast cancer have now been identified by RDA. These loci are presumed to carry tumor suppressor genes or oncogenes, respectively. Among the loci we have found deleted are those encoding the PTEN gene and the p16/ARF (alternate reading frame) tumor suppressors. Virtually all of the loci known to be amplified in breast cancer have also been identified by RDA, including ErbB2, c-Myc, and the cyclin D loci. Six deletion loci and an equal number of amplified loci remain that are uncharacterized and in which we are searching for genes.

Unfortunately, the regions of homozygous deletion and amplification are quite large, often greater than a megabase upon initial characterization. Therefore, the first step in characterizing these loci entails "epicenter" mapping, a process that delineates the minimum region that is commonly deleted in cancers. To achieve this, it is necessary to search through large archives of stored breast cancer biopsies. For this purpose, we have developed a technique for "immortalizing" and amplifying the DNA from small tumor samples (Lucito et al. 1998). The immortalized DNAs can then be analyzed for gene copy gains or loss, which we currently do by quantitative polymerase chain reaction (Q-PCR).

Once the epicenter has been determined, a variety of techniques will be employed for searching for genes, including exon trapping, mining the existing databases for expressed sequences, and a new method for hybridization screening that we have developed called rapid isolation of cDNAs by hybridization (RICH). In the latter procedure, cDNAs from libraries are identified by their ability to hybridize to a given large-insert chromosomal vector such as a BAC (bacterial artificial chromosome) or YAC (yeast artificial chromosome) (Hamaguchi et al. 1998).

Another method for gene searching entails DNA sequencing and sequence analysis. In a collaboration with Richard McCombie's lab at CSHL we have finished the sequencing of the PTEN locus. PTEN appears to arise from a gene-poor region of the genome. Nevertheless, we are using these studies to model methods for gene finding from large contiguous tracts of genomic sequence.

Genomic Microarrays

R. Lucito, J. West

Although RDA was an advance over existing methods for finding lesions in cancer cells, we need to accelerate the pace of cancer gene discovery. Several opportunities have moved our genomic studies into the arena of microarrays. DNA microarrays are spatially organized collections of probes on a surface ("chip") that are used for measuring DNA concentrations of complementary sequences by hybridization (DeRisi et al., *Nat. Genet. 14:* 457 [1996]; Chee et al., *Science 274:* 610 [1996]). We are beginning to use this technology to perform RDA on a chip. Eventually, we expect that this approach will allow us to scan the genome for amplifications and deletions more thoroughly, vastly more rapidly, and in a more quantitative manner than ever before. This application of microarrays to genomics is made feasible by the successful implementation of representations, which allows us to sample the genome reproducibly, yielding DNA populations with reduced nucleotide complexity and hence improved hybridization kinetics. With the help of Raju Kucherlapati, Geoffery Childs, and Aldo Massimi, from the Albert Einstein School of Medicine, we have constructed and hybridized our first pilot chips. Andy Reiner of CSHL's Bioinformatics division is directing the software development needed to interpret and analyze the data. We expect that the genomic chips we develop will also have application to cancer and genetic clinical diagnosis.

Applications of RDA to Spontaneous Genetic Disease

L. Serina, E. Hatchwell

In principle, RDA can be used to detect genetic rearrangements. Such events occur in cancer, but they also sometimes occur as spontaneous genetic lesions in the germ line of a parent. When these germ-line mutations are transmitted to offspring, "sporadic" genetic disease, such as some forms of neurofibromatosis, Duchenne's muscular dystrophy, William's Syndrome, and a host of other apparent anomalies such as autisms may result. We have begun to apply RDA to families of children with suspected sporadic genetic disease. Success could open a new chapter in the study of human genetic disorders.

Although we have encountered rearranged regions with RDA in cancers, the method was not optimized for that task. We are now testing a variety of improved methods for the "corepresentation" of progeny and parental DNAs that are designed to find restriction endonuclease fragments in the progeny that are not present in either parent. In principle, such differences should arise predominantly by spontaneous mutation. At present, we have been tackling technical obstacles that are formidable but can be overcome.

PUBLICATIONS

Hamaguchi M., O'Connor E.A., Chen T., Parnell L., McCombie R.W., and Wigler M.H. 1998. Rapid isolation of cDNA by hybridization. *Proc. Natl. Acad. Sci.* **95:** 3764–3769.

Lucito R., Nakimura M., West J.A., Han Y., Chin K., Jensen K., McCombie R., Gray J.W., and Wigler M. 1998. Genetic analysis using genomic representations. *Proc. Natl. Acad. Sci.* **95:** 4487–4492.

Myers M.P., Pass I., Batty I.H., Van Der Kaay J., Stolarov J.P., Hemmings B.A., Wigler M.H., Downes C.P., and Tonks N.K. 1998. The lipid phosphatase activity of PTEN is critical for its tumor suppressor function. *Proc. Natl. Acad. Sci.* **95:** 13513–13518.

Webb C.P., Van Aelst L., Wigler M.H., and Vande Woude G.F. 1998. Signaling pathways in Ras-mediated tumorigenicity and metastasis. *Proc. Natl. Acad. Sci.* **95:** 8773–8778.

In Press

Dong D.I., Liu R., Sherlock R., Wigler M.H., and Nestler H.P. 1999. Molecular forceps from combinatorial libraries prevent the farnesylation of Ras by binding to its carboxyl terminus. *Chem. Biol.* **6:** 133–141.

Tu H. and Wigler M. 1999. Genetic evidence for Pak1 autoinhibition and its release by Cdc42. *Mol. Cell. Biol.* **19:** 602–611.

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.

- David Helfman's laboratory studies how specific actin assemblies are organized and regulated and how alterations in actin filament assembly contribute to aberrant cell growth. They are interested in understanding how the assembly of different cytoskeletal structures is regulated by extracellular signals and how cellular contractility contributes to signaling cascades that lead to focal adhesion formation and the regulation of adhesion-dependent signal transduction.
- Nouria Hernandez and her colleagues work on basal mechanisms of transcription using two systems, the human snRNA genes and the HIV-1 LTR. The snRNA promoters assemble RNA polymerases II and III transcription initiation complexes with many of the same transcription factors; one interest in this system is to identify the determinants that ensure recruitment of the correct RNA polymerase. The HIV-1 promoter directs the synthesis of full-length transcripts and, in addition, short transcripts. In this case, their main interest is to understand the biochemical mechanisms that govern the formation of short transcripts.
- Shiv Grewal's group focuses on the epigenetic control of transcription and recombination. They have shown that an epigenetic imprint controlling transcriptional repression in the fission yeast is stable not only during mitosis, but also during meiosis, and is chromosomally inherited. In addition, their work suggests that propogation of the repressed state occurs through the self-templated assembly of chromatin during DNA replication.
- Tatsuya Hirano's laboratory continues to take biochemical approaches to the study of mitotic chromosome condensations and segregation. They have revealed a mechanism by which the chromosome condensation machinery is activated by the master mitotic kinase cdc2. In addition, a eukaryotic complex required for sister chromatid cohesion and a bacterial version of condensation protein have been purified and characterized to reveal insights into the evolution of chromosome architecture and dynamics.
- David Spector's group focuses on structural and function organization of the mammalian cell nucleus. During the past year, they have demonstrated that the carboxy-terminal domain of the large subunit of RNA polymerase II is involved in the targeting of pre-mRNA splicing factors to active sites of transcription. In addition, they have purified and biochemically characterized interchromatin granule clusters (ICGs), a nuclear structure enriched in 75 proteins including pre-mRNA splicing factors. By utilizing a novel mass spectrometry strategy and peptide microsequencing, 33 known proteins, many linked to pre-mRNA splicing, and several uncharacterized proteins have been identified in the ICG fraction.
- Nick Tonks' laboratory studies the physiological function of the protein tyrosine phosphatase (PTP) family of signal transducing enzymes. They developed "substrate-trapping" mutant forms of these enzymes, which form stable complexes with target substrates in a cellular context, and used them to identify physiological substrates of several members of the PTP family. This has revealed that, contrary to popular belief, the PTPs display exquisite substrate selectivity in a cellular context and thus may be specific regulators of cellular signaling events. In addition, they have shown that PTEN, the tumor suppressor from human chromosome 10q23, is a phosphatase that dephosphorylates the lipid second messenger phosphatidylinositol 3,4,5-trisphosphate (PIP3). It is the ability to recognise PIP3 as substrate that is essential for the tumor suppressor function of PTEN.
- Linda Van Aelst and her colleagues focus on the identification of signaling pathways mediating the effects of Ras and Rac on cell adhesion, invasiveness, and metastasis. They have shown that the effector pathways mediating the tumorigenic and metastatic functions of Ras can be segregated and that the activation of the Raf/Mek/MAPK pathway is essential for the acquisition of the metastatic phenotype. They have demonstrated a role for Rac in the cytoskeletal rearrangements associated with invasiveness and in T-cell adhesion. Finally, they have been characterizing the Ras-binding protein AF-6 and have found that it interacts with profilin, a G-actin-binding protein that regulates actin filament assembly. This interaction may provide a link between cell junctions and the actin cytoskeleton.

EPIGENETIC CONTROL OF GENE EXPRESSION

S. Grewal R. Krimper

Research in our laboratory focuses on the fundamental question of how differentiated cells establish and maintain stable patterns of gene expression. Although gene-specific control mechanisms participate in this process, heritable changes in transcription are often suggested to occur via epigenetic (heritable changes without alterations in DNA) modifications such as DNA methylation or stable changes in the architecture of the chromosomes. In addition to creating distinct metastable transcriptional domains, these epigenetic alterations can also influence interchromosomal or intrachromosomal recombination, avoiding potentially deleterious effects on the integrity of the genome. The underlying mechanisms by which differentiated cells establish and maintain stable states of gene expression are not clear. To address these fundamental questions of cellular differentiation and genome integrity, we study the mating-type switching system of the fission yeast *Schizosaccharomyces pombe*, where sophisticated tools of biology can be applied. Fission yeast uses a gene repression mechanism known as "silencing" to maintain the cell identity. Mating-type loci *mat2* and *mat3*, which serve as donors for *mat1* alleles during mating-type switching, are silenced even though they contain genetic information identical to that of the expressed *mat1-P* or *mat1-M* alleles, respectively. In addition, silencing also extends to adjoining areas, including an interval between the donor loci, called the *K*-region. The *K*-region, which shares homology with centromeric repeats, also exhibits severe recombination suppression. We recently found that transcriptional silencing, recombination suppression, and efficiency of mating-type switching are mechanistically related and controlled by an epigenetic mechanism. In our strains, which carry replacement of a part of the *K*-region with the *ura4*$^+$ marker gene ($K\Delta$::*ura4*$^+$), *ura4*$^+$ expression, recombination, and mating-type switching are variegated. The Ura$^-$ efficiently switching (*ura4-off*) and Ura$^+$ inefficiently switching (*ura4-on*) epigenetic states are chromosomally inherited during mitosis and meiosis. Recent observations suggest that the *ura4-off* and *ura4-on* states represent "closed" and "open" chromatin structures, respectively, at the *mat2/3* region. Although several of the *cis*- and *trans*-acting factors that affect stability of the epigenetic states have been identified, almost nothing is known about the nature of the epigenetic "imprint" governing propagation of the silenced state. In addition, the question of how chromosomal structures acting as imprints are duplicated at a replication fork remains unanswered. Through analysis of the transcriptionally repressed domain in the mating-type region, we are addressing the following questions: (1) What are the key molecular events that occur during conversion of the expressed state into the silenced state and vice versa? (2) What are the requirements (*trans* and/or *cis*) for the inheritance of a gene expression state? (3) Is assembly or disassembly of the silenced state restricted to a particular phase(s) of the cell cycle?

MECHANISM OF INHERITANCE OF THE EPIGENETIC STATES

During the last year, we continued the experiments initiated while I was in Dr. Amar Klar's laboratory at the National Cancer Institute in Frederick, and together we have made significant progress in understanding the nature of epigenetic imprint regulating propagation of the silenced state. By using a combination of genetics and molecular biology techniques, we have shown that propagation of each state is likely to occur by the self-templated assembly of the chromatin in the mating-type region. The transient presence of multiple copies of *swi6*, which encodes a chromodomain (a motif found in proteins associated with chromatin organization) protein, altered the imprint at the *mat* locus and resulted in heritable conversion of the *ura4-on* state to the *ura4-off* state. Our results suggest that Swi6 may be a dosage-critical component essential for resetting the epigenetic memory.

Evidence from other laboratories suggests that histone deacetylation leads to assembly of "compact" chromatin, which prevents access to transcription factors, whereas acetylation makes "open" chromatin, allowing access to the transcription machinery. In this regard, analysis of the status of histone acetylation at the $K\Delta$::*ura4*$^+$ gene showed that histones are acetylated in *ura4-on* cells but not in *ura4-off* cells. Remarkably, the Swi6-induced transition from *ura4-on* to *ura4-off* is associated with a heritable change in

the acetylation status. Thus, the effect of Swi6 is likely to be linked to heritable changes in chromatin structure at the silent mating-type region. Consistent with this idea, we find that mutations in two histone deacetylase homologs, *clr3* and *clr6*, disrupt the stable propagation of the epigenetic states.

The role of Swi6 in establishment of the imprint at *mat2/3* was confirmed by using a strain with three copies of *swi6* (*swi6*$^+$-333 allele). The *ura4-off* cells of this strain, whose *mat* locus is marked with a mutation in the tightly linked *his2* gene, were crossed to another *ura4-on* strain (*his2*$^+$; one copy of *swi6*) and subjected to tetrad analysis. Of the four different segregation patterns shown in Figure 1, we found that classes II through IV were novel and are not observed in similar crosses using standard *swi6*$^+$-containing strains. When *swi6*$^+$*-333* cosegregates with *his2*, the usual 2 Ura$^+$:2 Ura$^-$ segregation was seen (class I). However, in tetrads in which *swi6*$^+$*-333* cosegregates with *his2*$^+$ (which originally marked the *ura4-on* epiallele), non-Mendelian segregation patterns were observed: 1 Ura$^+$:3 Ura$^-$ (class II) or 0 Ura$^+$:4 Ura$^-$ (class III; Fig. 1). The gain of an imprint in classes II and III suggests that expression of multiple *swi6* copies converts *ura4-on* to *ura4-off* by changing the imprint at the *mat* locus. The effect of *swi6* multiple copies can also assert itself prior to meiosis during diploid mitotic growth (class IV). These data led us to conclude that the imprinting event and a self-replicating chromatin structure are one and the same. Therefore, the unit of inheritance in this system is composed of DNA plus the associated epigenetic set of chromatin structure. We are continuing to work on the Swi6-induced change in the imprint and addressing the following key questions: (1) Does the Swi6-induced *ura4-on* to *ura4-off* transition occur only during a specific phase of the cell cycle? (2) Is the change in the imprint associated with alteration in timing of replication, spatial proximity of sequences and complexes, or both? (3) What are the other requirements (*trans* and/or *cis*) for the establishment and propagation of the imprint?

IDENTIFICATION AND CHARACTERIZATION OF NOVEL FACTORS AFFECTING PROPAGATION OF EPIGENETIC STATES

The linkage groups previously implicated in silencing (*clr1-clr4, clr6, swi6,* and *rik1*) either were identified in indirect searches designed to uncover mutants deficient in mating-type switching or were isolated as mutations affecting silencing of donor mating-type loci. On the basis of our observation that *swi6*$^+$*-333* induced an increase in the *ura4-on* to *ura4-off* transition rate, we have designed and carried out a more direct scheme to systematically identify genes that might affect the establishment as well as propagation

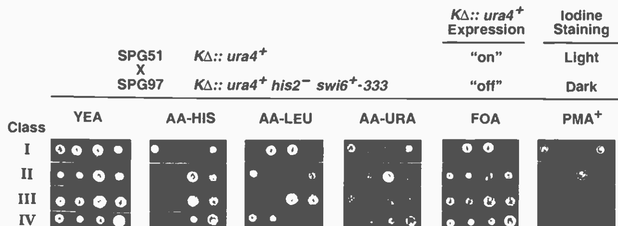


FIGURE 1 The presence of multiple copies of *swi6* causes novel non-Mendelian segregation of *K*Δ::*ura4*$^+$ epialleles in genetic crosses. Two copies of the *LEU2*-based plasmid containing *swi6* were integrated in tandem at the *swi6*$^+$ locus (*swi6*$^+$-333 allele) of the *ura4-on* strain. The resultant *ura4-off* strain (SPG97) was crossed to the *ura4-on* derivative of SPG51 to construct diploids. Diploids were sporulated and subjected to tetrad analysis. Segregation of the *swi6*$^+$*-333* allele was followed by its linkage to *LEU2*. Similarly, segregation of *K*Δ::*ura4*$^+$ epialleles was followed by their linkage to the respective *his2* marker. Examples of the different segregation patterns obtained are shown. Growth on AA-URA or FOA indicates *K*Δ::*ura4*$^+$ expression, and intensity of iodine staining (PMA$^+$ panel) indicates efficiency of mating-type switching.

of the epistates. This approach ensures identification of essential genes that might not have been identified previously. The *ura4-on* cells spontaneously change to *ura4-off* at a low rate of 8.1×10^{-4} per cell division. A fission yeast genomic library cloned in the pWH5 vector (~3–4 copies/cell) was transformed into *ura4-on* cells, and colonies showing an increased rate of conversion to the *ura4-off* state were sought. By using this approach, we have identified at least four new genes of unknown functions. Genetic and biochemical analyses of these genes and their functions will undoubtedly be helpful in understanding the mechanism of epigenetic inheritance.

In another effort to identify factors specifically affecting the inheritance of epigenetic states, we searched for mutants that could establish and maintain transcriptional repression yet displayed a reduction in the heritability of the repressed state. Colonies were selected that showed an increased rate of *ura4-off* to *ura4-on* transition, rather than complete derepression of *ura4*$^{+}$. Nine isolates that unambiguously showed increased papillation on uracil-lacking medium were selected for further study. The effect of the mutations seems likely to be epigenetic, as no rearrangements of the mating-type region were observed. Two of the nine mutations had a temperature-sensitive phenotype as well. We are in the process of dividing these mutants into different complementation groups. Taken together with the previously described genes, this class is expected to fill in many gaps left in the present picture which is composed almost entirely of genes that are strongly required for both mitotic propagation and interphase stability of the imprint.

T. Misteli, P. Mintz

S. Zhang, H. Palka

PUBLICATIONS

Grewal S.I.S., Bonaduce M.J., and Klar A.J.S. 1998. Histone deacetylase homologs regulate epigenetic inheritance of transcriptional silencing and chromosome segregation in fission yeast. *Genetics* **150:** 563–576.

R. Krimber, S. Grewel

THE CYTOSKELETON IN NORMAL AND TRANSFORMED CELLS

D.M. Helfman C. Berthier C. Chen J. Grossman A.J. Rai M. Selvakumar A. Vaahtokari Y.-C. Wang

We are interested in understanding how specific actin assemblies are organized and regulated and how alterations in actin filament assembly contribute to aberrant cell growth. Actin filaments have an important role in muscle contraction, cell movements, cell division, intracellular transport, regulation of cell shape, adhesion, and adhesion-mediated signaling. In particular, we are studying dynamic and stable macromolecular assemblies of distinct actin structures that are characteristic of specific cell types, e.g., stress fibers, lamellipodia, filopodia, and the contractile ring in fibroblasts or sarcomeres in skeletal muscle, in order to determine what proteins are required for the assembly and regulation of specific structures. We are also interested in understanding how the assembly of different cytoskeletal structures is regulated by extracellular signals, and how cellular contractility contributes to signaling cascades that lead to focal adhesion formation and regulation of adhesion-dependent signal transduction. Below is a description of our studies during the past year.

Regulation and Role of Cell Contractility in Adhesion-dependent Signaling

D. Helfman, C. Berthier [in collaboration with A. Bershadsky, Weizmann Institute of Science, Rehovot, Israel]

Contractility of nonmuscle cells has been hypothesized to be intimately associated with adhesion and adhesion-dependent signaling, leading to the formation of focal contacts, tyrosine phosphorylation, and signals required for cell growth and survival. To investigate the role of cell contractility in adhesion-dependent signaling, we have studied the effects of nonmuscle caldesmon on cellular contractility, actin cytoskeleton organization, and focal adhesion formation in fibroblasts. Caldesmon is an actin-, myosin-, tropomyosin-, and Ca^{++}-calmodulin-binding protein known to inhibit the actin-activated ATPase activity by phosphorylated myosin II, and it can block myosin-II-driven motility. Transient transfection of nonmuscle caldesmon prevents myosin-II-dependent cell contractility, induces disruption of stress fibers, and decreases the number and size of tyrosine-phosphorylated focal adhesions. In addition, expression of caldesmon interferes wih Rho-induced formation of stress fibers and focal adhesions, as well as with focal adhesion induction by microtubule disruption. These results demonstrate a role for nonmuscle caldesmon in the physiological regulation of actomyosin contractility and adhesion-dependent signaling, and they further demonstrate the involvement of contractility in focal adhesion formation and signal transduction. Disruption of adhesion-dependent signaling is a characteristic feature of neoplastic transformation; such cells often display anchorage-independence, growing and dividing in suspension without extracellular matrix contact. On the basis of our results demonstrating an ability of caldesmon to regulate adhesion-dependent signaling, we suggest that its interaction with specific tropomyosins could be important in maintaining the normal signaling function in the cell. Studies are in progress to determine how contractility leads to enhanced focal adhesion formation and what roles tropomyosin and caldesmon play in the signaling cascades associated with adhesion-mediated signal transduction events.

Dynamic Distribution of Tropomyosin Isoforms in Fibroblasts

C. Berthier

Nonmuscle tropomyosin (TM) isoforms belong to a conserved family of cytoskeletal proteins that are thought to have a role in the stabilization of filamentous actin and to act in cooperation with caldesmon to regulate actomyosin-based contractility. Our studies focus on the specific localization and role of TM isoforms in fibroblasts. The dynamic distribution of

fibroblast TM isoforms was investigated using stable cell lines of NIH-3T3 fibroblasts expressing fusion proteins of TM isoforms and green fluorescent protein (GFP) by time-lapse recordings and confocal analysis. Our results show that distinct actin-containing structures such as stress fibers, the contractile ring, and filopodia exhibit preferential incorporation of different isoforms. All isoforms incorporate into actin stress fibers. However, the low-molecular-weight TM-4 is preferentially present in filopodia. During cytokinesis, TM-4 and TM-5 associate with the contractile ring throughout the cell division process, whereas TM-1 and TM-2 are present only at the late stages. The results of these studies demonstrate that TM isoforms show specific localization to dynamic structures and suggest that they have specific roles in the regulation of actomyosin contractility.

Identification and Characterization of a Novel Conserved Phosphoprotein Involved in Intracellular Vesicle Transport

A.J. Rai

We have identified a novel protein from human nonmuscle cells. The cDNA sequence predicts a protein of 136 amino acids containing a transmembrane domain and a coiled-coil region. Computer database searches identified homologs in rodents, zebrafish, and *Drosophila*. Antibodies raised to the protein reveal a punctate cytoplasmic localization, as well as a strong juxtanuclear concentration. This staining pattern is characteristic of vesicles that emanate from the Golgi complex. Furthermore, the protein is phosphorylated, and perturbations in signal transduction pathways appear to alter its phosphorylation state and subcellular distribution. Work is in progress to delineate the identity of the vesicular subcompartment and its role in intracellular transport.

Binding of hnRNP H to an Exonic Splicing Silencer Is Involved in the Regulation of Alternative Splicing of the Rat β-TM Gene

C. Chen

In the rat β-tropomyosin (β-TM) gene, exons 6 and 7 are alternatively spliced in a mutually exclusive manner. Exon 6 is included in mRNA encoding nonmuscle TM-1, whereas exon 7 is used in mRNA encoding skeletal muscle β-TM. Previously, we demonstrated that a six-nucleotide mutation at the 5′ end of exon 7, designated as *ex-1*, activated exon 7 splicing in nonmuscle cells. In this study, we showed that the activating effect of this mutation is not the result of creating an exonic splicing enhancer (ESE) or disrupting a putative secondary structure. The sequence in exon 7 acts as a bona fide exonic splicing silencer (ESS), which is specifically bound by a *trans*-acting factor. Isolation and peptide sequencing revealed that this factor is heterogeneous nuclear ribonucleoprotein H (hnRNP H), a member of the hnRNP family. Binding of hnRNP H correlates with the ESS activity. Furthermore, addition of antibodies that specifically recognize hnRNP H to the splicing reactions or partial depletion of hnRNP H from nuclear extract activates exon 7 splicing in vitro, and this effect can be reversed by addition of purified recombinant hnRNP H. These results indicate that hnRNP H participates in exclusion of exon 7 in nonmuscle cells. The involvement of hnRNP H in the activity of an ESS may represent a prototype for the regulation of tissue- and developmental-specific alternative splicing.

Exonic Splicing Enhancers Contribute to the Use of Both 3′ and 5′ Splice Site Usage of Rat β-TM Pre-mRNA

M. Selvakumar

The rat β-TM gene encodes two tissue-specific isoforms that contain the internal mutually exclusive exons 6 (nonmuscle/smooth muscle) and 7 (skeletal muscle). We previously demonstrated that the 3′ splice site of exon 6 can be activated by introducing a nine-nucleotide polyuridine tract at its 3′ splice site, or by strengthening the 5′ splice site to a U1 consensus binding site, or by joining exon 6 to the downstream common exon 8. Examination of sequences within exons 6 and 8 revealed the presence of two purine-rich motifs in exon 6 and three purine-rich motifs in exon 8 that could potentially represent ESEs. In this report, we carried out substitution mutagenesis of these elements and showed that some of them have a critical role in splice site usage of exon 6 in vitro and in vivo. Using UV cross-linking, we have identified SF2/ASF as one of the cellular factors that bind to these motifs.

Furthermore, we show that substrates which have mutated ESEs are blocked prior to A complex formation, supporting a role for SF2/ASF binding to the ESEs during the commitment step in splicing. Using pre-mRNA substrates containing exons 5 through 8, we showed that the ESEs within exon 6 also play a part in cooperation between the 3′ and 5′ splice sites flanking this exon. The splicing of exon 6 to 8 (i.e., 5′ splice site usage of exon 6) was enhanced with pre-mRNAs containing either the polyuridine tract in the 3′ splice site or the consensus sequence in the 5′ splice site around exon 6. We showed that the ESEs in exon 6 are required for this effect. However, the ESEs are not required when both the polyuridine and consensus splice site sequences around exon 6 were present in the same pre-mRNA. These results support and extend the exon definition hypothesis and demonstrate that sequences at the 3′ splice site can facilitate use of a downstream 5′ splice site. In addition, the data support the hypothesis that ESEs can compensate for weak splice sites, such as those found in alternatively spliced exons, thereby providing a target for regulation.

Characterization of an Activity in Myogenic Cells That Stimulates Splicing of β-TM Exon 7

Y.-C. Wang

Exons 6 and 7 of rat β-TM pre-mRNA are alternatively spliced in a tissue-specific manner. Exon 7 is included in mature mRNA only in skeletal muscle cells. Previous studies suggested that the 3′ splice site of exon 7 is repressed in nonmuscle cells. We have developed an in vitro splicing complementation assay to study how muscle cells activate the 3′ splice site of exon 7. Nuclear extracts prepared from mouse myogenic cells, including BC3H1 and C2C12 cells, can complement a human 293 cell (nonmuscle) nuclear extract to activate exon 7, whereas nuclear extracts from mouse or human nonmuscle cells cannot. Addition of HeLa cell SR proteins to BC3H1 (but not 293 cell nuclear extract) stimulates the splicing of exons 5–7. This muscle-specific activity can be separated from known SR proteins by ammonium sulfate precipitation. Fractionation of the whole BC3H1 nuclear extract through a CsCl density gradient reveals a peak of stimulatory activity for exon 5–7 splicing. Besides this muscle-specific stimulatory activity, the results also suggest a possible involvement of two SR proteins, SRp30 and SRp20, in activating the 3′ splice site of exon 7. Collectively, these data demonstrate the presence of an activator in myogenic cells that promotes the inclusion of a muscle-specific exon in β-TM pre-mRNA.

PUBLICATIONS

Grossman J.S., Meyer M.I., Wang Y.-C., Mulligan G.J., Kobayashi R., and Helfman D.M. 1998. The use of antibodies to the polypyrimidine tract binding protein (PTB) to analyze the protein components that assemble on alternatively spliced pre-mRNAs that use distant branch points. *RNA* **4:** 613–625.

Temm-Grove C.J., Jockusch B.M., Weinberger R.P., Schevzov G., and, Helfman D.M. 1998. Distinct localizations of tropomyosin isoforms in LLC-PK1 epithelial cells suggests specialized function at cell-cell adhesions. *Cell Motil. Cytoskeleton* **40:** 393–407.

In Press

Chen C.D. and Helfman D.M. 1999. Donor site competition is involved in the regulation of alternative splicing of the rat β-tropomyosin pre-mRNA. *RNA* **5:** 290–301.

Chen C.D., Kobayashi R., and Helfman D.M. 1999. Binding of hnRNP H to an exonic splicing silencer is involved in the regulation of alternative splicing of the rat β-tropomyosin gene. *Genes Dev.* **13:** 593–606.

Helfman D.M., Berthier C., Grossman J., Leu M., Ehler E., Perriard E., and Perriard J.C. 1999. Nonmuscle tropomyosin-4 requires coexpression with other low molecular weight isoforms for binding to thin filaments in cardiomyocytes. *J. Cell Sci.* **112:** 371–380.

Helfman D.M., Levy E.T., Berthier C., Shtutman M., Elbaum M., and Bershadsky A.D. 1999. Caldesmon inhibits nonmuscle cell contractility and interferes with the formation of focal adhesions. *Mol. Biol. Cell.* (in press).

Lou H., Helfman D.M., Gagel R.F., and Berget S.M. 1999. Polypyrimidine tract-binding protein positively regulates inclusion of an alternative 3′-terminal exon. *Mol. Cell. Biol.* **19:** 78–85.

Selvakumar M. and Helfman D.M. 1999. Exonic splicing enhancers contribute to the use of both 3′ and 5′ splice site usage of rat β-tropomyosin pre-mRNA. *RNA* **5:** 378–394.

Stamm S., Casper D., Hanson V., and Helfman D.M. 1999. Regulation of the neuron-specific exon of clathrin light chain B. *Mol. Brain Res.* **64:** 108–118.

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

N. Hernandez T.L. Kuhlman E. Ford R.W. Henry B. Ma V. Mittal D.J. Morrison P.S. Pendergrast L.M. Schramm S. Sepehri Y. Sun X. Zhao

We work on RNA polymerase II and III transcription in two model systems, the human small nuclear RNA (snRNA) genes and the HIV-1 long terminal repeat. The human snRNA genes have very similar promoters, but some of them are transcribed by RNA polymerase II and others are transcribed by RNA polymer-ase III. Both the RNA polymerase II and III snRNA promoters contain an enhancer referred to as the distal sequence element (DSE), which is characterized by the presence of an octamer sequence responsible for recruiting the widely expressed POU domain transcription factor Oct-1. Both types of promoters also contain a common basal promoter element referred to as the proximal sequence element (PSE), which is responsible for recruiting a multisubunit complex we call $SNAP_c$. The RNA polymerase III snRNA promoters contain a second basal element, namely, a TATA box, which is responsible for recruiting the TATA-box-binding protein (TBP). The TATA box is absent in the RNA polymerase II snRNA promoters, and indeed in the context of the PSE, the TATA box determines RNA polymerase III specificity.

The human immunodeficiency virus type-1 (HIV-1) promoter directs the synthesis of two types of RNA molecules: full-length transcripts, whose synthesis is activated by the viral *trans*-activator Tat, and short transcripts, whose synthesis is activated by a DNA element called the inducer of short transcripts (IST). The synthesis of short transcripts does not require any viral factors, but the cellular factors required have not been identified. A candidate for a cellular factor involved in the synthesis of short transcripts is FBI-1, because FBI-1 binds to wild-type IST but not to mutated ISTs that are unable to direct the synthesis of short transcripts.

We want to understand how snRNA promoters assemble RNA polymerase II and III initiation complexes, and in particular how RNA polymerase specificity is determined. We are also interested in the mechanisms by which Oct-1, and more particularly the Oct-1 POU domain, mediates transcription activation of snRNA promoters. In addition, we want to understand how IST functions, and what part FBI-1 might play for short transcript formation or HIV-1 transcription in general. In the last year, we have assembled a functional $SNAP_c$ from recombinant subunits. This recombinant $SNAP_c$ constitutes an invaluable tool to understand how a multisubunit complex mediates the protein-protein interactions required to (1) recruit members of both the RNA polymerase II and RNA polymerase III general transcription machinery and (2) respond to transcription activators. Indeed, as described below, by generating recombinant $SNAP_c$s bearing single-amino-acid changes, we have been able to identify the target for activation by the Oct-1 POU domain within $SNAP_c$.

Assembly of a Functional Core Promoter Complex ($SNAP_c$) Shared by RNA Polymerases II and III

R. W. Henry, V. Mittal, B. Ma, R. Kobayashi, N. Hernandez

In coimmunoprecipitations of in-vitro-translated proteins, we had previously found that SNAP190 associates with SNAP45 and that SNAP50 associates with SNAP43. We were, however, unable to detect association between the two pairs of proteins. This suggested that we might be missing a $SNAP_c$ subunit. Indeed, we have now isolated cDNAs encoding SNAP19, the last subunit of $SNAP_c$. Inclusion of in-vitro-translated SNAP19 with the other in-vitro-translated $SNAP_c$ subunits results in the formation of a complex containing all five subunits. In addition, we could gener-

ate a recombinant $SNAP_c$ capable of binding specifically to the PSE by coinfection of insect cells with five recombinant baculoviruses expressing each of the $SNAP_c$ subunits.

The availability of recombinant $SNAP_c$ allowed us to determine whether the very same $SNAP_c$ can function for RNA polymerase II and III transcription. Depletion of a transcription extract with anti-SNAP43 antibodies inhibited snRNA gene transcription. Significantly, transcription by both RNA polymerases II and III could then be regained by addition of recombinant $SNAP_c$. Thus, the same complex mediates the assembly of two types of transcription initiation complexes, namely, RNA polymerase II and RNA polymerase III initiation complexes.

Protein-Protein Interactions among $SNAP_c$ Subunits

B. Ma, N. Hernandez

To understand the architecture of $SNAP_c$, we are defining which parts of the various $SNAP_c$ subunits interact with other members of $SNAP_c$ as determined by coimmunoprecipitation of in-vitro-translated proteins. Such information will allow us to build a "minimal" $SNAP_c$ lacking sequences dispensable for the integrity of the complex, which will be tested for DNA binding and transcription activity.

Characterization of Partial $SNAP_c$s

V. Mittal, N. Hernandez

Basal promoter elements in RNA polymerase I, II, and III promoters recruit multisubunit complexes. This is probably because such complexes can support multiple and flexible interactions with other players of the transcription machinery, including activators, repressors, and other members of the general transcription machinery. Because we can reconstitute $SNAP_c$ from recombinant subunits, and because it mediates the assembly of both RNA polymerase II and III initiation complexes, $SNAP_c$ constitutes an invaluable system to study how a multisubunit complex coordinates the assembly of two different initiation complexes, and how it responds to activators such as Oct-1.

To study this question, we are using the baculovirus expression system. Infection of insect cells with various subsets of $SNAP_c$ subunits allows the reconstitution of partial $SNAP_c$s, which are then tested for RNA polymerase II and III basal and activated transcription.

The Oct-1 POU Domain Activates snRNA Gene Transcription by Contacting a Region in the $SNAP_c$ Largest Subunit That Bears Sequence Similarities to the Oct-1 Coactivator OBF-1

E. Ford, N. Hernandez [collaboration with M. Strubin, University of Geneva, Switzerland]

We had previously shown that Oct-1 and $SNAP_c$ bind cooperatively to probes containing an octamer motif and a PSE and that this cooperative binding results in transcription activation in vitro. We had also shown that the largest subunit of $SNAP_c$, SNAP190, is capable of associating with Oct-1 POU bound to an octamer motif and that this association is sensitive to the same single-amino-acid substitution in Oct-1 POU (E7R) that affects cooperative binding of Oct-1 POU and $SNAP_c$ to probes containing both an octamer motif and a PSE. This raised the possibility that SNAP190 constitutes the target for transcription activation by the Oct-1 POU domain.

Although it is clear that one mechanism by which activators effect transcription activation is through recruitment of the basal transcription machinery, it has been difficult to identify with certainty the natural targets of various activators. It is therefore of considerable interest to define the target of the Oct-1 POU domain within the basal transcription machinery of snRNA genes in molecular detail. For this purpose, we first narrowed down the SNAP190 region required for interaction with octamer-bound Oct-1 POU to a small 44-amino-acid region. In so doing, we developed a generally applicable method involving in vivo selection in yeast to identify, with near single-amino-acid resolution, the borders of the domain required for interaction.

We changed several basic amino acids within the

minimal SNAP190 domain capable of interacting with octamer-bound Oct-1 POU to glutamic acids and assembled mutant recombinant $SNAP_c$s. These were then tested for cooperative binding with wild-type Oct-1 POU on probes containing an octamer motif and a PSE and for transcription activation. Strikingly, the $SNAP_c$ mutant K900E was unable to bind cooperatively with Oct-1 POU and could not sustain transcription activation, even though it was capable of sustaining basal transcription. Thus, a single-amino-acid change within the largest subunit of $SNAP_c$ debilitated transcription activation but had no effect on basal transcription.

Protein-protein interactions are best demonstrated by the generation of altered specificity interactions, in which a mutation in one partner that disrupts the interaction can be compensated for by a second mutation in the other partner that restores the interaction. Remarkably, SNAP190 K900E was capable of binding cooperatively with Oct-1 POU carrying the E7R mutation. As described above, E7R disrupts the interaction of Oct-1 POU with wild-type SNAP190. Together, these results identify a direct target for activation by the Oct-1 POU domain and show that activation is mediated by a protein-protein interaction.

OBF-1 is a B-cell-specific coactivator that associates with Oct-1 bound to an octamer motif and increases transcription from immunoglobulin promoters. Remarkably, although a comparison with the BLAST program of full-length SNAP190 with sequences in the databases failed to identify any related sequence in OBF-1, a direct comparison of the minimal SNAP190 region required for association with octamer-bound Oct-1 POU revealed a striking region of similarity with the first 60 amino acids of OBF-1, which are known to be required for interaction with octamer-bound Oct-1. Apart from the Oct-1 POU interacting regions, OBF-1 and SNAP190 do not share any obvious sequence similarity, nor are they known to share any functional role. Thus, two proteins that differ in their tissue distribution, their function, and their primary structure nevertheless share a common Oct-1 POU-binding domain.

Characterization of a Human RNA Polymerase III Holoenzyme

S. Sepehri, N. Hernandez

RNA polymerase II is known to exist in the cell as part of very large complexes. Some of these complexes contain a number of general transcription factors as well as factors required to mediate response to an activator. The same is true for RNA polymerase III. We are purifying this complex to determine its composition.

Factors Required for snRNA Transcription by RNA Polymerase III

L. Schramm, N. Hernandez

Basal RNA polymerase III transcription of the human snRNA genes requires $SNAP_c$, TBP, RNA polymerase III, and other, unidentified, components. The goal of this project is to identify new factors involved in RNA polymerase III transcription of snRNA genes.

Transcription from Chromatin Templates

X. Zhao, N. Hernandez

We are studying the transcription of snRNA genes wrapped into chromatin templates. We are especially interested in determining whether chromatin allows transcription activation by the Oct-1 POU domain and cooperative binding of $SNAP_c$ and Oct-1 POU to their natural targets in the human U6 promoter.

Factors Required for snRNA Transcription by RNA Polymerase II

T.L. Kuhlman, N. Hernandez [in collaboration with H. Cho and D. Reinberg, the Howard Hughes Medical Institute and the University of Medicine and Dentistry of New Jersey, Piscataway]

The general transcription factors required for RNA polymerase II transcription from a typical TATA-box-containing mRNA promoter such as the adenovirus 2

major late promoter have all been purified to near homogeneity, and most of them have been cloned. RNA polymerase II transcription from snRNA promoters requires $SNAP_c$, but surprisingly nothing is known about which of the general transcription factors are required.

We performed depletion experiments using antibodies directed against the various general transcription factors. In cases where we observed a reduction in transcription, we then tested whether complementation of the depleted extract with the missing recombinant factor restored transcription. Our results indicate that most of the general transcription factors are required for RNA polymerase II transcription of snRNA genes. Thus, although the factors that recognize the core elements of RNA polymerase II mRNA and snRNA-type promoters differ, they mediate the recruitment of many common general transcription factors.

Isolation of cDNAs Corresponding to FBI-1

D.J. Morrison, P.S. Pendergrast,
R. Kobayashi, N. Hernandez

We have obtained cDNAs encoding FBI-1 and have characterized the DNA-binding properties of this protein. FBI-1 contains a POZ domain at its amino terminus and four Krüppel-type zinc fingers at its carboxyl terminus. The carboxyl terminus is sufficient for specific binding, and FBI-1 can form homomers through its POZ domain and, in vivo, through its zinc finger domain as well. In addition, FBI-1 associates with Tat, suggesting that this interaction may have a role in Tat activation or in Tat repression of the short transcripts.

Function of FBI-1

P.S. Pendergrast, N. Hernandez

We are continuing our effort to define directly the function of FBI-1, both by in vivo transcription assays and protein-protein interaction assays.

PUBLICATIONS

Ford E., Strubin M., and Hernandez N. 1998. The Oct-1 POU domain activates snRNA gene transcription by contacting a region in the $SNAP_c$ largest subunit that bears sequence similarities to the Oct-1 coactivator OBF-1. *Genes Dev.* **12:** 3528–3540.

Henry R.W., Ford E., Mital R., Mittal V., and Hernandez N. 1998. Crossing the line between RNA polymerases: Transcription of human snRNA genes by RNA polymerases II and III. *Cold Spring Harbor Symp. Quant. Biol.* **63:** 111–120.

Henry R.W., Mittal V., Ma B., Kobayashi R., and Hernandez N. 1998. SNAP19 mediates the assembly of a functional core promoter complex ($SNAP_c$) shared by RNA polymerases II and III. *Genes Dev.* **12:** 2664–2672.

Pessler F. and Hernandez N. 1998. The HIV-1 inducer of short transcripts activates the synthesis of 5,6-dichloro-1-β-D-benzimidazole-resistant short transcripts *in vitro*. *J. Biol. Chem.* **273:** 5375–5384.

Wong M.W., Henry R.W., Ma B., Kobayashi R., Klages N., Matthias P., Strubin M., and Hernandez N. 1998. The large subunit of basal transcription factor $SNAP_c$ is a Myb domain protein that interacts with Oct-1. *Mol. Cell. Biol.* **18:** 368–377.

In Press

Kuhlman T.C., Cho H., Reinberg D., and Hernandez N. 1999. The general transcription factors IIA, IIB, IIF, and IIE are required for RNA polymerase II transcription from the human U1 small nuclear RNA promoter. *Mol. Cell. Biol.* **19:** 2130–2141.

Morrison D.J., Pendergrast P.S., Stavropoulos P., Colmenares S.U., Kobayashi R., and Hernandez N. 1999. FBI-1, a factor that binds to the HIV-1 inducer of short transcripts (IST), is a POZ domain protein. *Nucleic Acids Res.* **27:** 1251–1262.

HIGHER-ORDER CHROMOSOME DYNAMICS

T. Hirano M. Hirano A. Losada
K. Kimura T. Yokochi

The assembly of mitotic chromosomes is a fundamental process that ensures the faithful segregation of genetic information during eukaryotic cell division. Our laboratory is interested in understanding the molecular mechanisms underlying this dynamic structural change of chromosomes. Recent studies have shown that members of a unique class of chromosomal ATPases, known as SMC (structural maintenance of chromosome) proteins, have central roles in this process. By using a cell-free extract derived from *Xenopus laevis* eggs, we have identified two distinct SMC protein complexes, 13S condensin and 14S cohesin, which are key players in chromosome condensation and sister chromatid cohesion, respectively. Our efforts are now being focused on the structural and functional characterization of these two eukaryotic SMC protein complexes. SMC proteins are also conserved in bacteria and archaea and appear to fulfill similar cellular functions. To understand the evolutionarily conserved mechanism of SMC action, we are also working on a "primitive" SMC protein from the bacterium *Bacillus subtilis*. During the past year, great advances have been made in all three areas of research in our laboratory.

Condensin Regulation

K. Kimura, M. Hirano, T. Hirano [in collaboration with R. Kobayashi, Cold Spring Harbor Laboratory]

13S condensin is a chromosome condensation protein complex that we originally identified and purified from *X. laevis* egg extracts. 13S condensin is a five-subunit protein complex that consists of two different SMC subunits (XCAP-C and -E) and three non-SMC subunits (XCAP-D2, -G, and -H). When purified from mitotic extracts, 13S condensin can introduce positive supercoils into relaxed circular DNA in the presence of ATP and topoisomerase I. During the past year, we have obtained evidence that the positive supercoiling activity of 13S condensin is regulated by mitosis-specific phosphorylation of the XCAP-D2 and XCAP-H subunits. The phosphorylation of these two "regulatory" subunits is under the control of Cdc2 which governs the initiation of mitosis, as judged from immunodepletion and add-back experiments. Furthermore, in vitro phosphorylation and peptide-mapping experiments indicated that Cdc2 is likely to be the physiological kinase that directly phosphorylates and activates the condensin complex. By using phosphoepitope-specific antibodies, we were able to map three phosphorylation sites by Cdc2 in the carboxy-terminal domain of XCAP-D2. Our results provide for the first time a direct functional link between Cdc2 and one of its major downstream events, chromosome condensation.

Condensin Mechanism

K. Kimura, T. Hirano [in collaboration with N. Cozzarelli, University of California, Berkeley]

The energy-dependent positive supercoiling in the presence of topoisomerase I is likely to be an important activity of the chromosome condensation protein complex. We still do not know, however, how this reaction works or how this activity contributes to the compaction of chromatin fibers. To get further insight into the mechanism of condensin action, we set up a new functional assay in collaboration with Nick Cozzarelli's group. We found that when 13S condensin was incubated with a nicked circular DNA in the presence of a type II topoisomerase, a specific type of knot (i.e., positive trefoil knot) was generated. The knotting reaction, like the supercoiling reaction, required ATP hydrolysis and mitosis-specific phosphorylation of condensin subunits. These results suggest that 13S condensin actively compacts and organizes DNA by introducing a global positive writhe, rather than by a local overwinding or wrapping of DNA.

Cohesin and Sister Chromatid Cohesion

A. Losada, T. Yokochi, M. Hirano, T. Hirano

Sister chromatid cohesion is another important process for the faithful segregation of chromosomes in mitosis. The linkage between duplicated sister chromatids is established during S phase and maintained throughout the G_2 phase of the cell cycle. We have found that an SMC protein complex distinct from 13S condensin plays a crucial part in sister chromatid cohesion. This complex (termed 14S cohesin) consists of two SMC subunits (SMC1 and SMC3 types) and at least three additional subunits. One of the non-SMC subunits was identified to be the *Xenopus* homolog of budding yeast Scc1p/Mcd1p implicated genetically in sister chromatid cohesion. In the cell-free extracts, 14S cohesin associated with interphase chromatin during S phase, but most of the complex dissociated from chromatin at the onset of mitosis. Immunodepletion of 14S cohesin during interphase caused defects in sister chromatid cohesion in subsequent mitosis, whereas condensation was unaffected. We hypothesize that the cohesin complex is primarily involved in interphase-specific cohesion that holds sister chromatids together along their entire length. Upon entry in mitosis, the cohesin complex is released from the chromatids and mitosis-specific cohesion may be reestablished at the centromeric regions by a mechanism that remains to be determined.

We found that human tissue culture cell extracts also contain a protein complex whose size and subunit composition are very similar to those of the *Xenopus* 14S cohesin complex. We have established a purification scheme of this human cohesin complex and are currently trying to identify biochemical activities associated with this cohesion machine. Comparing and contrasting activities of 14S cohesin and 13S condensin should provide us with an integrated molecular picture of how higher-order chromosome dynamics are regulated throughout the eukaryotic cell cycle. These studies should also reveal a common molecular mechanism that might underlie the two apparently distinct processes: cohesion and condensation.

Mechanics of a Prokaryotic SMC Protein

M. Hirano, T. Hirano

Evolutionary conservation of SMC proteins among bacterial and archaeal species reflects their fundamental function in DNA dynamics. To understand the "basics" of SMC action, we have purified a prokaryotic SMC protein from the gram-positive bacterium *B. subtilis*. Unlike eukaryotic versions, the *B. subtilis* SMC protein (BsSMC) is a simple homodimer with no associated subunits. We found to our surprise that it binds preferentially to single-stranded DNA (ssDNA). The ATPase activity of BsSMC was stimulated by ssDNA more efficiently than by double-stranded DNA. A DNA spin-down assay revealed that in the presence of ATP, BsSMC forms large nucleoprotein aggregates in an ssDNA-specific manner. We also found that BsSMC undergoes conformational changes upon binding to ATP and ssDNA as judged from limited proteolysis experiments. Taking these results together with recent genetic data, we propose that the energy-dependent aggregation of ssDNA might represent a primitive type of chromosome condensation that occurs during segregation of bacterial chromosomes. We anticipate that comparative studies of the bacterial and eukaryotic SMC proteins will provide deep insights into the evolution of higher-order chromosome architecture as well as the evolution of SMC proteins per se.

PUBLICATIONS

Hirano M. and Hirano T. 1998. ATP-dependent aggregation of single-stranded DNA by a bacterial SMC homodimer. *EMBO J.* **17:** 7139–7148.

Hirano T. 1998. SMC protein complexes and higher-order chromosome dynamics. *Curr. Opin. Cell Biol.* **10:** 317–322.

Kimura K., Hirano M., Kobayashi R., and Hirano T. 1998. Phosphorylation and activation of 13S condensin by Cdc2 in vitro. *Science* **282:** 487–490.

Losada A., Hirano M., and Hirano T. 1998. Identification of *Xenopus* SMC protein complexes required for sister chromatid cohesion. *Genes Dev.* **12:** 1986–1997.

In Press

Hirano T. 1999. SMC-mediated chromosome mechanics: A conserved scheme from bacteria to vertebrates? *Genes Dev.* **13:** 11–19.

CELL BIOLOGY OF THE NUCLEUS

D.L. Spector T. Misteli
P. Mintz
T. Howard

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 are under way with the excellent technical expertise of Tamara Howard.

RNA Polymerase II Mediates Targeting of Splicing Factors to Transcription Sites In Vivo

T. Misteli, D.L. Spector

In eukaryotic cells, mRNAs are synthesized by a large RNA polymerase II holoenzyme containing the large subunit of RNA polymerase II and accessory transcription factors. In mammalian cells, the large subunit of RNA polymerase II contains a carboxy-terminal domain (CTD) consisting of 52 heptad repeats of the sequence YSPTSPS. Biochemical evidence has indicated that splicing factors as well as other RNA processing factors physically interact with the CTD of the large subunit of RNA polymerase II. During the past year, we have used live-cell microscopy to investigate the cell biological role of the CTD in the targeting of splicing components to transcription sites in intact nuclei. We find that the CTD mediates the recruitment and targeting of pre-mRNA splicing factors to transcription sites in vivo. Deletion of the CTD prevents the accumulation of splicing factors at a newly formed site of transcription and blocks pre-mRNA splicing. The inhibitory effect was general since deletion of the CTD resulted in the failure of not only SR proteins, but also core Sm proteins and the small nuclear RNAs (snRNAs) to accumulate at the site of transcription (Fig. 1). The presence of the CTD is crucial for targeting of splicing factors to sites of transcription since in CTD-less cells, splicing factors failed to accumulate at the transcription site despite the fact that they contained a level of RNA similar to that of cells expressing the large subunit with the wild-type CTD. This indicates that the mere presence of RNA is not sufficient for the targeting and accumulation of splicing factors to nascent RNA and that the CTD of the large subunit of RNA polymerase II is instrumental in the recruitment process. We have also mapped the domains of SR proteins that are required for the interaction with the large subunit of RNA polymerase II. We find that the RS domain is required for the interaction in several SR proteins and that the interaction of all SR proteins tested with the large subunit is independent of transcriptional activation of the cell. In the case of SF2/ASF-ΔRS, which has been shown to bind RNA with the same affinity as SF2/ASF-WT, but does not bind the CTD efficiently, this mutant protein did not accumulate at the site of transcription. These results argue that the CTD of RNA polymerase II is involved in the targeting of pre-

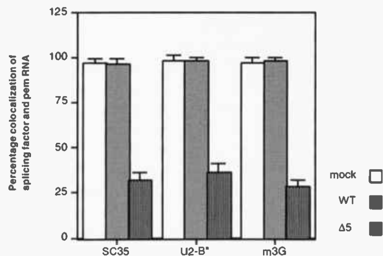


FIGURE 1 HeLa cells expressing pem RNA were examined for their ability to recruit splicing factors to the site of pem RNA transcription in the presence or absence of the RNA polymerase II CTD. In cells expressing the wild-type CTD or in mock transfected cells, splicing factors were recruited nearly 100% of the time to the transcription site. However, in cells expressing a CTD with only five heptapeptide repeats, splicing factors were recruited to the site of transcription only 25–30% of the time.

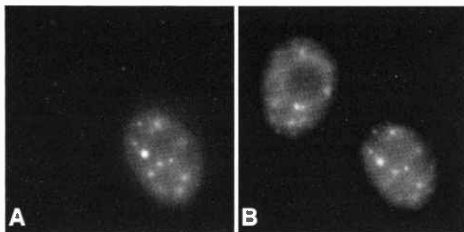


FIGURE 2 HeLa cells transiently expressing a plenty-of-proline's/YFP fusion protein (*A*) show the protein to be colocalized with an endogenous pre-mRNA splicing factor (*B*, lower nucleus) in a speckled nuclear distribution pattern. The upper cell in *B* was not transfected.

mRNA splicing factors to active sites of transcription. The general effect on splicing is consistent with the possibility that splicing components are present in the nucleus as complexes rather than single proteins, and it is thus tempting to speculate that the CTD is associated with prespliceosomal complexes or with spliceosomal subunits.

Purification and Biochemical Characterization of Nuclear Speckles

P. Mintz, D.L. Spector [in collaboration with S. Patterson, Amgen Inc.]

The mammalian cell nucleus is a highly organized structure that contains many internal nuclear domains including the nucleolus, interchromatin granule clusters, perichromatin fibrils, coiled bodies, PML bodies, and gems. Immunocytochemical studies have shown that pre-mRNA splicing factors are localized in a speckled nuclear pattern that corresponds to interchromatin granule clusters (IGCs) and perichromatin fibrils (PFs). Although PFs are thought to represent RNA transcripts, little to no transcription is associated with the majority of IGCs. One function of IGCs is to provide pre-mRNA splicing factors to sites of transcription. To elucidate additional roles of IGCs in nuclear function, we have developed a biochemical cell fractionation procedure to purify the IGCs from mouse liver nuclei. Samples have been taken from each stage in the biochemical fractionation and examined by electron microscopy for purity and morphological characterization. The purified interchromatin granules measured 18–27 nm in diameter, similar to their in situ counterparts. Immunogold labeling was performed on the IGC fraction with monoclonal antibody 3C5, which recognizes a family of SR proteins, to verify that it contained splicing factors. The immunogold particles labeled the IGCs. The protein composition of the purified IGCs was analyzed by one- and two-dimensional gel electrophoresis. We have identified approximately 300 proteins in the purified IGC fraction by two-dimensional gel electrophoresis; 75 proteins are enriched in this fraction. The IGC fraction was further immunopurified with the anti-3C5 monoclonal antibody. Immunoblotting was performed on the purified IGC fraction in order to identify known marker proteins. Several splicing and nonsplicing factors including $U2AF^{65}$, $U2AF^{35}$, and the five SR proteins (SRp20, SRp30a/b, SRp40, SRp55, SRp75) were enriched within the IGC fraction. The purified IGCs were analyzed by mass spectrometry in order to identify all of its constituents. Thus far, we have identified 27 known proteins and several expressed sequence tags. Using one of the peptide sequences, we identified a full-length cDNA encoding a protein called plenty-of-proline's. When this cDNA was fused to green fluorescent protein and transiently expressed in cells, the fusion protein localized to speckles (Fig. 2). We are currently in the process of developing monoclonal and polyclonal antibodies to the purified IGC fraction and the enriched proteins. By biochemically characterizing the IGCs, we hope to elucidate the biological roles of this nuclear compartment.

PUBLICATIONS

Huang S., Deerinck T.J., Ellisman M.H., and Spector D.L. 1998. The perinucleolar compartment and transcription. *J. Cell Biol.* **143:** 35–47.

Lorenz P., Baker B.F., Bennett C.F., and Spector D.L. 1998. Phosphorothioate antisense oligonucleotides induce the formation of nuclear bodies. *Mol. Biol. Cell.* **9:** 1007–1023.

Misteli T., Cáceres J.F., Clement J.Q., Krainer A.R., Wilkinson M.F., and Spector D.L. 1998. Serine phosphorylation of SR proteins is required for their recruitment to sites of transcription in vivo. *J. Cell Biol.* **143:** 297–307.

Misteli T. and Spector D.L. 1998. The cellular organization of gene expression. *Curr. Opin. Cell Biol.* **10:** 323–331.

PROTEIN TYROSINE PHOSPHATASES AND THE CONTROL OF CELLULAR SIGNALING RESPONSES

N.K. Tonks A.M. Bennett, R.L. Del Vecchio, A.J. Garton, M.J. Gutch, K.R. LaMontagne, J.P. Liu, M.P. Myers, H.L. Palka, K.A. Pennino, A.A. Samatar, Y. Shen, L. Zhang, S.H. 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 or 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, Anton Bennett, Mike Gutch, and Andy Garton completed their postdoctoral studies. Anton Bennett took up a faculty position in the Department of Pharmacology, Yale University, Mike Gutch joined EPS, Inc., a scientific publication company, as Manager of Science and Medical Projects, and Andy accepted a position as a Senior Research Scientist at OSI Pharmaceuticals, Inc., New York. In addition, Ken LaMontagne completed his Ph.D. and joined the laboratory of Dr. Judah Folkman at Children's Hospital, Boston, to perform postdoctoral studies. We were joined by Helena Palka, Yu Shen, and Lifang Zhang as postdocs and J.P. Liu worked as a technician in our laboratory during the year.

IDENTIFICATION OF PTP SUBSTRATES: DEVELOPMENT OF "SUBSTRATE-TRAPPING" MUTANT PTPs

The identification of substrates of PTPs is an essential step toward a complete understanding of the physiological function of members of this enzyme family. In 1995, in collaboration with David Barford (Oxford University), we determined the crystal structure of PTP1B in a complex with a phosphotyrosyl peptide substrate. Using this structure to drive an analysis by site-directed mutagenesis, we generated a form of PTP1B that maintains a high affinity for substrate but does not catalyze dephosphorylation effectively, i.e., we converted an extremely active enzyme into a "substrate trap." Furthermore, the residue that is mutated to generate the substrate-trapping mutant is the invariant catalytic acid (Asp-181 in PTP1B) that is conserved in all members of the PTP family. Therefore, this has afforded us a unique approach to identification of physiological substrates of PTPs in general. 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 become locked in a stable, "dead-end" complex. Potential substrates can be identified by immunoblotting lysates of cells expressing the mutant PTP with antibodies to pTyr to reveal proteins whose phosphorylation state is altered as a consequence of expression of the mutant. In addition, the complex between the mutant PTP and the pTyr substrate can be isolated by immunoprecipitation, and associated proteins can be identified by immunoblotting or, on a larger scale, by primary sequence determination. We have now initiated such an approach to substrate identification with intriguing results. The major take-home message from this work is that members of the PTP family display exquisite substrate specificity in a cellular context.

PTP-PEST

Previously, we had shown that the cytosolic protein tyrosine phosphatase PTP-PEST displays a remarkable degree of selectivity for tyrosine-phosphorylated $p130^{cas}$ as a substrate, both in vitro and in intact cells. We demonstrated that the highly specific nature of the interaction between PTP-PEST and $p130^{cas}$ appears to result from a combination of two distinct substrate recognition mechanisms; the catalytic domain of PTP-

PEST contributes specificity to the interaction with p130^{cas}, whereas the SH3 domain-mediated association of p130^{cas} and PTP-PEST dramatically increases the efficiency of the interaction. We have developed the study further by investigating the physiological role of PTP-PEST using Rat1 fibroblast-derived stable cell lines which we have engineered to overexpress PTP-PEST. These cell lines exhibit normal levels of tyrosine phosphorylation of the majority of proteins, but they have significantly lower levels of tyrosine phosphorylation of p130^{cas} than control cells. Initial cellular events occurring following integrin-mediated attachment to fibronectin (cell attachment and spreading) are essentially unchanged in cells overexpressing PTP-PEST; similarly, the extent and time course of MAP kinase activation in response to integrin engagement are unchanged. In contrast, the reduced phosphorylation state of p130^{cas} is associated with a considerably reduced rate of cell migration and a failure of cells overexpressing PTP-PEST to accomplish the normally observed redistribution of p130^{cas} to the leading edge of migrating cells. Furthermore, cells overexpressing PTP-PEST demonstrate significantly reduced levels of association of p130^{cas} with the Crk adaptor protein. Our results suggest that one physiological role of PTP-PEST is to dephosphorylate p130^{cas}, thereby controlling tyrosine-phosphorylation-dependent signaling events downstream from p130^{cas} and regulating cell migration.

PTPH1

PTPH1, which was first characterized in my laboratory, features a long noncatalytic amino-terminal segment characterized by the presence of a domain that has homology with band 4.1. PTPH1 is mostly associated with membrane structures in cells, and this association is mediated by the amino-terminal segment of the molecule. Furthermore, we have shown previously that the amino-terminal segment of PTPH1 exerts an inhibitory effect on activity of the enzyme in vitro, suggesting the potential for direct modulation of activity at defined locations in the cell. In addition, we had observed that up to 50% of PTPH1 is complexed with the 14-3-3 adaptor protein in cells in a manner that is dependent on phosphorylation of serine residues in PTPH1 and is regulated by mitogenic signals. However, the biological function of PTPH1 remained unclear.

When we expressed PTPH1 in NIH-3T3 cells under the control of a tetracycline-repressible promoter, we observed that the wild-type enzyme dramatically inhibited cell growth, whereas a catalytically impaired mutant showed no effect. To identify the direct target of PTPH1 in the cell, we applied our strategy of expression of a substrate-trapping mutant form of the enzyme, in which the invariant general acid aspartate residue is changed to alanine (D811A in PTPH1). The PTPH1-D811A mutant specifically trapped a 97-kD tyrosine-phosphorylated protein, which was determined to be valosin-containing protein (VCP, also named p97 or yeast CDC48) from various cell lysates in vitro. However, when expressed in mammalian cells, the D811A mutant was observed to contain high levels of pTyr and did not trap substrates. The recovery of pTyr in the D→A mutant appeared to be dependent on catalytic activity, since the inactive C→S mutant, in which the essential nucleophilic cysteine residue is mutated, did not incorporate phosphate. We focused our attention on the tyrosine residue (Y676) that forms one side of the active site cleft. Mutation of Y676 to phenylalanine (Y676F) in the PTPH1-D811A mutant led to a marked reduction in pTyr content. Furthermore, this double mutant specifically trapped VCP in vitro. Like wild-type PTPH1, this double mutant also inhibited cell proliferation. Moreover, induction of wild-type PTPH1 resulted in specific dephosphorylation of VCP without changing the overall phosphotyrosine profile of the cells. VCP is a member of a family of proteins that have been implicated in endoplasmic reticulum (ER)/Golgi membrane fusion, vesicle trafficking, organelle biogenesis, and cell cycle control. VCP has been found to be tyrosine-phosphorylated during mitosis and upon T-cell activation. Furthermore, in yeast, mutation of the tyrosine residue in CDC48 which is equivalent to the major phosphorylation site of VCP results in growth defects. Our data suggest that PTPH1 may exert its effects on cell proliferation via dephosphorylation of VCP as substrate, thus implicating tyrosine phosphorylation as an important regulator of VCP function. Furthermore, these studies reveal an important refinement to our strategy for identifying physiological substrates of the PTPs. Upon expression of several members of the PTP family, the D→A mutants are recovered in a form that contains high levels of pTyr and in which substrate trapping is impaired. We anticipate that the combination of the D→A mutation with conversion of the active site tyrosine residue to phenyl-alanine will yield effective substrate-trapping mutant forms of all of these PTPs and thus expand the scope of this approach.

PTEN

Since their initial characterization 10 years ago, many PTPs have been linked to the inhibition of cell proliferation. This led to the proposal that these enzymes may

function as tumor suppressors. However, it was not until 1997 that the identification of PTEN revealed the first clear-cut example of a tumor suppressor PTP. PTEN is one of the most frequently deleted tumor suppressor genes, especially in prostate cancer, endometrial cancer, melanomas, and glioblastomas. In addition to playing a part in sporadic cancers, germ-line transmission of PTEN mutations gives rise to a variety of familial cancer syndromes, such as Cowden Disease. These disorders are characterized by the formation of multiple, benign tumors and an increased susceptibility to malignant cancers. The frequent loss of PTEN in different types of malignancies indicates that it has a critical role in cancer surveillance in a wide variety of tissues. Therefore, the study of PTEN is expected to reveal important insights into the complex process of tumorigenesis and may lead to the discovery of new or improved treatments for a variety of cancers.

Previously, we had shown that PTEN possesses intrinsic phosphatase activity and displays selectivity for highly acidic substrates. Furthermore, mutations in PTEN, identified in primary tumors, tumor cell lines, and tissue explants from patients with Cowden Disease, result in the ablation of phosphatase activity in vitro, suggesting that phosphatase activity is required for PTEN to function as a tumor suppressor. One exception to this rule is a mutation that was isolated from two independent Cowden Disease kindreds. This mutation, in which a glycine in the conserved catalytic signature motif is mutated to a glutamate (G129E), did not significantly ablate the phosphatase activity of PTEN in vitro. Although it retained phosphatase activity using artificial substrates, this mutation must ablate the tumor suppressor function of PTEN as it is the causative ablation in a cancer predisposition syndrome. Therefore, this mutation becomes a powerful tool with which to ascertain which activities ascribed to PTEN are important for its tumor suppressor function (Fig. 1).

We have extended our analysis of this important enzyme to identify its physiological substrates. Despite the precedents showing that the PTPs recognize protein targets, our observations led to the proposal that the physiological substrates of PTEN may actually be nonproteinaceous and focused our attention on the inositol phospholipids. Inositol phospholipids were attractive candidates because, in addition to their highly acidic nature, they function as second messengers that regulate the morphological and hyperproliferative responses that underlie cellular transformation. Additionally, these lipids have also been shown to inhibit apoptosis, a naturally occurring form of cell death that is often triggered by, and counteracts, the effects of hyperproliferative stimuli. In fact, we discovered that PTEN dephosphorylates a specific inositol phospholipid, phosphatidylinositol 3,4,5-trisphosphate (PIP_3). PIP_3 is normally produced by the activation of PI3-kinase in response to a variety of stimuli, including most growth and survival factors, and functions to activate a wide variety of signaling pathways by recruiting signaling molecules, such as the protein kinase PKB/Akt, to the plasma membrane. PTEN exhibited exquisite selectivity for PIP_3 to the point of dephosphorylating only a single site in the inositol ring—the site phosphorylated by PI3-kinase. Importantly, the G129E Cowden Disease mutation resulted in the specific loss of the ability of PTEN to dephosphorylate PIP_3. Since this mutation, which ablates the tumor suppressor function of PTEN, retained activity against proteinaceous substrates, it was clear that the lipid phosphatase activity of PTEN, rather than its protein phosphatase activity, is required for its function as a tumor suppressor. Additionally, tumor cell lines, which have lost PTEN expression through mutation or deletion of the endogenous alleles, have increased levels of PIP_3 and of activated PKB/Akt. Reconstitution of PTEN expression in these cells lowered both the levels of PIP_3 and of activated PKB/Akt, indicating that PTEN also functions as PIP_3 phosphatase in intact cells. Most importantly, reconstitution of PTEN expression in prostate cancer cell lines resulted in the inhibition of PIP_3-dependent survival signals and the induction of apoptosis in these cells. Significantly, the lipid-phosphatase-deficient mutant of PTEN did not induce apoptosis in these cells, further emphasizing the requirement of the lipid phosphatase activity of PTEN to inhibit the tumor-promoting signals emanating from PIP_3. These data demonstrate that the lipid phosphatase activity of PTEN is required for it to function as a tumor suppressor and provide a molecular basis for Cowden Disease, as loss of the lipid phosphatase activity of PTEN leads to the accumulation of the products of PI3-kinase and results in the neoplastic pathology characteristic of the disease.

PUBLICATIONS

Brady-Kalnay S.M., Mourton T., Nixon J.P., Pietz G.E., Kinch M., Chen H., Brackenbury R., Rimm D.L., Del Vecchio R.L., and Tonks N.K. 1998. Dynamic interaction of PTPμ with multiple cadherins *in vivo*. *J. Cell Biol.* **141:** 287–296.

Gutch M.J., Flint A.J., Keller J., and Tonks N.K. 1998. The *Caenorhabditis elegans* SH2 domain-containing tyrosine phosphatase, PTP-2, participates in signal transduction

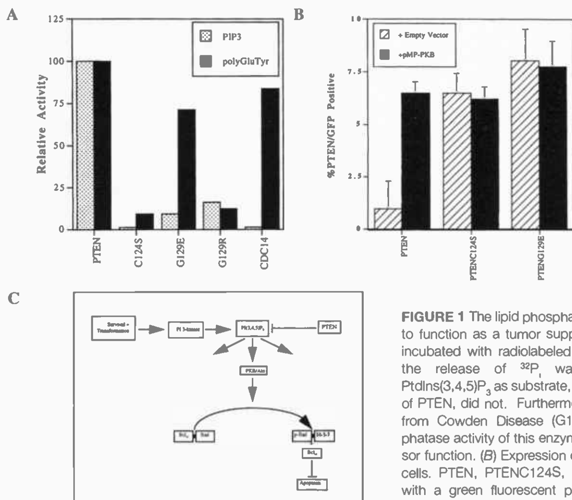


FIGURE 1 The lipid phosphatase activity of PTEN is required for it to function as a tumor suppressor. (*A*) Recombinant PTEN was incubated with radiolabeled polyGluTyr and PtdIns(3,4,5)P_3 and the release of $^{32}P_i$ was measured. PTEN recognized PtdIns(3,4,5)P_3 as substrate, whereas cdc14, the closest homolog of PTEN, did not. Furthermore, a point mutant of PTEN isolated from Cowden Disease (G129E) revealed that the lipid phosphatase activity of this enzyme is important for its tumor suppressor function. (*B*) Expression of PTEN induces apoptosis in LnCaP cells. PTEN, PTENC124S, or PTENG129E were cotransfected with a green fluorescent protein (GFP) expression vector into LnCaP cells. The cotransfections also included either expression vector for a constitutively active form of PKB/Akt (*black bars*) or a control empty vector (*striped bars*). Expression of PTEN resulted in a decrease in the number of viable cells recovered, and this loss could be rescued by coexpression of the activated form of PKB/Akt. This indicates that PTEN inhibits the PKB/Akt-dependent survival signals. Significantly, the lipid phosphatase-defective mutant (G129E) did not effect cell viability, indicating that the lipid phosphatase activity of PTEN is required for this effect. Transfected cells were identified as GFP-positive cells or by immunofluorescence microscopy following staining with antibodies to the transfected PTEN. Transfection efficiency was assessed by determining the percentage of GFP/PTEN-positive cells (total number of cells was determined by counting nuclei [stained with DAPI]). Data are expressed as the mean transfection efficiency (±S.D. n = 3). (*C*) Model of PTEN function in antagonizing the growth/survival signals stimulated by PI 3-kinase.

during oogenesis and vulval development. *Genes Dev.* **12:** 571–585.

LaMontagne, Jr. K.R., Hannon G., and Tonks N.K. 1998. Protein tyrosine phosphatase PTP1B suppresses p210 bcr-abl-induced transformation of Rat1 fibroblasts and promotes differentiation of K562 cells. *Proc. Natl. Acad. Sci.* **95:** 14094–14099.

LaMontagne, Jr. K.R., Flint A.J., Franza, Jr. B.R., Pendergast A.M., and Tonks N.K. 1998. Protein tyrosine phosphatase 1B dephosphorylates and antagonizes signalling by the oncoprotein tyrosine kinase p210 bcr-abl in vivo. *Mol. Cell Biol.* **18:** 2965–2975.

Li J., Simpson L., Takahashi M., Miliaresis C., Myers M.P., Tonks N., and Parsons R. 1998. The *PTEN/MMAC1* tumor suppressor induces cell death that is rescued by the Akt/Protein kinase B oncogene. *Cancer Res.* **58:** 5667–5672.

Myers M.P., Pass I., Batty I.H., Van der Kaay J., Stolarov J.P., Hemmings B.A., Wigler M.H., Downes C.P,. and Tonks N.K. 1998. The lipid phosphatase activity of PTEN is critical for its tumor suppressor function. *Proc. Natl. Acad. Sci.* **95:** 13513–13518.

Pannifer A.D.B., Flint A.J., Tonks N.K., and Barford D. 1998. Visualization of the cysteinyl-phosphate intermediate of a protein tyrosine phosphatase by X-ray crystallography. *J. Biol. Chem.* **273:** 10454–10462.

Tiganis T., Bennett A.M., Ravichandran K.S., and Tonks N.K. 1998. Epidermal growth factor receptor and the adaptor protein p52^{shc} are specific substrates of T cell protein tyrosine phosphatase. *Mol. Cell Biol.* **18:** 1622–1634.

In Press

Bianchi C., Sellke F., DelVecchio R., Tonks N.K., and Neel B.G. 1999. Receptor type protein tyrosine phosphatase μ (RPTPμ) is expressed in specific vascular endothelial beds *in vivo*. *Exp. Cell Res.* (in press).

Garton A.J. and Tonks N.K. 1999. Regulation of fibroblast motility by the protein tyrosine phosphatase PTP-PEST. *J. Biol. Chem.* **274:** 3811–3818.

Garton A.J., Flint A.J., and Tonks N.K. 1999. Identification of substrates for protein tyrosine phosphatases. In *Protein phosphorylation: A practical approach* (ed. D.G. Hardie), 2nd edition. Oxford University Press. (In press.)

SIGNAL TRANSDUCTION

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

Our research interest is the study of the signal transduction pathways involved in cell growth control and morphogenesis. In particular, one major research project is to investigate the role of the Ras and Rac proteins in signal transduction. Ras and Rac are members of the Ras and Rho subfamilies of small GTPases which function as molecular switches cycling between an inactive guanine nucleotide diphosphate (GDP)-bound state and an active guanine nucleotide triphosphate (GTP)-bound state. These proteins have been shown to have a central role in the signal transduction pathways that mediate important biological phenomena such as transformation, senescence, adhesion, invasiveness, metastasis, and development. Our major objective is to uncover the molecular mechanisms by which the Ras and Rac proteins exert these effects. Another project ongoing in our laboratory is the functional characterization of $p62^{dok}$, a constitutively tyrosine phosphorylated, Ras-GAP-associated protein, detected in chronic myelogenous leukemia (CML) progenitor cells.

Ras SIGNALING PATHWAYS

Ras proteins have a crucial role in the ethiology of human cancers. Activating mutations in *ras* genes are commonly found in a variety of human tumors, and, in addition, oncogenic mutants of Ras confer both tumorigenic and metastatic properties to rodent cells in culture, providing a system for determining the molecular events necessary for tumor progression. Abundant evidence suggests a crucial role for the Raf-MEK-MAPK (mitogen-activated protein kinase) pathway in Ras-mediated transformation; however, more recent studies have implicated Raf-independent pathways which contribute to mammalian cell transformation. These include the PI3-kinase/Rac and RalGDS/Ral (Ral guanine nucleotide dissociation stimulator) pathways. Less clear are the Ras-mediated signaling pathways key to the acquisition of the metastatic phenotype. To gain more information, we tested effector domain mutants of oncogenic *ras* (which are deficient in specific effector function and therefore unable to activate specific downstream signaling) for their abilities to mediate tumorigenic and metastatic phenotypes in athymic nude mice when expressed in NIH-3T3 cells. The use of these Ras effector loop mutants (RasV12S35, RasV12G37, and RasV12C40) showed that all mutants displayed comparable tumorigenic properties, but that only the mutant which retained its ability to activate the Raf/MEK/MAPK pathway, RasV12S35, induced tumors in the experimental metastasis assay. Furthermore, we found that direct activation of the MEK/MAPK pathway, using either a constitutively active mutant of MEK or the oncogene *mos*, which is a potent activator of MEK, was sufficient to induce metastasis, whereas R-Ras, which is able to activate PI3-kinase but not MAPK was tumorigenic but nonmetastatic. Finally, we observed that the subcutaneous tumors and lung metastases derived from RasV12S35-transformed cells displayed an increased level of MAPK activity as compared to parental cells, whereas cells explanted from RasV12G37- and RasV12C40-induced tumors did not show any changes in MAPK activity. Taken together, these results indicate that whereas Ras-mediated tumorigenicity can arise independently from MAPK activation, experimental metastasis requires constitutive activation of the MAPK pathway, and that the Ras effector pathways mediating the tumorigenic and metastatic activities can be segregated. We cannot, however, exclude that in addition to Raf, other effectors differentially regulated by the above-described Ras effector loop mutants may contribute to the metastatic phenotype. In addition, since an experimental metastasis assay was used, the metastatic phenotype relates to later stages of metastasis (e.g., extravasation and establishment and/or secondary growth) and not to the early steps (e.g., intravasation) in which the Rho-GTPases are likely to play a part (see below). These studies have been performed in collaboration with G. Webb and G. Vande Woude (National Cancer Institute).

In addition to the above-mentioned Ras effectors, we identified AF-6 as a novel Ras-binding protein. AF-6 has been previously described as a fusion partner for ALL1 in acute lymphoblastic leukemias. The AF-6 protein contains a combination of interesting homology regions. At its very amino terminus reside two puta-

tive Ras-binding motifs, which we proved to be essential for Ras binding. These are followed by U104 and DIL motifs, domains that are found in the head portions of actin and microtubule-based motor proteins. Located further to the carboxyl terminus is a PDZ domain. Recent studies showed that AF-6 interacts with members of the Eph receptor tyrosine kinases through its PDZ domain. Furthermore, the carboxy-terminal remainder of AF-6 harbors several proline-rich clusters that may also function as docking sites for other molecules. Although the biochemical function of AF-6 remains to be defined, a picture is emerging that suggests a role for AF-6 in the establishment and/or maintenance of cell-cell contacts. In epithelial cells, AF-6 colocalizes with the cell adhesion component ZO-1 at tight junctions, whereas in cells lacking tight junctions, the two proteins colocalize with adherens junctions. Furthermore, AF-6 has been reported to interact directly with ZO-1. We recently found that the carboxy-terminal portion of AF-6 interacts with profilin, a G-actin-binding protein that regulates actin filament assembly. It is thus an intriguing possibility that AF-6 serves as a multi-adaptor protein linking components of tight junctions and/or adherens junctions with the actin cytoskeleton. At present, the functional relationship between Ras and AF-6 remains to be unraveled. To address this question in a more comprehensive system, we took refuge to *Drosophila* as a model system. A *Drosophila* homolog of AF-6 known as Canoe was first identified in a genetic search for unknown components of Notch signaling, a pathway that in a multitude of developmental processes determines various cell fates. Notably, the Canoe protein harbors the same homology regions in the same order as AF-6. In fact, we found that the domain containing the predicted Ras-binding motifs in Canoe is sufficient to bind to Ras. In collaboration with Ulrike Gaul (Rockefeller, New York), we recently observed that expression of Canoe under GMRGAL4 control (which drives expression in all cells posterior to the morphogenetic furrow in the developing eye) leads to excessive neural determination of precursor cells in the eye disc and that Canoe appears to influence eye differentiation in a dose-dependent manner. Further studies are under way to assess the relationship between Ras and Canoe.

RAC SIGNALING PATHWAYS

During the past years, it has become clear that the Rho family member, Rac, participates in a wide variety of biological activities, including cytoskeletal organization, transcriptional activation, and proliferation, and more recently adhesion, invasiveness, and neuronal development. Depending on the cell type and the extracellular matrix, Rac can promote either cell adhesion or cell migration. We recently showed that expression of a constitutively activated mutant form of Rac, RacV12, triggers the adherens of a Jurkat T-cell line on immobilized fibronectin in an integrin-dependent manner and that adhesion is associated with dramatic cell spreading, cytoskeletal rearrangements, and integrin clustering. Expression of RacV12, however, does not alter the expression level nor activation state of the integrins, indicating that the contribution of Rac to T-cell adhesion involves events following receptor occupancy, such as cell spreading and integrin clustering, rather than alterations of integrin affinity or cell surface expression.

A major challenge has been the identification of downstream effector pathways by which Rac mediates the above activities. We have taken three different approaches to obtain more insight in the signaling pathways mediating these effects. As a first approach, we made use of the yeast two-hybrid system to isolate Rac-interacting proteins. We succeeded in the isolation of three novel Rac-interacting proteins and provided evidence for a role of one of them, designated POR1, in cytoskeletal rearrangements. The other proteins are currently under investigation. Second, we devised a system to dissect genetically the different functions of Rac. This was accomplished through the use of Rac effector domain mutants, which we obtained in a yeast two-hybrid screen for mutant forms of Rac which are differentially impaired in their ability to bind different Rac effector proteins. These results indicate that the growth-promoting activity of Rac is not dependent on signals contributed by the Jun N-terminal kinase (JNK) MAP kinase or SRF pathways. Activation of JNK and SRF (serum response factor) is also not essential for Rac's effect on T-cell adhesion; the latter is, however, dependent on the short-term cytoskeletal rearrangements triggered by Rac and appears to involve a lipid kinase. As a third approach to identify targets of Rac—in particular those underlying invasiveness—we intend to perform cDNA-RDA (representational difference analysis) on pairs of cDNAs obtained from a breast carcinoma cell line (T47D) expressing RacV12 under an inducible promoter. The choice of this particular cell line is based on the fact that activation of RacV12 in T47D cells was previously shown to confer an invasive potential to these cells. RDA is a polymerase chain reaction (PCR)-based subtractive hybridization technique

which selectively amplifies the differences between two DNA populations. We will be performing RDA on the cDNAs obtained from T47D cells induced and not induced for RacV12 expression to isolate the cDNAs that are differentially expressed. These experiments will be performed in collaboration with Rob Lucito and M. Wigler here at the Laboratory.

FUNCTIONAL CHARACTERIZATION OF p62^{DOK}

Another research project ongoing in our laboratory is the functional characterization of p62^{dok}, a protein that was observed to be constitutively phosphorylated on tyrosine in primary *lin*$^-$ Ph$^+$ chronic-phase CML blasts. CML is characterized by the presence of the fusion protein p210$^{bcr\text{-}abl}$ which has an elevated tyrosine kinase activity relative to normal c-Abl. Furthermore, p62^{dok} was found to form an in vivo complex with p120 Ras-GAP. It is an intriguing possibility that p62^{dok} is a critical immediate substrate of p210$^{bcr\text{-}abl}$ and that its constitutive phosphorylation plays a part in a complex physiological disorder manifested in the chronic phase of CML.

We obtained further evidence that p62^{dok} is a direct substrate of p210$^{bcr\text{-}abl}$ and interacts directly with the SH2-SH3-SH2 domain of Ras-GAP. R. Kobayashi here at the Laboratory succeeded in mapping the tyrosine sites on p62^{dok} responsible for GAP binding. To obtain more information on the function of p62^{dok} and its relative contribution to the p210$^{bcr\text{-}abl}$-induced aberrant phenotype in hematopoietic cells, we searched for p62^{dok}-interacting proteins and constructed a variety of truncation mutants with the goal to obtain dominant-negative mutants of p62^{dok} that may interfere with p210$^{bcr\text{-}abl}$ signaling. Our coimmunoprecipitation experiments using antibodies raised against p62^{dok} and Mo7/p210$^{bcr\text{-}abl}$ cells revealed the presence of at least five additional proteins with molecular masses of 64, 66, 68, 120, and 210 kD. The 120- and 210-kD bands were identified as Ras-GAP and p210$^{bcr\text{-}abl}$, respectively. The identity of the other proteins remains to be defined; we do know, however, that these proteins are not Zap70, Slp76, Lck, or Syk components in T-cell signaling. We intend to purify these proteins and clone their respective cDNAs. Additionally, we are currently assessing the effects of p62 truncation mutants on p210$^{bcr\text{-}abl}$-induced proliferation and differentiation in hematopoietic cell lines as well as the growth properties of p62$^{-/-}$ cells (which we retrieved from P. Pandolfi, Memorial Sloan-Kettering Cancer Center, New York) and potential altered signaling in these cells. These studies are being performed in collaboration with R. Kobayashi and N. Tonks (CSHL) and B. Clarkson and P. Pandolfi.

PUBLICATIONS

Antonarakis, S.E. and Van Aelst L. 1998. Mind the GAP, Rho, Rab and GDI. *Nature Genet.* **19:** 106–108.

Bagrodia S., Taylor S.J., Jordon K.A., Van Aelst L., and Cerione R.A. 1998. A novel regulator of p21-activated kinases. *J. Biol. Chem.* **273:** 23633–23636

D'Souza-Schorey C., Boettner B., and Van Aelst L. 1998. Rac regulates integrin mediated spreading and increased adhesion of T lymphocytes. *Mol. Cell. Biol.* **18:** 3936–3946

Lin A.W., Barradas M., Stone J.C., Van Aelst L., Serrano M., and Lowe S.W. 1998. Premature senescence involving p53 and p16 is activated in response to constitutive MEK/MAPK mitogenic signaling. *Genes Dev.* **12:** 3008–3019.

Webb C.P., Van Aelst L., Wigler M.H., and Vande Woude G.F. 1998. Signaling pathways in Ras-mediated tumorigenicity and metastasis. *Proc. Natl. Acad. Sci.* **95:** 8773–8778.

In Press

Boettner B. and Van Aelst, L. 1999. Rac and Cdc42 effectors. *Progr. Mol. Subcell. Biol.* **22:** 136–158.

STRUCTURE AND GENOMICS

The Structure and Genomics Section continued to expand its activities and interactions with other CSHL scientists. This group continues its work to apply both classical experimental approaches and computational approaches toward a new understanding of the correlation between structure and function in both proteins and nucleic acids.

The Structural area has seen several important advances.

- Rui-Ming Xi's lab has made considerable advances in understanding the structure of the proteins in the splicing complex.
- Leemor Joshua-Tor's lab has determined the structure of bleomycin hydrolase, and this has led to a new understanding of the function of this protein.
- Peter Nestler's lab showed the potential of screening combinatorial libraries with peptide epitopes to isolate binding regions.
- The Kobayashi lab, in collaboration with Yuri Lazebnick, used mass spectrometry to identify a protein identified with oncogene activity as being the apoptotic protease activity factor I.
- Andy Neuwald's lab, using a computational approach to protein structure, has developed new algorithms to better compare protein sequences and has used this to study a group of ATPases.

The Genomics area has expanded with the addition of Dr. Lincoln Stein.

- Lincoln Stein has been developing tools to allow the display of complex genome databases to biologists via the World Wide Web. His first use of these tools has been to display the database ACEDB, which contains the complete genome sequence of *Caenorhabditis elegans*, the first animal to be completely sequenced.
- The Zhang lab has expanded their efforts in predicting genes from genome sequence and has also begun efforts to find other types of signals in sequences, such as promoters.
- The McCombie lab has continued its genome analysis efforts, sequencing more than 2 million bases of DNA, including parts of *Arabidopsis* and the region encoding the human tumor suppresser PTEN.

DNA Sequencing Lab entrance at Hershey

STRUCTURAL BIOLOGY

L. Joshua-Tor	T. Messick	D. van Aalten
	P. O'Farrell	D. Vaughn
	J. Rosenbaum	C. Chong (URP)

We study the molecular basis of cell regulatory processes by 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 to characterize properties that can be correlated to protein structure and information from molecular biology and genetics in a collaborative effort to study protein function.

Our current efforts center around two distinct themes. The first is the study of an evolutionarily conserved family of oligomeric self-compartmentalizing intracellular proteases, the bleomycin hydrolases (cylinases). The second theme involves structural studies of protein complexes involved in gene expression. We are also looking at the effect of molecular motions on various biological processes.

During the last year, Tom Malone, who has been the Crystallographic Systems Manager for both structure groups, departed the Laboratory. Ming Wang has taken his place and now runs the X-ray and computer facilities for the Structural Biology Center.

Bleomycin Hydrolases

Bleomycin hydrolase (BH) is a 300-kD cysteine protease with unusual structural and biological features. It was discovered because of 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; homologs exist in bacteria, yeast, birds, and mammals, with high sequence identity among the different forms. The yeast form negatively regulates the galactose metabolism system and also binds single-stranded DNA and RNA with high affinity. Nucleic-acid-binding activity was also reported for other members of the family.

Previously, we determined the crystal structure of the yeast BH, Gal6. The structure reveals several unique features of these proteases. It is a hexameric ring-barrel structure with the active sites embedded in a central cavity. This cavity is 22 Å wide at the openings on either end, and widens to 45 Å in the interior of the protein. The only access to the active sites is through the cavity, and thus a protein substrate would need to be at least partially unfolded or degraded in order to reach them. 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 recent work on the yeast BH, Gal6, has shown that it acts as a carboxypeptidase on its carboxyl terminus to convert itself to an aminopeptidase and peptide ligase. The carboxyl terminus anchors the substrate into the active site and confers "positional" specificity. This model also serves to explain the unique ability of BH/Gal6 to inactivate bleomycin.

Human Bleomycin Hydrolase

P. O'Farrell, L. Joshua-Tor [in collaboration with
F. Gonzalez, W. Zheng, and S.A. Johnston,
University of Texas-Southwestern Medical Center]

In an effort to understand the mechanism of action of the enzyme, and with the long-term goal of designing an effective inhibitor and a cleavage-resistant bleomycin, we have determined the crystal structure of wild-type human bleomycin hydrolase (hBH) at 2.6 Å resolution and of an active site mutant C73S at 1.85 Å. As expected from sequence identity, the structure of hBH is very similar to the structure of the yeast BH, Gal6. However, there is a striking difference between the two forms. The central channel, which has a very prominent positive charge in the yeast enzyme, is slightly negative in the human protein (Fig. 1). We have also determined that hBH does not have the strong single-stranded DNA- or RNA-binding activity of the yeast protein. This may reflect a somewhat different, or perhaps altered, role in their respective biological functions. As mentioned, the clinical relevance of hBH has recently been augmented by its possible association with Alzheimer's disease. The structure suggests that the location of the putative disease-linked variant could affect interactions with another protein, which in turn may modulate the peptidase activity of hBH by repositioning the carboxyl terminus.

If indeed there is an involvement of hBH in some Alzheimer's disease pathology, the structural and functional similarities and differences between the yeast and human forms may prove useful in delineating the disease mechanism. In addition, the human structure may now serve as the basis for a rational design of inhibitors of BH activity to augment the effectiveness of bleomycin in cancer treatment.

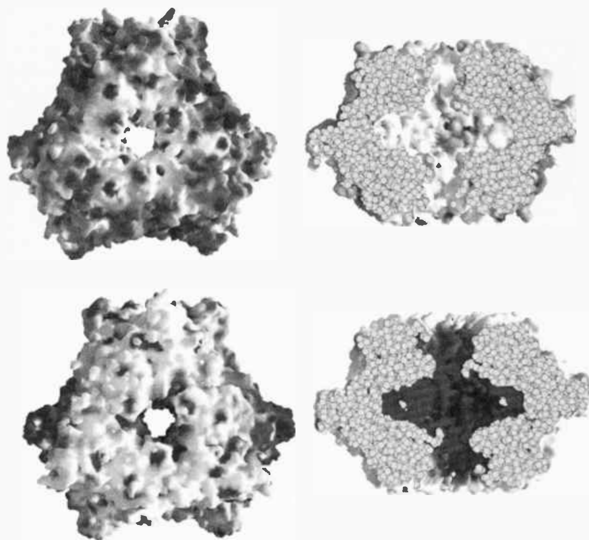


FIGURE 1 The central channel in hBH is close to neutral in charge, whereas the yeast BH, Gal6, has a very prominent positive charge as shown by the darker surface. *(Top)* hBH; *(bottom)* Gal6. *(Left panels)* Top view down the threefold axis, *(right panels)* a slice through the protein, highlighting the difference in charge between the interiors of the human and yeast proteins.

A Structural Snapshot of Base Pair Opening in DNA

D.M.F. van Aalten, L. Joshua-Tor [in collaboration with G. Verdine, Harvard University]

The response of double-helical DNA to torsional stress may be a driving force for many processes acting on DNA. The 1.55-Å crystal structure of a duplex DNA oligonucleotide $d(CCAGGCCTGG)_2$ with an engineered cross-link in the minor groove between the central guanine bases depicts how the duplex can accommodate such torsional stress. We have captured in the same crystal two very different conformational states. One duplex (A) contains a strained cross-link that is stabilized by calcium ion binding in the major groove, directly opposite the cross-link. This strain results from an eclipsed bond for one of the ethano bridges in the tether that forms the ethyldisulfide cross-link and very tight packing of the cross-link in the minor groove. In the other duplex (B), the strain in the cross-link is relieved as both ethano bridges are in a staggered conformation and the cross-link is loosely packed in the groove. The net result is that in going from molecule A to B, the tether is elongated and thrust forward in the direction of the bases by approximately 2.5 Å. Thus, the entire disulfide cross-link in molecule B is fully extended. This pushes the guanine at the end of the cross-link into the major groove. However, because the far end of this guanine base remains attached to the DNA backbone, the base is constrained to swivel rather than slide forward. The cytosine swivels the other way, partially flipping out from the helix, with a partial rupture of the base pair, retaining only one hydrogen bond. This conformation is subsequently trapped by a stabilizing hydrogen-bonding interaction with a neighboring molecule. The consequence of this is therefore partial extrusion of a cytosine accompanied by helix bending. The sequence used is the target sequence for the *Hae*III methylase, and this partially flipped cytosine is the same nucleotide targeted for extrusion by the enzyme. Molecular dynamics simulations of these structures show an increased mobility for the partially flipped-out cytosine.

Crystal Structure of Carboxypeptidase A Complexed with D-Cysteine

D.M.F. van Aalten, C.R. Chong, L. Joshua-Tor

D-Penicillamine is used as a treatment for Wilson's disease and arthritis. Recent experiments have shown that it is a potent inhibitor of zinc proteases, such as carboxypeptidase A. It appears to inhibit carboxypeptidase by causing the active site zinc to dissociate from the protease. D-cysteine, which differs from D-penicillamine by removal of two methyl groups on the β-carbon, inhibits carboxypeptidase by formation of a ternary complex in which the zinc ion remains bound in the active site. We solved the crystal structure of carboxypeptidase A complexed to D-cysteine at 1.75 Å resolution. This is the first structure of a thiol inhibitor bound to carboxypeptidase. The thiolate of D-cysteine has replaced the active site water to coordinate the zinc ion. In addition to the zinc-sulfur bond, the inhibitor is tethered to the active site by eight hydrogen bonds, five of which are protein residues. In contrast, L-cysteine would not be capable of forming all of these interactions concurrently, which may explain its reduced affinity. In addition, the two additional methyl groups of D-penicillamine would result in steric clashes with primary and secondary zinc ligands. This may explain why this drug acts presumably by extracting zinc from the active site rather than simply blocking substrate access to the active site.

PUBLICATIONS

Joshua-Tor L. and Johnston S.A. 1998. Bleomycin hydrolase . In *Handbook of proteolytic enzymes* (ed. A.J. Barrett et al.), pp. 634–638. Academic Press, London.

van Aalten D.M.F., Grotewold E., and Joshua-Tor L. 1998. Essential dynamics from NMR clusters: Dynamic properties of the Myb DNA-binding domain and a hinge-bending enhancing variant. *Methods* **14:** 318–328.

Zheng W., Johnston S.A., and Joshua-Tor L. 1998. The unusual active site of Gal6/bleomycin hydrolase can act as a carboxypeptidase, aminopeptidase, and peptide ligase. *Cell* **93:** 103–109.

In Press

O'Farrell P.A., Gonzalez F., Zheng W., Johnston S.A., and Joshua-Tor L. 1999. Crystal structure of human bleomycin hydrolase, a self-compartmentalizing cysteine protease. *Structure* (in press).

PROTEIN CHEMISTRY

R. Kobayashi N. Kashige
N. Poppito
K. Wanat

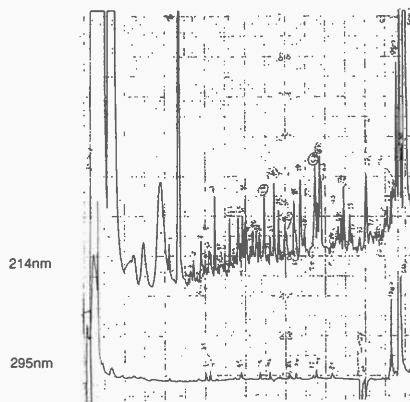
The Human Genome Project is well on its way to completion, and thus we are focusing more and more on studying protein function. To do so, we are developing all kinds of methods for microcharacterization of protein, including protein sequencing and posttranslational modification analysis.

Oncogene-dependent Apoptosis Is Mediated by Caspase-9

R. Kobayashi, N. Poppito, K. Wanat [in collaboration with Y. Lazebnik, Cold Spring Harbor Laboratory]

Collaboration with other scientists at Cold Spring Harbor Laboratory continues to be a major activity in our laboratory. We use the matrix-assisted laser desorption ionization time-of-flight mass spectrometer (MALDI-TOF-MS) for identification of proteins in a database. Dr. Yuri Lazebnik's group here at the Laboratory studies apoptosis, and they have purified an oncogene-generated activity (OGA). The OGA was purified from a cell line that expresses the *E1A* oncogenes, and when added to the untransformed extract, it activated caspases, thus mimicking the effects of *E1A* expression in cells. It was therefore suggested that OGA is a link between oncogene expression and the apoptotic machinery. To determine the function of OGA, we use our in-gel digestion protocol, followed by regular chemical sequencing and mass mapping using MALDI-TOF-MS. About 15 pmoles of the OGA

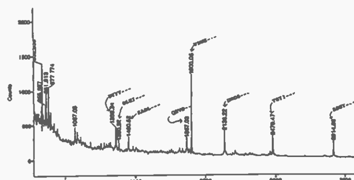
HPLC peptide map of OGA



Peptide fragments identified by Edman degradation

DNDSYVSFYNALLHEGYKDLAALLHDGIPVVSSSS
YVVPVESSLGK
SFRYYLHDLQ
LLEIK
QFFLNLEDPQEDMEVIVK
TVWHIQFTADEK
VWNIITGNK
IHVSPDFK

Mass mapping with MALDI-MS



Peptide fragments identified by mass spectrometry

Sequence	mass Found	mass Expected
DYYTDLSILQK	1359.34	1359.52
GLEILSLFVNMK	1380.52	1380.69
KADLPEQAHSIIK	1450.52	1450.68
QEVDNGMLYLEWINK	1867.03	1869.11
VRNEPTQQQRAAMLIK	1900.05	1900.22
DNDSYVSFYNALLHEGYK	2136.22	2136.29
TSYIMDHMISDGFLTISEEEK	2479.47	2479.75
SSSYDYEALDEAMSISVEMLREDIK	2914.88	2915.18

FIGURE 1 Two methods to identify OGA.

was purified and digested by lysylendopeptidase. For analysis, the digest was divided for high-performance liquid chromatography peptide mapping (95%) and mass spectrometry mass mapping (5%). Both methods were able to identify the OGA as apoptotic protease activating factor I (APAF-I) (Fig. 1).

Biochemical Characterization of p62^{dok}, a Constitutively Tyrosine-phosphorylated Protein in CML

N. Kashige

We continue to study the tyrosine-phosphorylated p62^{dok} in chronic myelogenous leukemia (CML) in the presence of p210$^{bcr\text{-}abl}$ tyrosine kinase. Human CML is characterized by the Philadelphia chromosome, a translocation in which the breakpoint cluster region (*bcr*) gene on chromosome 22 becomes fused to the *abl* tyrosine kinase proto-oncogene on chromosome 9. As a result, a chimeric 210-kD Bcr-Abl protein is produced. In CML, p62^{dok} is constitutively tyrosine-phosphorylated by p210$^{bcr\text{-}abl}$ and only tyrosine phosphorylated p62^{dok} binds to Ras-GAP. Since Ras-GAP is a negative regulator of Ras, p62^{dok} may have an important role in the Ras signaling pathway. We continued tyrosine phosphorylation site analysis of p62^{dok} and identified five essential phosphotyrosine residues for Ras-GAP association.

We are currently analyzing the effect of p62^{dok} and its mutants on the Ras-GAP activity in vitro.

PUBLICATIONS

Dallas P.B., Cheney I.W., Liao D.-W., Bowrin V., Byam W., Pacchione S., Kobayashi R., Yaciuki P., and Moran E. 1998. The p300/CBP-related protein, p270, is a component of mammalian SWI/SNF complexes. *Mol. Cell. Biol.* **18:** 3596–3603.

Di Cristofano A., Carpino N., Dunant N., Friedland G., Kobayashi R., Strife A., Wisniewski D., Clarkson B., Pandolfi P.P., and Resh M.D. 1998. Molecular cloning and characterization of p56^{dok2} defines a new family of RasGap-binding proteins. *J. Biol. Chem.* **273:** 4827–4830.

Emili A., Kobayashi R., and Ingles C.J. 1998. A novel yeast protein influencing the response of RNA polymerase II to transcriptional activators. *Proc. Natl. Acad. Sci.* **95:** 11122–11127.

Fearnhead H.O., Rodriguez J., Govek E.-E., Guo W., Kobayashi R., Hannon G., and Lazebnik Y.A. 1998. Oncogene-dependent apoptosis is mediated by caspase-9. *Proc. Natl. Acad. Sci.* **95:** 13664–13669.

Grossman J.S., Meyer M.I., Wang Y.C., Mulligan G.J., Kobayashi R., and Helfman D. 1998. The use of antibodies to the polypyrimidine tract binding protein (PTB) to analyze the protein components that assemble on alternatively spliced pre-mRNAs that use distant branch points. *RNA* **4:** 613–625.

Henry R.W., Mittal V., Ma B., Kobayashi R., and Hernandez N. 1998. SNAP19 mediates the assembly of a functional core promoter complex ($SNAP_c$) shared by RNA polymerases II and III. *Genes Dev.* **12:** 2664–2672.

Kimura K., Hirano M., Kobayashi R., and Hirano T. 1998. Phosphorylation and activation of 13S Condensin by Cdc2 in vitro. *Science* **282:** 487–490.

Liao H.J., Kobayashi R., and Mathews M.B. 1998. Activities of adenovirus virus-associated RNAs: Purification and characterization of RNA binding proteins. *Proc. Natl. Acad. Sci.* **95:** 8514–8519.

Lutz C.S., Cooke C., O'Connor J.P., Kobayashi R., and Alwine J.C. 1998. The snRNP-free U1A (SF-A) complex(es): Identification of the largest subunit as PSF, the polypyrimidine-tract binding protein-associated splicing factor. *RNA* **12:** 1493–1499.

Nomura K.H., Kobayashi R., Hirabayashi Y., Fujisue-Sakai M., Mizuguchi S., and Nomura K. 1998. Involvement of blood-group-B-active trisaccharides in Ca^{2+}-dependent cell-cell adhesion in the *Xenopus* blastula. *Dev. Genes Evol.* **208:** 9–18.

Saginario C., Sterling H., Beckers C., Kobayashi R., Solimena M., Ullu E., and Vignery A. 1998. MFR, a putative receptor mediating the fusion of macrophages. *Mol. Cell. Biol.* **11:** 6213–6223.

Silva E., Ullu E., Kobayashi R., and Tschudi C. 1998. Trypanosome capping enzymes display a novel two-domain structure. *Mol. Cell. Biol.* **18:** 4612–4619.

Tugal T., Zou-Yang X.H., Gavin K., Pappin D., Canas B., Kobayashi R., Hunt T., and Stillman B. 1998. The Orc4p and Orc5p subunits of the *Xenopus* and human origin recognition complex are related to Orc1p and Cdc6p. *J. Biol. Chem.* **273:** 32421–32429.

Wong M.W., Henry R.W., Ma B., Kobayashi R., Klages N., Matthias P., Strubin M., and Hernandez N. 1998. The large subunit of basal transcription factor $SNAP_c$ is a Myb domain protein that interacts with Oct-1. *Mol. Cell. Biol.* **18:** 368–377.

Yan H., Chen C.-Y., Kobayashi R., and Newport J. 1998. Replication focus-forming activity 1 and the Werner syndrome gene product. *Nature Genet.* **19:** 375–378.

Zelko I., Kobayashi R., Honkakoski P., and Negishi M. 1998. Molecular cloning and characterization of a novel nuclear protein kinase in mice. *Arch. Biochem. Biophys.* **352:** 31–36.

SEQUENCE-BASED ANALYSIS OF COMPLEX GENOMES

W.R. McCombie	M. de la Bastide	L. Spiegel	R. Shah	M. Shekher
	K. Habermann	R. Preston	I. Swaby	L. Parnell
	L. Gnoj	K. Schutz	A. Matero	N. Dedhia
	E. Ning Huang	M.D. Vil	M. Rodriguez	A. O'Shaughnessy

For several years, we have been building an infrastructure that will allow us to use high-throughput DNA sequencing to analyze large genomic regions. In 1998, we made significant progress and reached several milestones. With our sample tracking software in place, and a reorganization of our laboratory to maximize efficiency, we have continued to greatly increase our sequencing capacity.

In the area of production sequencing, we expanded our capacity in 1998 by adding a second shift of production sequencing. This has allowed us to more fully utilize our laboratory space and sequencing instruments, and thus to increase our production from about 800 reactions per week run on gels at the end of 1997 to about 3500 per week by the end of 1998. In addition, we were able to increase our success rate slightly during this scale-up. This has had a large impact in our production of finished, highly accurate sequence to GenBank.

Figure 1 shows the amount of finished sequence we have submitted to GenBank from 1996 to 1998. In calendar year 1997, we submitted just over 700 kb of finished sequence to GenBank. In calendar year 1998, we submitted more than 2 million bases of finished sequence. This increase in finished sequence coupled with that in our raw sequence generation has allowed us to extend our contribution to projects not requiring finished sequence.

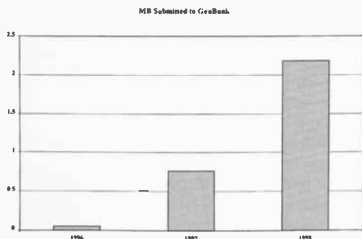


FIGURE 1 Output of finished sequence submitted to GenBank.

The net effect of this rise in capacity has been the simultaneous increase of our contribution to several projects. We have substantially increased our *Arabidopsis* genomic sequencing. Most of our increased output in 1998 was directed at this project. In late 1998, we also began to scale up two human sequencing projects. One project in association with the Wigler lab is to sequence regions of the human genome deleted in breast and other types of cancers.

The second is a pilot project to sequence a region of human chromosome 18. This infrastructure has allowed us to carry out both of these major new projects, as well as to continue several smaller projects in a very efficient manner.

Arabidopsis Genome Sequencing

M. de la Bastide, K. Habermann, L. Gnoj, E. Ning Huang, L. Spiegel, R. Preston, K. Schutz, M.D. Vil, R. Shah, I. Swaby, A. Matero, M. Rodriguez, M. Shekher, A. O'Shaughnessy, L. Parnell, N. Dedhia, W.R. McCombie [in collaboration with R. Martienssen, CSHL, R.K. Wilson and M. Marra, Washington University School of Medicine, and E. Chen, PE Applied Biosystems]

Our maps of sequence-ready clones for the region of the *Arabidopsis* genome that we targeted for sequencing were completed in 1998. This allowed us to ramp-up our sequence production both here at CSHL and in the laboratory of our collaborators at the Washington University Genome Sequencing center.

In 1998, our consortium produced 4.5 million bases of finished *Arabidopsis* genomic sequence. Of this amount, our group at CSHL produced about 1.7 Mb. Much of this sequence is present in a highly contiguous region of chromosome IV. The status of this project can be found on our Web page at the URL www.cshl.org/protorab.

Arabidopsis Sequence Annotation

L. Parnell, W.R. McCombie [in collaboration with R. Martienssen, CSHL, C.M.E. Schueller, H.-W. Mewes and K.F.X. Mayer, Munich Institute of Protein Science]

As we have increased the rate of sequence annotation, we have found it necessary to modify the way in which we analyze and annotate the sequence we generate. This change has been most acute in the way we analyze our *Arabidopsis* sequence. We are continuing to work with our collaborators at the Munich Institute for Protein Science to develop approaches to annotation that are both informative and scaleable to keep pace with the increase in sequencing rates.

As an example, we had been using as many as seven different gene prediction programs and comparing the results of these programs with those of database searches to predict the presence and boundaries of genes. It has become impossible to keep up with the rate of sequencing using this annotation strategy. We have, however, learned from the use of all these methods a considerable amount about the strengths and weaknesses of the programs. Comparing predictions with known or experimentally presumptive structures based on cDNA or EST data enabled us to minimize the number of programs employed. Although it does not provide as much accuracy as when we use all of the programs, it does allow us to approximate the quality of the annotation in a time frame that is reasonable given our sequencing rate.

As the amount of sequence has increased, we have been able to begin carrying out statistical analyses on the results of our analysis. While this is ongoing, several interesting points can be made. Figure 2 shows the comparison of the genomic sequence to the current *Arabidopsis* EST database. Interestingly, although not surprisingly, a large percentage of the genes predicted from genomics sequence are not found in the EST database. Some of these are almost certainly false-positive predictions. However, it is likely that the great majority of them represent real genes not in the EST database. For instance, many are similar to known genes from other species. Interestingly, a few of the predicted genes each matches many ESTs.

We can also begin to break down the genes found according to function. This is shown in Figure 3. Many of the genes are involved in so-called housekeeping functions, such as metabolism. However, a large percentage are involved in fairly specialized complex functions such as signal transduction. There is clearly much remaining in the *Arabidopsis* genome to sequence and analyze. This initial sample is large enough that it will not likely change in any major way as far as the representation of types of gene functions found.

We are in the final stages of completing chromosomes IV and V. Chromosome IV will likely be the first chromosome to be sequenced from a plant. The entire genome is slated for completion by the end of the year 2000. This sequence, coupled with the large-scale functional analyses being done in a variety of labs, including Rob Martienssen's lab, will dramatically change the way *Arabidopsis* research is conducted,

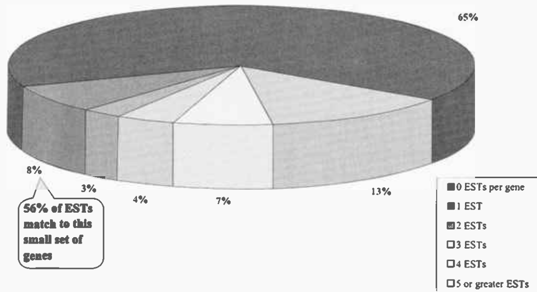


FIGURE 2 Comparison of genomic sequence to the current *Arabidopsis* EST database. Most genes have no EST counterpart.

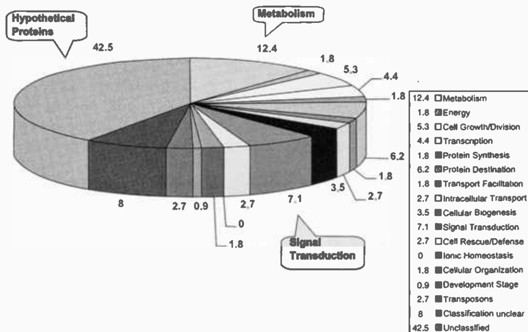


FIGURE 3 Breakdown of genes according to function.

as well as our understanding of the complex patterns of growth, development, and regulation that higher plants exhibit.

Gene Trap Sequencing

K. Schutz, L. Gnoj, W.R. McCombie [in collaboration with R. Martienssen, Cold Spring Harbor Laboratory]

In addition to determining the structure of the *Arabidopsis* genome, it is important that we use this information to advance our understanding of the function of the genes in this plant. One way that this is being carried out is through the systematic generation of transposon insertions in the *Arabidopsis* genome and the sequencing of the sites of insertion of those transposons.

The Martienssen laboratory is generating insertions in *Arabidopsis* on a large scale, and in a collaboration with them, we are sequencing those insertion sites. In 1998, we sequenced about 2100 insertion sites and will continue to sequence these insertions in 1999. These data are being used to create a database of transposon insertions. The completion of the *Arabidopsis* genome sequence will allow precise mapping of the position of any of these insertions within the genome.

Development of a Method to Filter Repetitive DNA from Libraries Generated from Plant Genomes

K. Schutz, L. Parnell, N. Dedhia, W.R. McCombie [in collaboration with P. Rabinowicz and R. Martienssen, Cold Spring Harbor Laboratory]

The genomes of most higher eukaryotes, especially higher plants, contain a large amount of repetitive DNA. In fact, the genomes of higher plants are composed mostly of these repetitive DNA sequences. In model genomes such as those of *Arabidopsis* and rice, efforts are under way to sequence the entire genome. In larger genomes, such as corn and wheat (about 20-fold and 100-fold larger than *Arabidopsis*, respectively), this is not possible. However, it might be possible to obtain much of the sequence information using various "skimming" or partial sequencing strategies, without completely sequencing the genome. We have explored these strategies in the past for human genome analysis, and they are even more relevant for plant genome analysis. The difficulty even more than with the human genome is that much of the effort in skimming plant genomes would go into sequencing repetitive DNA. We have been testing a method that would remove most of the repetitive DNA from libraries made from plant genomic DNA. The libraries were plated in the Martienssen lab on

Escherichia coli, which restricts methylated DNA. Since most of the repetitive DNA is highly methylated, it is not grown on this host. As a control, the same libraries were plated on *E. coli* strains containing mutations in the methylation restriction lines. Our lab sequenced several hundred clones isolated from each of the *E. coli* host strains and worked with the Martienssen lab to analyze these sequences for their gene content and repetitive element content. The results showed that plating on the appropriate host could reduce the number of clones containing repeats by 10–20-fold over controls. This approach has considerable promise for sequence-based gene detection by skimming in plant genomes.

Sequencing of Tumor Suppressor Deletion Loci

M. de la Bastide, K. Habermann, L. Gnoj, E. Ning Huang, L. Spiegel, R. Preston, K. Schutz, M.D. Vil, R. Shah, I. Swaby, A. Matero, M. Rodriguez, M. Shekher, A. O'Shaughnessy, L. Parnell, N. Dedhia, W.R. McCombie
[in collaboration with R. Lucito, M. Hamaguchi, and M. Wigler, Cold Spring Harbor Laboratory]

There are a sizable number of genes whose deletion or damage creates tumors or modifies their progression. Many of these are unknown. We have joined an ongoing effort with the Wigler lab to identify and characterize these genes. Using RDA, the Wigler lab has been mapping these loci in the human genome. We have begun using our high-throughput DNA sequencing to assist in identifying and characterizing these genes. This is being done in several ways depending on the size of the loci and the other available information. In areas where the loci are relatively large, we are using sequence skimming at low redundancy. This will potentially identify genes in the area as well as generate sequence data that can be used to further identify probes from the region to facilitate more precise localization of the region of interest. In better-defined areas, we are carrying out more complete, finished sequence. This information then undergoes comprehensive analysis to identify genes in the area. Potential tumor suppressors can then be identified and confirmed.

In 1998, we completed the sequencing and analysis of the first of these regions. The tumor suppresser in this region had been previously identified as PTEN (Li et al., *Science 275:* 1943 [1997]). Sequencing of this region enabled us to obtain the complete genomic sequence of the PTEN gene. The region sequenced is about 200 kb. The coding region of PTEN is composed of nine exons spread over about 110,000 bases. In addition, there are several other genetic features found within this region. We are using the region to test methods for finding genes in genomic sequence at various levels of sequence coverage. For instance, we have tested what percentage of the PTEN exons are found by various computational gene-finding programs and are testing how those results are effected by reducing the amount of sequence data available (to simulate skimming of the region). This will allow us to implement strategies for the most rapid and efficient ways to screen large regions of genomic DNA for putative tumor suppressors.

PUBLICATIONS

Dedhia N.N. and McCombie W.R. 1998. Kaleidaseq: A Web-based tool to monitor data flow in a high throughput sequencing facility. *Genome Res.* **8(3):** 313–318.

Hamaguchi M., O'Connor E.A., Chen T., Parnell L., McCombie R.W., and Wigler M.H. 1998. Rapid isolation of cDNA by hybridization. *Proc. Natl. Acad. Sci.* **95(7):** 3764–3769.

Lucito R., Nakimura M., West J.A., Han Y., Chin K., Jensen K., McCombie R., Gray J.W., and Wigler M. 1998. Genetic analysis using genomic representations. *Proc. Natl. Acad. Sci.* **95(8):** 4487–4492.

COMBINATORIAL LIBRARIES

H.P. Nestler R. Liu
R. Sherlock
W. Cui (URP)

Combinatorial "One-bead–One-structure" libraries have opened a new dimension for the study of interactions of small molecules with chemical and biological ligands. We are employing such encoded combinatorial libraries to identify molecules that specifically interfere with the biological function of proteins of physiological importance at specific functional sites. Hereby, we pursue an approach that outlines a new paradigm for molecular biology, as we will attempt to prevent a biological process not by inhibiting the enzyme connected with this process, but by masking the substrate with a synthetic molecule. The second aspect of our research is the identification of substrates and subsequently of inhibitors of enzymes, especially proteases and kinases. Binding studies of proteins with molecules in combinatorial libraries are rather straightforward, but the identification of enzyme substrates is difficult because of the deactivation of enzymes on most library supports. Finding new substrates for enzymes will not only open ways to design compounds inhibiting these enzymes, but also help to elucidate the pathways these enzymes are involved in.

Molecular Forceps from Combinatorial Libraries That Bind Ras Proteins

R. Liu, R. Sherlock, H.P. Nestler [in collaboration with D.L. Dong and M.H. Wigler, Cold Spring Harbor Laboratory]

Our initial studies have shown that simple molecules displaying two or more short peptide or peptide-like chains (which we termed "molecular forceps") can provide reasonable affinities and selectivities toward peptide substrates. We are using libraries of molecular forceps to identify molecules that are able to bind to the carboxyl terminus of Ras proteins (also termed "CaaX box") and prevent its farnesylation. The farnesylation of Ras has been shown to be a crucial step in the activation of Ras as a signal transduction protein, and pharmaceutical trials have shown that molecules which inhibit the farnesyl transferase (FTase), the farnesylating enzyme, are able to stop tumor growth. The approach we follow outlines a new paradigm for molecular biology, as we will attempt to prevent a biological process not by inhibiting the enzyme connected with this process, but by masking the substrate with a synthetic molecule. To efficiently target our molecular forceps toward the carboxyl terminus of Ras, we use the isolated peptide sequence instead of the whole protein as substrate for the screening of our libraries. The molecules selected against the peptide epitope should bind Ras site-specifically at the CaaX box. Although precedence exists that antibodies can be raised against peptide epitopes in this fashion, it is unclear whether this approach is suitable for the selection of synthetic molecules. The carboxyl terminus of Ras is a suitable test case for this novel approach as it is conformationally not restricted and should therefore be readily accessible for the molecular forceps we intend to generate.

In our assays of a library that contained two-armed and four-armed molecular forceps with the octapeptide derived from the CaaX box of Ras and the whole Ras protein, we observed molecular forceps with four peptide arms as interacting molecules. Each of the molecules that had been selected against the peptide binds with micromolar affinities selectively to the Ha-Ras protein, with some specificity over other proteins such as ovalbumin or bovine serum albumin, supporting our assumption that we can screen libraries with isolated peptide epitopes to identify forceps binding to the full-length protein. Furthermore, two of the peptide binders not only bind to the Ras protein, but also slow down the farnesylation of Ha-Ras with IC_{50} values of 100 μM and 400 μM. None of the protein binders or of nonbinding forceps prevent the farnesylation in the micromolar range. These findings suggest that some molecular forceps—although not all of them—selected for binding to peptide epitopes recognize this epitope on the full-length protein, whereas forceps selected against the protein bind at other locations of the protein surface. Beyond that,

the retardation of farnesylation by the stronger acting forceps is sensitive to the peptide used as substrate for the FTase: The farnesylation of a peptide derived from the CaaX box of Ras is inhibited with an IC_{50} of 100 μM, whereas the farnesylation of a peptide derived from Ki-Ras B is inhibited with an IC_{50} greater than 1 mM (75% farnesylation efficiency at a concentration of 1 mM) and the farnesylation of a hybrid peptide is inhibited with an IC_{50} of approximately 500 μM. The farnesylation of a peptide derived from the carboxyl terminus of lamin B is not affected at concentrations of up to 1 mM. The same selectivity pattern is observed using protein substrates for the farnesylation: The farnesylation of the Ha-Ras peptide is significantly inhibited, whereas the farnesylation of other substrates is only slightly affected.

These patterns, the correlation of inhibitory activity with binding strength, and the fact that our forceps molecules do not bind to the FTase prove that the inhibition is not achieved through inhibition of the enzyme, but through trapping of the substrate. This mode of action by a synthetic molecule is without precedence in the literature and may suggest a new approach to modulate enzymatic transformations.

Inhibitors of Ras Farnesylation

R. Liu, R. Sherlock, H.P. Nestler [in collaboration with A. Mielgo, C. Scolastico, and C. Gennari, Università di Milano, Italy, and D.L. Dong, Cold Spring Harbor Laboratory]

Ras proteins have a central role in normal cellular physiology and pathophysiology and are commonly found in human tumors. Ras proteins are initially synthesized in the cytoplasm where they undergo a series of posttranslational modifications at their carboxy-terminal sequence. The processed proteins become localized to the cell membrane, a step that is essential to their functioning. The farnesylation cascade has been considered to be a logical target for the development of cancer therapeutics. In fact, inhibitors of the FTase can induce morphological reversion of cells transformed by Ras at concentrations that do not arrest normal cell growth.

Structural studies on the FTase revealed that the enzyme has two substrate recognition pockets in its active site to bring together the two partners of the farnesylation reaction, farnesyl pyrophosphate (FPP) and the CaaX-containing proteins. Although compounds that compete with FPP have been used to inhibit FTase, peptides and peptide mimics that compete with the protein in the CaaX-box-binding site show more potential for inhibitory activity and tuning of selectivity. Structural investigations of substrates and inhibitors suggest an active conformation containing a peptide turn. In the course of studies on factors determining the conformation of peptides, we investigated peptide-turn-inducing bicyclic lactams. It was our expectation that the incorporation of the reverse-turn mimics into an FTase substrate sequence could lead to potent FTase inhibitors.

When we incorporated the bicyclic lactams as building blocks into the farnesylation substrate sequence, we found that molecules with a free amino-terminal site did not have the expected activity, whereas molecules carrying a lipophilic group at this site had activities one order of magnitude weaker than commercially available inhibitors. Appropriate modification of the side chains displayed on the lactam skeleton should allow us to improve the activity of our inhibitors.

Substrates and Inhibitors for Proteases and Kinases

R. Liu, W. Cui, H.P. Nestler [in collaboration with S. Ressel, U. Piarulli, and C. Gennari, Università di Milano, Italy, and P. Chan and Todd Miller, SUNY, Stony Brook]

In recent years, the importance of cysteine proteases for physiological events and the determination of cell fate have become of major interest. The quest for new mechanism-based inhibitors of these enzymes yielded a variety of new structural elements. We have been investigating a class of peptide analogs, vinylogous sulfonamides, that contain a functional moiety suitable for inhibition of cysteine proteases. When we incorporated these analogs into short peptides, we observed weak inhibition of cathepsin L, a protease involved in inflammatory processes. We are now using combinatorial libraries to find peptide sequences that are preferred substrates of cathepsin L. Incorporating our vinylogous sulfonamides into such sequences should yield potent inhibitors.

Although binding studies of proteins with mole-

cules in combinatorial libraries are rather straightforward, the identification of enzyme substrates is quite difficult because of the deactivation of enzymes on most library supports. Although we observe the binding of proteins to peptides on commonly used plastic supports, the proteins seem to precipitate on these supports and thus are deactivated. Our search for a suitable support for enzymatic assays led us to controlled pore glass (CPG) beads, the same material that is used for oligonucleotide synthesis. We have developed a fluorescence-based assay to identify protease substrates in encoded "One-bead–One-structure" libraries. We are also using libraries on the same CPG support to find substrates for physiologically important tyrosine kinases. For the screening of these libraries, we use an automicroradiographic screening that we established a few years ago. The knowledge of the sequence specificity for these kinases should help to elucidate the cellular substrates by sequence homology.

PUBLICATIONS

Nestler H.P. and Liu R. 1998. Combinatorial libraries: Studies in molecular recognition. *Combin. Chem. High Through-put Screen* **1:** 113–126.

In Press

Dong D.L.-Y., Liu R., Sherlock R., Wigler M.H., and Nestler H.P. 1999. Molecular forceps from combinatorial libraries prevent the farnesylation of Ras by binding to its carboxy-terminus. *Chem. Biol.* **6:** 133–141.

Liu R., Dong D.L.-Y., Sherlock R., Nestler H.P., Gemnari C., Mielgo A., and Scolastico C. 1999. Novel reverse-turn mimics inhibit farnesyl transferase. *Bioorg. Med. Chem. Lett.* (in press).

Liu R., Sherlock R., and Nestler H.P. 1999. Using the copper-catalyzed benzidine blue reaction for the detection of binding of hexahistidine-tagged proteins and peptides to combinatorial library beads. In *Innovation and perspectives in solid phase synthesis & combinatorial libraries 1998* (ed. R. Epton). Mayflower Scientific Ltd., Kingswin-ford, United Kingdom. (In press.)

P. Nestler at West Side School night

Y. Zhang

J. Rosenbaum, P. O'Fearghail

SEQUENCED-BASED PREDICTION OF PROTEIN STRUCTURE AND FUNCTION

A.F. Neuwald A. Poleksic

Our work focuses on the development and application of computational methods for predicting protein structure and function from multiple sequence data. Specifically, this involves the development and optimization of statistical models representing particular protein families, and the design of procedures based on these models to detect and align distantly related sequences. Because the goal of our research is to discover important biological features of protein sequences, the development of new methods is closely linked to the analysis of specific protein families or superfamilies.

Modeling, Detection, and Alignment of Protein Families

A.F. Neuwald, A. Poleksic

The detection and alignment of structurally and functionally related proteins require a biologically meaningful statistical model of the specific family under investigation. Conversely, developing such a model requires an understanding of both the similarities and the differences between related proteins. For this reason, the design of statistical models has been closely linked to the study of structural and sequence divergence in various protein superfamilies, such as the α,β-hydrolase-fold superfamily, for which about 2000 highly diverse sequences and about 20 distinct structures are available. This has led to improvements in database search sensitivity, in multiple sequence alignment procedures, and in related methods that aid interpretation of sequence and structural similarities.

Several approaches have been taken to improve the database search sensitivity of our programs. These include the incorporation of weighting schemes to adjust for sequence redundancy, modified log-likelihood scores that model insertions and deletions within and between conserved regions of an alignment, and the application of a jackknife test for statistical significance. The jackknife test is important for detecting and eliminating false positives from an evolving alignment model during automated iterative database searches (as are used by the PROBE and PSI-BLAST programs). Measures of statistical significance for modified log-likelihood scores were devised in collaboration with J.L. Spouge at the National Center for Biotechnology Information (NCBI). To better understand fundamental principles involved in the detection of distant relationships, an information theoretical analysis of database search problems was conducted in collaboration with W.J. Wilbur at the NCBI. This analysis has led to a measure of statistical significance for aggregate scores obtained by combining several individual scores reflecting distinct properties.

Many of the improvements in multiple sequence alignment procedures involve the design of new Gibbs sampling operations. Some of these operations are linked to fundamental changes in the underlying statistical model. These include sampling of single or multiple domains within a single polypeptide chain, sampling of short insertions and deletions within conserved regions (earlier methods only aligned contiguous sequence blocks), and incorporation of residue substitution matrices. Other operations help speed up convergence to an optimal alignment (as defined by the underlying statistical model) by facilitating escape from suboptimal alignments. These operations include, for instance, operations to split and fuse aligned regions and more efficient recombination operations within the genetic algorithm component of Gibbs sampling. These operations function in a manner analogous to that of chemical catalysts, which drive a reaction to thermodynamic equilibrium by facilitating escape from kinetic traps.

Other approaches for speeding up the Gibbs sampling procedures were also devised. For example, "simulated annealing" is now applied after convergence to locate the optimal alignment and, consequently, to better detect subtly conserved patterns in the sequences. Similarly, machine learning procedures were implemented to help the program apply the correct number and sequence of particular sampling operations. Better assessment of convergence has been obtained through incorporation of routines to measure the "distance" between independently generated mul-

tiple alignments. This is needed, given the stochastic nature of Gibbs sampling procedures, to find the optimum alignment and, consequently, to detect structurally accurate, biologically meaningful sequence similarities such as described in the example below. The interpretation of multiple sequence alignment data was facilitated through development of a statistically based method for detecting and highlighting conserved residues within an alignment. This facilitates identification of patterns having important functional and structural significance.

The methods described here, although currently implemented only within our own programs, can also be incorporated into other procedures. In particular, we are collaborating with the NCBI to incorporate related improvements into the PSI-BLAST program. Thus, although these methods are currently unavailable to the biological community, they will eventually be made available through our own programs (which have not yet been published or released) and through the services provided by the NCBI.

Analysis of a Class of Chaperone-like ATPases

A. Neuwald [in collaboration with
L. Aravind and E.V. Koonin, National
Center for Biotechnology Information, NIH]

The methods described in the previous section were applied (in combination with other computational approaches) to an analysis of replication factor C (RFC)-related proteins, which function in the initiation of DNA replication. Included among these is one protein of known structure, the *Escherichia coli* DNA polymerase III δ′ subunit (polIII δ′), which provides structural information relevant to the family as a whole. Starting from these proteins, our analysis resulted in the detection and alignment of many other distantly related ATPases, collectively designated the AAA$^+$ class. These include members of the AAA family (*A*TPases *a*ssociated with a variety of cellular *a*ctivities), whose activities include signal transduction, cytoskeletal interactions, mitotic spindle formation, cell cycle control, peroxisome biogenesis, assembly of mitochondrial membrane proteins, protein degradation by the 26S proteasome, and transcription. Fortunately, the three-dimensional structure of the hexameric complex for one of the AAA proteins, the D2 ATPase module of *N*-ethylmaleimide-sensitive factor (NSF), was recently determined, which allowed us to glean further structural and functional insights into this class. Our analysis indicates that polIII δ′ and NSF-D2 are structurally related to ATPases involved in the initiation of DNA replication and transcription, to Lon and Clp family chaperones, and to other ATPases (some of which are described below) performing diverse functions.

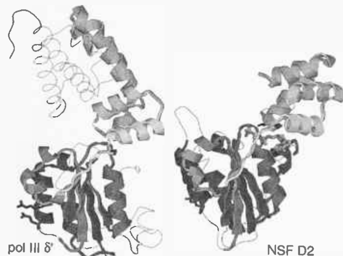
Many of the AAA$^+$ proteins are known to function as chaperones and others, although not known to be chaperones, are nevertheless involved in the assembly or remodeling of protein complexes. These include, for example, subunits of the origin recognition complex (ORC), Cdc6, MCM (minichromosome maintenance) DNA licensing factors, and RFC subunits, all of which are involved in successive steps in the initiation of DNA replication. It is feasible that these proteins possess chaperone-like remodeling activities considering that known AAA$^+$ chaperones have been found to be involved in the assembly or initiation of protein-DNA complexes. For example, the ClpA chaperone facilitates binding of the bacteriophage replication initiator protein RepA to DNA, and the ClpX chaperone is involved in the initiation of bacteriophage Mu DNA replication. Similarly, AAA$^+$ proteins are also associated with the initiation of transcription. For example, the bacterial AAA$^+$ protein NtrC activates transcription from a distant enhancer by remodeling the closed complex between promoter DNA and RNA polymerase to an open complex. Known AAA$^+$ chaperones have also been associated with transcription.

The functions of other AAA$^+$ proteins are also likely to involve chaperone-like remodeling. For example, Rubisco activase, which couples ATP hydrolysis to the release of inhibitory sugar phosphates bound to Rubisco-active sites, appears to function as a chaperone. The dynein motor protein, which transports membranous organelles along microtubules, contains six AAA$^+$ ATPase modules. This suggests that it may form an assemblage resembling the NSF D2 hexamer and that it functions via chaperone-like remodeling.

Our improved procedures allowed construction of a structurally meaningful sequence alignment of this diverse class of ATPases. This is illustrated in Figure 1, which shows our alignment of NSF-D2 versus polIII δ′ (Fig. 1A) and the corresponding locations of these aligned regions in the structures (Fig. 1B). These regions correspond to two distinct structural components: an amino-terminal α,β-fold that is generally similar to other P-loop ATP-binding domains and carboxy-terminal structural elements that appear to be directly associated with chaperone activity (Fig. 1C). On the basis of the location of bound ATP relative to

A

B



C

FIGURE 1 (*A*) Sequence alignment of the NSF D2 ATPase module (NSF_CRIGR) with the DNA polymerase III δ′ subunit (1A5TX). (*B*) Locations of aligned regions in the corresponding structures. (*C*) Basic structural components of the NSF-D2 module. The image on the left corresponds to the α,β-fold P-loop domain. The center image corresponds to chaperone-specific structural components. The image on the right corresponds to the complete NSF D2 hexameric complex.

the chaperone-specific component, it appears that ATP binding or hydrolysis leads to coordinated conformational changes required for substrate remodeling. Furthermore, the spiral appearance of the chaperone-specific component (center image in Fig. 1C) suggests that these conformational changes may involve an inward, twisting motion. Possible mechanisms of action are suggested by comparing conserved positions in the alignment with residue locations in the NSF-D2 and polIII δ′ structures. This reveals several highly conserved residue positions that may function to directly link binding or hydrolysis of ATP to coordinated conformational changes.

PUBLICATIONS

Chung M.S., Neuwald A.F., and Wilbur W.J. 1998. A free energy analysis by unfolding applied to 125-mers on a cubic lattice. *J. Fold. Des.* **3:** 51–65.

Neuwald A.F. 1998. A probable solution to sequence-analysis problems. *Trends Biochem. Sci.* **23:** 365.

Neuwald A.F and Koonin E.V. 1998. Ataxin-2, global regulators of bacterial gene expression, and spliceosomal snRNP proteins share a conserved domain. *J. Mol. Med.* **76:** 3–5.

In Press

Liu J.S., Neuwald A.F., and Lawrence C.E. 1999. Markovian structures in biological sequence alignments. *J. Am. Stat. Assoc.* **94:** 1–15.

Neuwald A.F. 1999. The hexamerization domain of N-ethylmaleimide-sensitive factor: Structural clues to chaperone function. *Structure* **7:** R19–R23.

Neuwald A.F, Aravind L., Spouge J.L., and Koonin E.V. 1999. AAA+: A class of chaperone-like ATPases associated with the assembly, operation and disassembly of protein complexes. *Genome Res.* **9:** 27–43.

Ponting C.O., Neuwald A.F., and Aravind L. 1999. Sec14p-like domains in NF1 and Dbl-like proteins indicate lipid-regulation of Ras and Rho signaling. *Curr. Biol.* (in press).

Wilbur W.J. and Neuwald A.F. 1999. Information theory and search problems. *Comput. Chem.* (in press).

STRUCTURAL BIOLOGY

R.-M. Xu J. Jiang H. Shi
M. Hayashi Y. Zhang
K. Hendren (URP)

Our laboratory is concerned with understanding the molecular mechanism of protein-RNA interactions in pre-mRNA splicing and the molecular assembly of multiprotein complexes in DNA replication and cell cycle regulation. We use X-ray crystallography as our principal approach to address these questions.

PROTEIN-RNA INTERACTIONS IN PRE-mRNA SPLICING

Most eukaryotic genes are interrupted by introns. The primary transcript must be processed to remove the introns and to join the coding sequence into a contiguous mRNA molecule. Splicing of pre-mRNA involves an ordered assembly of components, including heterogeneous nuclear ribonucleoproteins (hnRNPs), small nuclear ribonuclear particles (snRNPs), and additional protein-splicing factors. The current focus in our laboratory is to determine the crystal structure of individual splicing factors. Our research activities during 1998 are described below.

Structural Studies of hnRNP A1. Human hnRNP A1 is a versatile nucleic-acid-binding protein that has been shown to be important in various aspects of mRNA metabolism and in telomere length regulation. We have previously determined the crystal structure of the amino-terminal RNA-binding domain of hnRNP A1 (UP1) alone and in complex with telomeric DNA. These structural studies revealed several unexpected features: (1) The two RNA-recognition motifs (RRMs) are antiparallel and held together in a rigid structure by two salt bridges and (2) specific protein-protein interaction may be important for sequence-specific recognition of telomeric DNA. Implication of these structural findings in pre-mRNA splicing is being investigated in collaboration with Adrian Krainer here at the Laboratory. Further structural studies aimed at understanding the structure and function of the carboxy-terminal region of hnRNP A1 are currently being pursued.

SF2-associated Protein p32. We have solved the crystal structure of human p32 at 2.25 Å resolution. p32 was originally isolated by copurification with the nuclear pre-mRNA splicing factor SF2/ASF, with which it appeared to be associated, although no evidence of a splicing function was obtained. Since the original study, it has become apparent that p32 is an evolutionarily conserved eukaryotic protein. In addition to its ability to bind to SF2/ASF in vitro, many additional properties or apparent functions of p32 have been reported. In particular, it can interact with many viral proteins, including human immunodeficiency virus type-1 (HIV-1) Tat and Rev, EBNA-1 of Epstein-Barr virus, ORF P of herpes simplex virus, and core protein V of adenovirus. Unexpectedly, immunofluorescence and subcellular fractionation studies have shown that p32 and its yeast homolog localize predominantly in the mitochondrial matrix. It has been reported that p32 functions in the maintenance of mitochondrial oxidative phosphorylation. There is some evidence that a small fraction of p32 protein localizes in the nucleus. Furthermore, the distribution of p32 appears to be altered upon adenovirus infection, with p32 migrating to the nucleus together with a viral core protein. These observations suggest that p32 has a bridging role in nucleus-mitochondrion interactions. In addition to trying to understand the structure and function of p32, we are also attempting to use p32 as a vehicle for the crystallization of pre-mRNA splicing factor SF2/ASF.

The crystal structure shows that p32 forms a doughnut-shaped trimer (Fig. 1), with an outer diameter of approximately 75 Å, an average inner diameter of about 20 Å, and a thickness of about 30 Å. Interestingly, the protein monomer has a novel fold! p32 is a very acidic protein: 23% of its residues are aspartic and glutamic acids. A dramatic feature of the p32 trimer is that the charge distribution on the protein surface is highly asymmetric, with a high negative charge distribution on one side of the doughnut surface and inside the channel. This polarity in charge distribution clearly suggests asymmetric functional roles for the two sides of the protein. On the basis of the structural findings, we propose that p32 may serve as a high-capacity divalent cation storage protein to modulate mitochondrial matrix cation concentration.

Yeast Splicing Factor Prp18p. Splicing of pre-mRNA proceeds in two sequential steps. The first step involves the excision of the 5′ exon and the formation of a lariat intermediate. In the second step, the intron is cleaved at the 3′ splice site and two exons are ligated together. Both steps require the participation of a large assembly of RNP particles and protein factors. Prp18p from *Saccharomyces cerevisiae* is associated with the U5/snRNP and is required for the second step of the splicing reaction.

We have isolated a large fragment of yeast Prp18p by controlled proteolysis. This proteolytic fragment corresponds to an 79-residue deletion at the amino terminus of the full-length Prp18p. The splicing activity of this fragment is nearly indistinguishable from the wild-type protein, judging from its ability to recover splicing activity in Prp18p-depleted yeast extract. This Prp18p fragment can readily be crystallized. The recombinant form of this fragment, termed Prp18Δ79, crystallized in space group $C222_1$ with cell dimensions of a = 49.94 Å, b = 79.17 Å, and c = 171.20 Å. There are two Prp18Δ79 molecules per asymmetric unit.

The crystal structure of Prp18Δ79 was solved using a single Os derivative, and it has been refined to 2.15 Å resolution. The refined structure has an R factor of 20.3% (R-free = 25.2%) and good stereochemistry. Prp18Δ79 forms a compact all-helical structure. The core of the structure consists of five α helices packed close together in a way dissimilar to other structures. Detailed structural analysis is still ongoing, but it is already clear that conserved residues form a distinct region on the protein surface that are likely to be important for protein-protein interactions in pre-mRNA splicing.

FIGURE 1 Ribbon representation of the p32 trimer, looking down the noncrystallographic threefold axis. The dotted lines show disordered segments in the structure. The three monomers that make up the trimer are colored in different shades of gray.

MULTIPROTEIN COMPLEXES IN CELL CYCLE CONTROL OF DNA REPLICATION

Initiation of DNA replication in eukaryotes is a tightly regulated process. Cell-cycle-dependent assembly of the prereplicative complex (pre-RC) coordinates the initiation of chromosomal replication with cell cycle progression. Many essential components of the pre-RC have been identified and characterized, including the origin recognition complex (ORC), the minichromosome maintenance (MCM) complex, and Cdc6p and Cdc45p. Both ORC and MCM are six-subunit protein complexes. To understand the molecular mechanism of origin recognition and pre-RC formation, we are pursuing structural studies of several of the pre-RC components, in collaboration with the Stillman laboratory here at Cold Spring Harbor.

PUBLICATIONS

Mayeda A., Munroe S.H., Xu R.-M., and Krainer A.R. 1998. Distinct functions of the closely related tandem RNA-recognition motifs of hnRNP A1. *RNA* **4:** 1111–1123.

In Press

Jiang J., Zhang Y., Krainer A.R., and Xu R.-M. 1999. Crystal structure of human p32, a doughnut-shaped acidic mitochondrial matrix protein. *Proc. Natl. Acad. Sci.* **96:** 3572–3577.

Ding J., Hayashi M., Zhang Y., Manche L., Krainer A.R., and Xu R.-M. 1999. Crystal Structure of the two-RRM domain of hnRNP A1 (UP1) complexed with single-stranded telomeric DNA. *Genes Dev.* (in press).

COMMUNITY ACCESS TO GENOME RESOURCES

L.D. Stein

The Human Genome Project and its related model organism sequencing projects are producing unprecedented amounts of information regarding the contents, structure, and function of the genome. However, without good software tools for storing, analyzing, and accessing this information, much of it is beyond the reach of the biological community.

My laboratory is actively developing tools to put these data at the fingertips of the community, by making it accessible via the World Wide Web and by providing software tools for manipulating and analyzing the information. Our major achievement during 1998 was the development and release of two interface libraries for the popular ACEDB database, one written for the Perl programming language and the other in Java. Use of these interfaces makes it possible for biologists to directly access genome data stored in ACEDB databases, including the complete genomes of yeast and *Caenorhabditis elegans*, as well as portions of the human genome. Genome sequencing and mapping information from multiple plant crops are also available in this form, including maize, millet, rice, and barley.

Fortuitously, the completion of the Perl interface library came just as the sequence of the *C. elegans* nematode genome was being completed. In collaboration with the *C. elegans* sequencing consortium, my laboratory created a series of Web sites that allows the community to query, browse, and display this rich data resource (see URLs http://stein.cshl.org/elegans.html and http://wormsrv1.sanger.ac.uk/cgi-bin/ace/simple/worm/). These Web pages were made available concomitantly with the publication of the paper announcing the completion of the *C. elegans* sequence in December 1998. In the first month of use, the pages

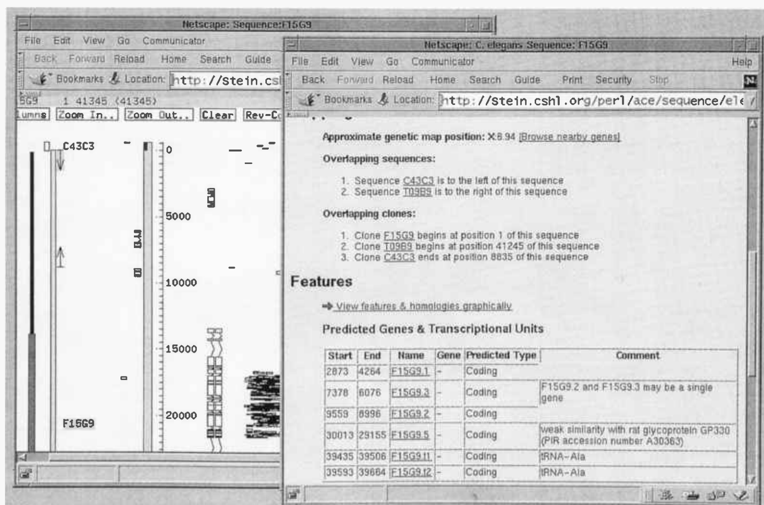


FIGURE 1 Web Browser screen shot of the *C. elegans* database.

saw over 60,000 accesses. Figure 1 shows a screen shot of the database as seen in a Web browser.

During 1999, this work will be extended to create a community annotation system for the *C. elegans* genome and other genomes maintained in ACEDB. This system will allow interested laboratories to add comments, corrections, and new information to the *C. elegans* sequence, allowing this information to be shared immediately with the community in electronic form. We will also be expanding our suite of free genome analysis tools, which currently includes tools for building genome maps and for comparing the genomes of related species.

PUBLICATIONS

Deloukas P. et al. 1998. A physical map of 30,000 human genes. *Science* **282:** 744–746.

Stein L.D. 1998. Introduction to human genome computing via the World Wide Web. In *Guide to human genome computing*, 2nd edition (ed. M. Bishop). Academic Press, San Diego.

Stein L.D. 1998. Of mice and men: Navigating public physical mapping databases. In *Bioinformatics: A practical guide to the analysis of genes and proteins* (ed. B.F.F. Ouellette and A.D. Baxevanis). Wiley, New York.

Stein L.D. 1998. *Web security: A step-by-step reference guide.* Addison-Wesley Longman, Reading, Pennsylvania.

Stein L.D. 1998. *The official guide to programming with CGI.pm.* Wiley, New York.

Stein L.D. and Thierry-Mieg J. 1998. Scriptable access to the *Caenorhabditis elegans* genome sequence and other ACEDB databases. *Genome Res.* **8:** 1308–1315.

Stein L.D., Cartinhour S., Thierry-Mieg D., and Thierry-Mieg J. 1998. JADE: An approach for interconnecting bioinformatics databases. *Gene* **209:** 39–43.

Wang D.G., Fan J.-B., Siao C.-J, Berno A., Young P., Sapolsky R., Ghandour G., Perkins N., Winchester E., Spencer J., Kruglyak L., Stein L., Hsie L., Topalogolu T., Hubbell E., Robinson E., Mittman M., Morris M.S., Shen N., Kilburn D., Rioux J., Nusbaum C., Rozen S., Hudson T.J., Lipshutz R., Chee M., and Lander E.S. 1998. Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome. *Science* **280:** 1077–1081.

J. Tabaska

A. Neuwald

COMPUTATIONAL GENOMICS

M.Q. Zhang R. Bari B. Chan
J. Feng J. Tabaska
I. Ioschikhes J. Zhu
Z. Ioschikhes

Our research interest continues to be the identification and characterization of genetic elements in nucleic acid sequences by computational means. As the Human Genome Project entered its large-scale sequencing phase, the development of 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. We have previously studied statistical characteristics of exons and introns in protein-coding regions and developed coding-exon prediction programs by applying multivariate statistical pattern-recognition techniques. This year, we began 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. In addition to structural genomics, we have also started looking into functional genomics where genome-wide expression data analysis has become our new challenge.

B. Chan returned to Harvard to continue work on his Ph.D. thesis in Tom Maniatis' laboratory, and J. Feng found a job at the American Physical Society. J. Zhu joined our laboratory from Georgia Tech., and R. Bari moved over from Holly Cline's laboratory as a part-time student; she has been helping all of the postdoctoral fellows with their research projects.

Continuation of Sp1 and CCAAT Site-containing Promoter Data Collection

I. Ioshikhes, J. Feng, M.Q. Zhang

This approach to promoter analysis is based on studying experimentally obtained distributions of promoter sequence elements. Since collection and annotation of the complete 5′ sequence database are very labor-intensive, we decided to begin by collecting smaller databases that are structurally homogeneous (promoters containing certain transcription factor elements [TFEs] simultaneously). We extended the analysis of the promoters containing the E2F/Sp1 transcription factor pair to other transcription factors, such as Sp1/NF-Y or, in a more general case, Sp1/CCAAT motifs. A database for a specific family of promoters is being built to facilitate statistical analysis. The database is close to completion and will be presented at our group Web site. We are currently completing a full annotation and will be making an exhaustive analysis of this database. We will use the neural network and other approaches for its analysis. Our goal is to use significant spatial correlation of specific transcription factor sites as a robust criterion for identification of functional promoters and for screening new genes under similar regulatory pathways. The database is accessible from http://www.cshl.org/mzhanglab/.

Periodical Distribution of Transcription Factor Sites in Promoter Regions and Connection with Chromatin Structure

I. Ioshikhes, M.Q. Zhang [in collaboration with E.N. Trifonov, Weizmann Institute]

Nucleosomes regulate transcriptional initiation when positioned in the promoter area. This may require correlation of transcription factor (TF) sites with nucleosome positions and phasing on the nucleosome surface. If this is the case, a periodic distribution of TF sites would be expected in the vicinity of promoters, with a nucleosomal period of 10.1–10.5 bp. We examined the distributions of putative binding sites of 323 different TFs on 1057 sequences of the Eukaryotic Promoter Database (EPD) (release 50) (Perier et al., *Nucleic Acids Res. 26:* 353 [1998]) and of 218 TFs on 673 sequences of the Lead Exon Database (LEDB) of human promoter sequences (Zhang 1998). We obtained a statistically

significant overrepresentation of TF sites distributed with a main period of 10.1–10.5 bp in the region –50 to +120 around the transcription start site and in a few locations nearby. Correlation of the positioning of TF sites with the nucleosomes is further reinforced by sequence-directed mapping of the nucleosomes, a method developed previously (Ioshikhes et al., *J. Mol. Biol. 262:* 129 [1996]).

Computational Identification of the Polyadenylation Site and the Last Exon of Human Genes

J. Tabaska, M.Q. Zhang

To expand the MZEF internal coding exon-finding program into a complete gene prediction system, we are developing a 3′-terminal exon detection module. As an intermediate step toward this goal, we have compiled a database of human poly(A) signal regions and have produced a program for prediction of human poly(A) signals. Stand-alone 3′-terminal exon recognition presents some special challenges, especially with regard to detection of the 3′ end of the gene. Most gene-finding programs—which generally attempt to simultaneously predict all of the exons in a gene—simply define the 3′ end as the first stop codon in the reading frame that extends through the predicted exons. When trying to find the 3′-terminal exon alone, as we are trying to do, there is generally too little coding information present to accurately determine the location of the stop codon. We are therefore using poly(A) signals, rather than stop codons, as markers for gene 3′ ends. The potential advantage is that our predicted genes will include information about mRNA 3′-untranslated regions, which are known to contain posttranscriptional regulatory signals.

Development of our 3′-terminal exon finder thus began with a study of poly(A) signals. To ensure that we were using the best possible data, we collected polyadenylated human expressed sequence tag (EST) sequences and matched them with known gene sequences. The resulting database of genes with known poly(A) sites was then used to train quadratic discriminant functions (QDFs) for poly(A) signal detection. These QDFs are implemented in our program, *polyadq*. In tests, *polyadq* substantially outperforms other poly(A) signal-finding programs.

Work is now under way to combine the poly(A) signal capablities of *polyadq* with a splice acceptor site QDF to produce a 3′-terminal exon-finding program. We are also compiling data on mouse 3′-terminal exons to produce poly(A) signal and terminal exon-finding programs for this organism.

SCPD: A Promoter Database of Yeast *Saccharomyces cerevisiae*

J. Zhu, M.Q. Zhang

To facilitate the study of yeast *S. cerevisiae* promoters on a genome scale, we have developed a yeast promoter database (SCPD), which collects two types of information, the experimentally mapped transcriptional factors' binding sites (TF) and transcriptional start sites (TSS). It also contains information on binding affinity and gene expression. Currently, SCPD contains 580 TF sites and 500 TSS. In addition, it provides a variety of computational tools: Retrieve promoter sequences, Search existing motif, Search putative regulatory elements, Group genes according to their function categories, K-tuple relative information, and Motif distribution. All programs work with multiple sequences. SCPD is accessible at http://www.cshl.org/mzhanglab/.

Identification of Cell-cycle-regulated Genes and Promoter Elements in Yeast *S. cerevisiae*

M.Q. Zhang [in collaboration with the Futcher Lab, Cold Spring Harbor Laboratory, and the Brown/Botstein Labs, Stanford]

We wanted to create a comprehensive catalog of yeast genes whose transcript levels vary periodically within the cell cycle. We used DNA microarrays and samples from yeast cultures synchronized by three independent methods: α-factor arrest, elutriation, and arrest of a *cdc15* temperature-sensitive mutant. Using periodicity and correlation algorithms, we identified 800 genes that meet an objective minimum criterion for cell cycle regulation. In separate experiments designed to examine the effects of inducing either the G_1 cyclin Cln3p or the B-type cyclin Clb2p, we found that the mRNA levels of more than half of these 800 genes respond to one or both of these cyclins. Furthermore, we analyzed our set of

cell-cycle-regulated genes for known and novel promoter elements and showed that several elements (or variations thereof) contain information predictive of cell cycle regulation. A full description and complete data sets are available at http://www.cshl.org/mzhanglab/.

Computational Analysis of Intronic Elements Involved in Alternative Splicing

B. Chan, M.Q. Zhang

Alternative splicing allows various isoforms of a gene to be produced from the same pre-mRNA transcript, and it is often associated with suboptimal splice sites; the reduced affinity of these sites for the splicing machinery allows neighboring splice sites to compete. Alternative splicing is regulated by a number of splicing factors that bind to specific RNA sequences known as enhancers. Splicing enhancers are found in both exons and introns. The majority of existing research has focused on the exons, but intronic elements have recently been emphasized as well, and enhancers located in introns are not restrained like their exonic counterparts by a need to avoid disrupting a protein-coding sequence. This study focused on intronic splicing enhancers. After the compilation, a dual approach was followed: We first searched for new instances of a published element using (1) the weight matrix method and (2) the repeat detection method and then we identified putative novel splicing enhancer elements in Genbank. This work will be continued as a Ph.D. thesis project in Tom Maniatis' laboratory at Harvard; the computer program developed is still being tested, and some putative enhancer elements will be followed up by experimental verification.

Identification of Functional Exonic Splicing Enhancer Motifs Recognized by Individual SR Proteins

M.Q. Zhang [in collaboration with H.-X. Liu and A.R. Krainer, at Cold Spring Harbor Laboratory]

Using an in vitro randomization and functional selection procedure, we have identified three novel classes of exonic splicing enhancers (ESEs) recognized by human SF2/ASF, SRp40, and SRp55, respectively. These ESEs are functional in splicing and are highly specific. For SF2/ASF and SRp55, in most cases, only the cognate SR protein efficiently recognizes an ESE and activates splicing. In contrast, the SRp40-selected ESEs function with either SRp40 or SRp55, but not with SF2/ASF. UV cross-linking/competition and immunoprecipitation experiments showed that SR proteins recognize their cognate ESEs in a nuclear extract by direct and specific binding. A motif search algorithm was used to derive consensus sequences for ESEs recognized by these SR proteins. Each SR protein yielded a distinct five- to seven-nucleotide degenerate consensus. These three consensus sequences occur at higher frequencies in exons than in introns and may thus help define exon-intron boundaries; they occur in clusters within regions corresponding to naturally occurring mapped ESEs. We conclude that a remarkably diverse set of sequences can function as ESEs. The degeneracy of these motifs is consistent with the fact that exonic enhancers evolved within extremely diverse protein-coding sequences and are recognized by a small number of SR proteins that bind RNA with limited sequence specificity.

Construction of the Web Service for Multiple DNA Sequence Alignment Tools

Z. Ioschikhes, M.Q. Zhang

To support our group as well as CSHL scientists for multiple DNA sequence alignment needs, a suite of computer programs is provided on the Internet which may be accessed at http://www.cshl.org/mzhanglab/. The following three programs are implemented on our local machine: (1) neural networks for multiple alignment of binding sites (Heumann et al., *Intelligent Sys. Mol. Biol. 2:* 1884 [1994]); (2) Original Gibbs Motif and Sites Samplers (the Gibbs Sampler stochastically examines candidate alignments in an effort to find the best alignment as measured by the maximum a posteriori log-likelihood ratio); and (3) Consensus and WConsensus programs (see Hertz, *Comput. Appl. Biosci. 6:* 81 [1990]). A fourth program, MIME (Bailey and Elkan, *Mach. Learn. 21:* 51 [1990]), is provided as a hot link. These programs determine con-

sensus patterns in unaligned sequences. The algorithm is based on a matrix representation of a consensus pattern.

GibbsDNA Sites and Motif Samplers for DNA sequence analysis have been developed on the basis of the original program, taking into account specific sequence features of DNA that differ from protein sequences. These features have been reflected in the following program options:

1. *Including both strands of DNA as a single sequence* allows searching for a common pattern on either direct or reverse complementary DNA strands.
2. *Including both strands of DNA as separate sequences* allows searching for common pattern on both the strands simultaneously.
3. *Aligning motifs that cannot contain words* allows excluding certain words (oligonucleotide motifs) from the pattern being searched.
4. *Aligning motifs that must contain a word* allows searching only for those motifs that are including certain word.
5. *Assuming that the pattern is symmetrical* is a feature designed specifically for search of palindrome motifs only.
6. *Setting distance (or distance range) between two motifs* allows simultaneous search of two motifs separated by a certain distance.

PUBLICATIONS

Chen T. and Zhang M.Q. 1998. POMBE: A fission yeast gene-finding and exon-intron structure prediction system for fission yeast. *Yeast* **14:** 701–710.

Liu H.-X., Zhang M.Q., and Krainer A.R. 1998. Identification of functional exonic splicing enhancer motifs recognized by individual SR proteins. *Genes Dev.* **12:** 1998–2012.

Spellman P.T., Sherlock G., Zhang M.Q., Iyer V.R., Anders K., Eisen M.B., Brown P.O., Botstein D., and Futcher B. 1998. Comprehensive identification of cell cycle regulated genes of the yeast *Saccharomyces cerevisiae* by microarray hybridization. *Mol. Biol. Cell.* **9:** 3273–3297.

Zhang M.Q. 1998. Identification of human gene core promoters in silico. *Genome Res.* **8:** 319–326.

Zhang M.Q. 1998. Identification of protein coding regions in *Arabidopsis thaliana* genome based on quadratic discriminant analysis. *Plant. Mol. Biol.* **37:** 803–806.

Zhang M.Q. 1998. Statistical features of human exons and their flanking regions. *Hum. Mol. Genet.* **7:** 919–932.

In Press

Ioshikhes I., Trifonov E.N., and Zhang M.Q. 1999. Periodical distribution of transcription factor sites in promoter regions and connection with chromatin structure. *Proc. Natl. Acad. Sci.* (in press).

Tabaska J.E. and Zhang M.Q. 1999. Detection of polyadenylation signals in human DNA sequences. *Gene* (in press).

Zhang M.Q. 1999. Promoter analysis of co-regulated genes in the yeast genome. *Comput. Chem.* (in press).

Zhu J. and Zhang M.Q. 1999. SCPD: A promoter database of yeast *Saccharomyces cerevisiae. Bioinformatics* (in press).

NEUROSCIENCE

Over the last half century, neuroscience research has been dominated by cellular studies. Technical advances in electrophysiological recording and cellular imaging have yielded important biological insights to developmental programs, synaptic transmission, neurotransmitter receptors, ion channels, action potentials, intracellular signaling pathways, and gene regulation. Most of the major discoveries, in fact, have been made.

The next frontier of neuroscience lies in integrative approaches: Gene function must be integrated with cells, cellular function with circuits, circuit function with neural systems, system function with behavior. How can this be accomplished? One approach is via genetic analysis. By manipulating genetic variants experimentally as independent variables, causal inferences can be drawn to dependent measures at various levels of analysis. Such a "vertical integration" allows us to "connect the dots" from gene function to biochemistry, cell biology, neuroanatomy and behavior—at any developmental stage.

As cellular neuroscientists become interested in the biological bases of learning and memory, they have focused on the study of molecular mechanisms of synaptic plasticity. From a neurogenetic perspective, however, a broader question can be asked. What genes in the human genome, when mutated, give rise to learning/memory disabilities? An adequate answer to this question will include the identification of (1) genes involved in cellular mechanisms of synaptic plasticity, (2) genes involved in the development of underlying neuronal architecture, and (3) genes involved in both neurodevelopment and neuronal function.

At the heart of this neurogenetic perspective is gene discovery, which now is driven with increasing force by the genome project(s) and the use of genetically amenable model systems. For the next few years, much of this effort will appear factory-like. Brute force will be required to identify genes and to connect them to particular biological functions. As more and more genes are "put on the table," however, vertical integration will accelerate and Kuhnian insight will emerge. With such comprehensive knowledge of brain function, an understanding of the biological bases of cognitive dysfunction will be within our grasp and effective therapies will emerge.

Neuroscience Entrance

ACTIVITY-DEPENDENT NEURONAL DEVELOPMENT

H.T. Cline K. Bronson, I. Cantallops, I. Cohen, L. Foa, K. Haas, A. Javaherian, J. Jay, Z. Li, I. Mahapatra (Eton Student), E. Nedivi, J. Newman, I. Rajan, D. Rosa, E. Ruthazer, W.C. Sin, G.-Y. Wu, D.-J. Zou

The role of neuronal activity in shaping circuits within the brain has been apparent since the pioneering work of Hubel and Weisel, who demonstrated that visual experience governs the formation of synaptic connections within the mammalian visual cortex. Despite the widespread recognition that the developing brain can be influenced by activity, we have little understanding of how neurons translate "experience" into modified neuronal structure or changes in synaptic strength. The focus of my laboratory is to determine the cellular mechanisms by which neuronal activity controls central nervous system (CNS) development and plasticity. We address this question using the retinotectal projection of the frog. We have applied several analytical approaches to the retinotectal system that allow us to assess and manipulate the coordinated development of neuronal morphology and synaptic function in the CNS. One approach is to use in vivo imaging during periods of up to 5 days of retinal axons or tectal neurons labeled with a fluorescent lipophilic dye, DiI. A second approach is to use a whole-brain preparation to take patch-clamp recordings from optic tectal neurons at different stages of maturation. A third approach is to use viral vectors to deliver genes of interest into postmitotic frog neurons.

Control of Visual System Plasticity by CaMKII

G.-Y. Wu, D.-J. Zou, D. Rosa, E. Ruthazer, K. Bronson, H.T. Cline

Developing neurons coordinate their morphological and functional development. We showed previously that calcium/calmodulin protein kinase type II (CaMKII) activity is sufficient to promote the maturation of retinotectal glutamatergic synapses in *Xenopus*. G.-Y. Wu tested whether CaMKII activity also controls morphological maturation of optic tectal neurons using in vivo time-lapse imaging of single neurons during periods of up to 5 days. We divided morphological development of tectal cells into three stages: Stage 1 neurons are newly differentiated, and they undergo axonogenesis with little elaboration of the dendritic arbor. Stage 2 neurons are in a phase of rapid dendritic growth. Stage 3 is a period of slower dendritic arbor growth. Short-interval observation indicates that stage-2 neurons are more dynamic than more mature stage-3 neurons.

CaMKII expression in tectal neurons correlates with the transition from rapidly growing stage-2 neurons to more stable stage-3 neurons. Elevating CaMKII activity in young neurons by viral expression of constitutively active CaMKII slowed dendritic growth to a rate comparable to that of mature neurons. CaMKII overexpression stabilized dendritic structure in more mature neurons, whereas pharmacological CaMKII inhibition increased their dendritic growth.

D.-J. Zou tested whether endogenous CaMKII activity in postsynaptic tectal cells regulates the elaboration of neuronal processes in the *Xenopus* tadpole retinotectal projection. He selectively infected postsynaptic tectal cells with recombinant vaccinia viruses that express CaMKII-specific inhibitory peptides. Using in vivo time-lapse imaging, he found that expression of CaMKII inhibitor peptides in postsynaptic tectal cells increased the elaboration of both tectal cell processes and presynaptic retinotectal axon arbors. The results suggest that endogenous postsynaptic CaMKII activity regulates the coordinated development of pre- and postsynaptic arbor structures.

To test the role of CaMKII in brain plasticity further, E. Ruthazer will determine whether CaMKII is necessary and sufficient for the development and maintenance of the retinotopic map within the optic tectum.

Role of Glutamate Receptor Activity in the Development of Tectal Cell Dendrites In Vivo

I. Rajan, K. Bronson, H.T. Cline

Glutamatergic retinotectal inputs mediated principally by *N*-methyl-D-aspartate (NMDA) receptors can be recorded from optic tectal neurons early during their morphological development in *Xenopus* tadpoles. As tectal cell dendrites elaborate, retinotectal synaptic responses acquire an α-amino-3-hydroxy-5-methyl-4-isoxazole (AMPA)-receptor-mediated synaptic component in addition to the NMDA component. I. Rajan tested whether glutamatergic activity was required for the elaboration of dendritic arbors in *Xenopus* optic tectal neurons. Using in vivo time-lapse imaging of single DiI-labeled neurons, she showed that the NMDA receptor (NMDA-R) antagonist, amino phosphono valeric acid (APV), blocked the early development of the tectal cell dendritic arbor, whereas the AMPA receptor (AMPA-R) antagonist, CNQX, or the sodium channel blocker, TTX, did not. The decreased dendritic development is due to failure to add new branches and extend preexisting branches. These observations indicate that NMDA-type glutamatergic activity promotes the initial development of the dendritic arbor. At later stages of tectal neuron development, when AMPA-R-mediated synaptic transmission is strong, both APV and CNQX decrease dendritic arbor branch length, consistent with a role for glutamatergic synaptic transmission in maintaining dendritic arbor structure. These results indicate that AMPA-R and NMDA-R can differentially influence dendritic growth at different stages of neuronal development, correlating with changes in the relative contribution of the receptor subtype to synaptic transmission.

Role of Glutamate Receptor Activity in the Development of Tectal Cell Dendrites and Retinal Axons In Vivo

I. Rajan, K. Bronson, H.T. Cline

Previous studies suggested that blocking retinotectal synaptic activity might increase the elaboration of retinal axon arbors, whereas the experiments mentioned above indicate that blocking rectinotectal synaptic activity can limit elaboration of the dendritic arbor. I. Rajan investigated the role of NMDA-R activity in the formation of both the presynaptic axon arbor and postsynaptic dendritic arbors in vivo by taking time-lapse confocal images of single DiI-labeled *Xenopus* retinotectal axons and optic tectal neurons in the presence and absence of the NMDA-R antagonist, APV. Retinotectal axons or tectal neurons were imaged at 30-minute intervals over 2 hours or twice during a 24-hour period. Retinal axons in animals exposed to APV in rearing solution show an increase in rates of branch additions and a decrease in branch lifetimes compared to untreated axons. Under the same experimental conditions, tectal neurons show a decreased rate of branchtip additions and retractions and a decrease in branch lifetimes. APV treatment over 24 hours had no apparent effect on axon arbor morphology, but it did decrease tectal cell dendritic development. These observations indicate that NMDA-R activity in postsynaptic neurons promotes the initial development of the dendritic arbor and stabilizes presynaptic axon arbor morphology.

Regulation of Dendritic Morphology by RhoA GTPases

Z. Li, W.C. Sin, H.T. Cline [in collaboration with L. Van Aelst, Cold Spring Harbor Laboratory]

The Rho family of small GTPases regulates actin cytoskeletal structure. To test whether this family of proteins affects neuronal structure during brain development in vivo, Z. Li and W.C. Sin have expressed dominant negative and constitutively active forms of the Rho GTPases in tectal neurons using vaccinia virus. In vivo imaging of single neurons shows that the three GTPases, Rac, Rho, and Cdc42, all affect neuronal structure in distinct ways. We will continue these studies to determine the signaling pathways controlling neuronal structural plasticity.

Candidate Plasticity Genes

I. Cantallops, L. Foa, K. Haas, J. Jay, E. Nedivi, J. Newman, I. Rajan, H.T. Cline, K. Bronson, G.-Y. Wu [in collaboration with P. Worley, Johns Hopkins University]

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 begun to investigate the potential function of two activity-induced genes *cpg15* and *homer* in brain development.

CPG15, a GPI-linked Signaling Molecule Induced by Synaptic Activity, Promotes Dendritic Growth In Vivo

E. Nedivi, G.-Y. Wu, K. Bronson, H.T. Cline

During CNS development, intricate connections between neurons are established with remarkable accuracy. Activity-independent and activity-dependent processes work in concert to guide afferent and efferent processes to their appropriate synaptic partners. E. Nedivi and G.-T. Wu showed that CPG15, a novel protein identified in a differential screen for genes regulated by synaptic activity, functions as a new growth factor. Using time-lapse imaging and virally mediated gene transfer, they demonstrated that CPG15 promotes dendritic arbor growth in vivo. CPG15 accelerates the growth of projection neurons, but has no effect on the growth rate of interneurons. They further showed that CPG15, a GPI-linked protein, controls growth of neighboring neurons through an intercellular signaling mechanism. Thus, CPG15 is a signaling molecule capable of translating neuronal activity into structural changes. Our findings posit the existence of a new class of activity-regulated growth factors, which by virtue of being membrane-bound, may permit exquisite spatial and temporal control of neuronal growth.

cpg15 Expression in Tectal Cell Increases Presynaptic Retinal Axon Arbor Growth In Vivo

I. Cantallops, K. Bronson, H.T. Cline

I. Cantallops tested the effect of tectal cell overexpression of *cpg15* on the development of retinotectal axons. She showed that viral expression of *cpg15* in postsynaptic neurons increases the elaboration of presynaptic retinal axons in vivo. These results concur with the experiments done by E. Nedivi and G.-Y. Wu documenting the effects of *cpg15* expression of tectal cell dendritic arbor development. The results suggest that CPG15 may be one of the mediators of activity-dependent developmental plasticity.

CPG15 Promotes Maturation of Retinotectal Synapses

K. Haas, H.T. Cline

The studies mentioned above indicate that CPG15 increases the morphological complexity of pre- and postsynaptic neurons in the visual system. To test whether CPG15 affects the development of electrophysiological properties of the visual system, K. Haas took whole-cell recordings from tectal neurons of animals infected with vaccinia virus expressing CPG15. He found that expression of CPG15 resulted in increased strength of glutamatergic retinotectal synaptic transmission. CPG15 promoted the maturation of glutamateric synapses in immature neurons. Expression of the truncated form of CPG15 lacking the GPI consensus sequence reduced the strength of synaptic transmission and prevented the normal maturational program seen in glutamatergic synapses from control animals.

Developmental Regulation of *cpg15* Gene and Protein Expression

A. Javaherian, E. Nedivi, J. Jay, H.T. Cline

To continue our analysis of CPG15 function in the developing nervous system, A. Javaherian cloned *cpg15* from *Xenopus*. The gene from *Xenopus* is highly homologous to *cpg15* from rat, cat, and humans. Comparison of the gene from *Xenopus* with mammalian species will allow us to test the function of conserved regions of the protein, under the assumption that the conserved domains are required for CPG15 function. In situ hybridization and antibody labeling were used to determine the developmental time course and distribution of CPG15 expression in the tadpole nervous system. The *cpg15* message is highly expressed in retinal ganglion cells, but no other cells in the retina; however, the protein appears to be preferentially targeted to the axons of retinal ganglion cells. Within the brain, the *cpg15* message and protein are widely expressed in differentiated neurons.

CPG15 protein expression is barely detectable in brains of adult frogs, indicating that it is developmentally regulated.

Analysis of Aberrant Axon Trajectories in Homer Expressing Neurons

I. Rajan, J. Jay, J. Newman, L. Foa, H.T. Cline
[in collaboration with P. Worley, Johns Hopkins]

homer is an activity-regulated gene induced in the hippocampus of rodents. To study the potential function of Homer in developmental plasticity, J. Jay and I. Rajan labeled the tadpole brain with antibodies against Homer. Homer is widely expressed throughout the developing nervous system. Injection of kainic acid, an analog of glutamate, increased Homer expression in tadpole brains. J. Newman and L. Foa have worked to clone the *Xenopus* homolog of *homer*. I. Rajan used vaccinia virus to express Homer in the brains of *Xenopus* tadpoles, combined with in vivo imaging of single neurons to test whether Homer expression modified neuronal morphological development. Axon guidance, as opposed to synaptogenesis, has been considered activity-independent. It therefore came as a surprise to find that expression of Homer resulted in stereotyped errors in axon guidance in tectal neurons. Dendrites appeared to be normal. The data suggest that *homer*, an activity-regulated gene, may be involved in the early stages of axonal guidance in *Xenopus*. Homer's association with mGluR1/5a and their coupling to IP3/Ca2$^+$ signal transduction suggest potential mechanisms by which this protein causes enhancement of aberrant axon trajectories.

PUBLICATIONS

Cline H.T. 1998. Topographic maps: Developing roles of synaptic plasticity. *Curr. Biol.* **8:** R836–839.

Constantine-Paton M. and Cline H.T. 1998. LTP and activity-dependent synaptogenesis: The more alike they are the more different they become. *Curr. Opin. Neurobiol.* **8:** 139–148.

Haas K., Cline H., and Malinow R. 1998. No change in NMDA receptor-mediated response rise-time during development: Evidence against transmitter spillover. *Neuropharmacology* **37:** 1393–1398.

Nedivi E., Wu G.-Y., and Cline H.T. 1998. Promotion of dendritic growth by a GPI-linked signaling molecule, CPG15, an activity-induced signaling molecule. *Science* **281:** 1863–1866.

Pinches E.M. and Cline H.T. 1998. The distribution of synaptic vesicle proteins within single retinotectal axons of *Xenopus* tadpoles. *J. Neurobiol.* **5:** 426–434.

Rajan I. and Cline H.T. 1998. Glutamate receptor activity is required for normal development of tectal cell dendrites *in vivo*. *J. Neurosci.* **18:** 7836–7846.

Wu G.-Y. and Cline H.T. 1998. Stabilization of dendritic arbor structure in vivo by CaMKII. *Science* **279:** 222–226.

In Press

Cline H.T. 1999. Development of dendrites. In *Dendrites* (ed. G. Stuart et al.). Oxford University Press, London. (In press.)

Cline H.T., Edwards J.A., Rajan I., Wu G.Y., and Zou D.J. 1999. In vivo imaging of CNS neuron development. In *Imaging: A laboratory manual* (ed. R. Yuste et al.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. (In press.)

Edwards J.A. and Cline H.T. 1999. Light-induced calcium influx into retinal axons is regulated by presynaptic nicotinic acetylcholine receptor activity in vivo. *J. Neurophysiol.* **81:** 895–907.

Rajan I., Witte S., and Cline H.T. 1999. NMDA receptor activity stabilizes presynaptic retinotectal axons and postsynaptic optic tectal cell dendrites in vivo. *J. Neurobiol.* **38:** 357–368.

SIGNAL TRANSDUCTION AND DIFFERENTIATION

G. Enikolopov J. Hemish, S. John, P. Krasnov, T. Michurina, J. Mignone, N. Nakaya, M. Packer, N. Peunova, R. Rajagopal, V. Scheinker, Y. Stasiv

The main interest of our laboratory remains the signals that link cell activity with cell differentiation. We focus on the role of nitric oxide (NO), a multifunctional second messenger, in tissue differentiation and organism development. Within the last several years, we found that NO acts as an essential negative regulator of cell proliferation in developing tissue, contributing to the control of organ size. We now study the role of NO in several systems of animal development, focusing on the genetic and molecular interactions of NO with the major pathways that control cell proliferation and differentiation.

Search for Genes Activated by NO

N. Nakaya

Although the physiological function of NO in the organism has been studied in much detail, very little is known about the transcriptional targets of NO. We decided to search for genes whose transcription is affected by short- or long-term NO signaling.

We used four approaches to isolate NO-induced genes: suppressive subtractive hybridization (SSH), selective amplification via biotin- and restriction-mediated enrichment (SABRE), immobilized cDNA arrays of several hundred identified genes (Atlas Gene Expression Arrays, Clontech), and arrays of 18,024 genes from the IMAGE project (Gene Discovery Arrays, Genome Systems). Attempts with SSH and gene arrays were particularly successful, and we have identified more than 100 genes whose transcription was induced or suppressed in response to NO. For many genes, the changes were confirmed by Northern or reverse transcriptase–polymerase chain reaction (RT-PCR) analysis. Among these NO-dependent genes, we found new genes and previously identified genes. Importantly, many genes were isolated in all four screening procedures, supporting the feasibility of this approach. Of most importance, some NO-activated genes fall into distinct groups, revealing signaling cascades necessary for various physiological functions of the cell. Although some of these groups were anticipated (stress response genes, cell-cycle-related genes, transcription factors), some were not and provided unforeseen insights to the nature of NO activity in the cell (genes related to cell adhesion, G-protein signaling, cytoskeleton, chemokine receptors, etc.). Thus, we were able to consider the action of NO in terms of particular cellular functions and signaling cascades, rather than induction of individual genes. For instance, observation of several targets of p53 induced in response to NO gave us a direct indication to search for functional interactions between NO and p53. Indeed, in collaboration with Scott Lowe here at the Laboratory, we found that $p53^{-/-}$ mouse embryonal fibroblasts do not respond to NO by induction of these target genes and are compromised in their ability to stop dividing in response to NO, that the p53 protein is accumulated in cells after exposure to NO, and that the p53 molecule undergoes characteristic posttranslational modifications in response to NO. We use the results of the screen to identify specific components of the cell cycle machinery that are activated by the NO signals.

Structure and Expression of the *Xenopus* NOS Gene

V. Scheinker, Y. Stasiv, N. Peunova

Using highly conserved regions from mammalian and *Drosophila* nitric oxide synthase (NOS) genes as probes, we cloned NOS cDNA from a *Xenopus* tadpole cDNA library. The nucleotide and deduced amino acid sequences of the cloned *XNOS1* gene show the highest similarity to the neuronal isoform of mammalian NOS.

The deduced XNOS1 protein contains the determinants which are crucial for NOS activity, including regions for binding FMN, FAD, NADPH, tetrahydrobiopterin, heme, and calmodulin, and a consensus site for phosphorylation by cAMP-dependent protein kinase. The amino terminus of XNOS1 contains a

PDZ motif which is crucial for association with PDZ domains of other proteins. When expressed in cultured cells, *XNOS1* cDNA produced a polypeptide of the expected size of 160 kD, similar to mammalian neuronal NOS and NOS from the frog brain. XNOS1 generated NO with high efficiency after transfection in cultured cells. Production of NO was dependent on the presence of calcium, calmodulin, and NADPH and was completely blocked by application of specific inhibitors of NOS.

In situ hybridization with the *XNOS1* probe shows that *XNOS1* RNA is present in the tadpole brain in a distinct pattern in neuron cell bodies in the optic tectum, thalamus, telencephalon, and spinal cord. Antibody staining showed the presence of *XNOS1* in the cell bodies and processes in the tadpole brain. Together, the gene structure, enzymatic properties, and distribution of XNOS1 indicate that we have cloned the ortholog of the mammalian neuronal isoform of NOS.

NO Is an Essential Negative Regulator of Cell Proliferation in *Xenopus* Brain

N. Peunova [in collaboration with H.T. Cline, Cold Spring Harbor Laboratory]

NO can suppress DNA synthesis and cell proliferation in cultured cells and in *Drosophila* imaginal discs. To examine the role of NO in vertebrate organogenesis, we studied the antiproliferative activity of NO synthase on brain development in *Xenopus laevis*. We used histochemical staining and in situ hybridization of a cloned neuronal isoform of *Xenopus* NOS (XNOS1; see previous section) to identify its expression pattern in the developing brain. We found that a zone of NOS-positive neurons lies adjacent to the zone of dividing neuronal precursors, suggesting that NO may be involved in cessation of proliferation of neuronal precursors. To test whether NO is essential for cell cycle arrest in *Xenopus* brain development, we introduced a polymer matrix impregnated with NOS inhibitors into the ventricle of the tadpole brain. These implants resulted in a dramatic increase in the number of proliferating cells in the tectum area and a net increase in the overall size of the tadpole brain. Using specific antibodies as markers of neuronal development, we found that the organization of the brain was grossly perturbed by excess proliferation of precursors that differentiated after NOS inhibitors were applied. Neurons that differentiated before application of NOS inhibitors appeared to be unaffected. Our results indicate that NO is an important negative regulator of cell proliferation during normal *Xenopus* tadpole brain development.

Transgenic Mice Expressing nNOS in the Adult Brain

M. Packer, S. John

We have developed transgenic mice overexpressing the neuronal nitric oxide synthase (nNOS) gene under the control of the calcium/calmodulin kinase IIα promoter. This promoter gives strong expression of the transgene from postnatal day 12 in specific regions of the fully formed brain of the mouse. This has the advantage that normal development of the brain will not be potentially affected by expression of the transgene. Spatial expression of the transgene is also an advantage because we can then follow the fate of specific cell types.

Full characterization of the nine lines of transgenic mice by PCR, RT-PCR, Northern blotting, in situ hybridization, histochemical and immunohistochemical staining, and NOS activity assay has shown that six of the nine lines express the nNOS transgene in the expected areas of the brain and that this expression continues in mice until at least 35 weeks of age. Phenotypically, these mice appear to be normal at a gross level.

We plan to use these mice to study the differentiation process of neural progenitor cells in the adult mouse brain. Two areas exist in the rodent brain where neurogenesis continues into adulthood. One of these areas, in particular, the subgranular zone of the dentate gyrus of the hippocampus, is a region where we get very high expression of our transgenic nNOS protein. These mice therefore are good models to test the role of NO in the control of proliferation and subsequent differentiation of these cells.

NO in the Developing Brain

J. Mignone

Antiproliferative properties of NO are used by the organism to control the cell number during differentiation and development. Numerous data indicate that

NO may have a similar role during mammalian brain development. We want to address this question by generating transgenic mice that express NOS genes in the developing brain. We created bicistronic constructs which express different isoforms of NOS and reporter genes under the control of general and neuron-specific promoters and tested their activity in cultured cells. We are now introducing these recombinants in the germ line and will search for changes in the proliferation patterns of neuronal precursors and distribution of early neuronal markers.

Molecular Mechanisms of NO Synthesis during *Drosophila* Development

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

Molecular characterization of the *Drosophila* NOS locus shows that *DNOS1* mRNA which encodes *Drosophila* NO synthase is dispersed over a minimum 40 kb of genomic DNA and consists of at least 20 exons with translation initiation and termination in exon 3 and exon 20, respectively. Previous studies in mammals demonstrated that several forms of nNOS mRNA are generated due to alternative splicing. We found that this mechanism of NOS regulation is present in *Drosophila* as well: Numerous alternatively spliced transcripts of *DNOS* were identified using RT-PCR and screening of cDNA libraries. In one of the alternatively spliced transcripts, one of the introns is not spliced out. This introduces a premature stop codon, and as a result, this transcript encodes a truncated protein DNOS4 with a unique carboxyl terminus. This protein lacks important domains and does not produce NO. However, it strongly inhibits DNOS1 activity in cotransfection experiments. One possibility is that it disrupts homodimerization of DNOS1 which is crucial for its enzymatic activity. The truncated protein forms homodimers in yeast two-hybrid system, and we are now testing to determine whether it can form heterodimers with DNOS1 protein. Our results suggest that DNOS4 may code for a natural negative regulator of *DNOS1* activity and that it can be used as a dominant negative regulator of DNOS1 when introduced to the *Drosophila* germ line. We have generated transgenic flies expressing DNOS4 under an eye-specific promoter to test this hypothesis.

Protein Targeting to Neuronal Cell Terminals

P. Krasnov

Synaptic vesicle biogenesis requires precise targeting of constituent proteins to the axon terminal. We searched for the targeting signals that determine delivery of synaptotagmin, a major synaptic vesicle protein, to the neurite terminal in neuronal cells. Using deletion and point mutagenesis of the synaptotagmin molecule, we identified a targeting signal in the carboxy-terminal domain that is necessary for the accumulation of synaptotagmin at the neurite terminal and found two amino acid residues which are indispensable for the correct targeting. When possible conformations of the synaptotagmin carboxy-terminal domain were modeled using the Macromodel program (P. Nestler and P. Krasnov), the crucial residues fell within the loop of a highly stable hairpin formed by the adjacent acidic and basic residues, suggesting that these residues may represent points of contact between the synaptotagmin molecule and proteins that determine its distribution in the neuronal cell. Using affinity chromatography, we isolated a protein that binds to the targeting signal. We identified it by microsequencing (R. Kobayashi and P. Krasnov) and are currently testing whether it interacts with the synaptotagmin molecule in neuronal cells in vivo.

PUBLICATIONS

Reilein A.R., Tint I.S., Peunova N.I., Enikolopov G.N., and Gelfand V.I. 1998. Regulation of organelle movement in melanophores by protein kinase A (PKA), protein kinase C (PKC), and protein phosphatase 2A (PP2A). *J. Cell Biol.* **142:** 803–813.

In Press

Kuzin B. and Enikolopov G. 1999. Nitric oxide acts as a negative regulator of cell prolin imaginal discs of *Drosophila*. *Cell Death Dif.* (in press).

Nishizaki T., Matsuoka T., Nomura T., Enikolopov G., and Sumikawa K. 1999. Arachidonic acid potentiates currents through Ca^{2+} permeable AMPA receptors by interacting with a CaMKII pathway. *Mol. Brain Res.* **67:** 184–189.

Xiong H., Kiyofumi Y., Han D., Nabeshima T., Enikolopov G., Carnahan J., and Nawa H. 1999. Mutual regulation between the intracellular messengers nitric oxide and brain-derived rodent neurotrophic factor in rodent neocortical cultures. *Eur. J. Neurosci.* **11:** 1567–1576.

TRANSMISSION AND PLASTICITY IN MAMMALIAN CENTRAL SYNAPSES

R. Malinow N. Dawkins, A. Barria, J. Esteban, Y. Hayashi, F. Kamenetz, Z. Mainen, M. Maletic-Savatic, A. Piccini, J.-C. Poncer, S. Shi, S. Zaman, N. Yamashita (visiting Japanese medical student)

We address 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 which are complex enough 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 transfection techniques. This allows us to examine the cell biological 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.

Delivery of Glutamate Receptors to Synapses during Development

J. Esteban [in collaboration with R. Petralia and R. Wenthold, National Institutes of Health]

The synapses we study (which are similar to and serve as a model for most excitatory synapses in the vertebrate brain) use glutamate as the neurotransmitter and have two types of postsynaptic glutamate-sensitive receptors: α-amino-3-hydroxy-5-methyl-4-isoxazole proprionate (AMPA) type and *N*-methyl-D-aspartate (NMDA) type. Roughly speaking, AMPA receptors (AMPA-Rs) transmit all presynaptic release events, whereas NMDA receptors (NMDA-Rs) require intense activity and trigger plasticity. We have used immunogold electron microscopy to test the hypotheses that (1) there are synapses containing NMDA-Rs and no AMPA-Rs, (2) such synapses are more prevalent early in development, and (3) there is an increase in AMPA-R-containing synapses with no change in the NMDA-Rs.

Since immunogold electron microscopy allows accurate quantification and precise anatomical localization of the proteins in question, these methods are optimal for the questions addressed. We find that a large fraction of synapses contain NMDA-R immunoreactivity with no AMPA-R immunoreactivity. These are likely to correspond to the pure NMDA responses detected electrophysiologically by our laboratory (Liao et al., *Nature 375:* 400 [1995]), and at present by several other laboratories, in various preparations, including the cerebral cortex, spinal cord, and optic tectum. As the physiology predicts, the prevalence of these synapses containing only NMDA-R protein decreases during development. These results provide a molecular basis for the physiological observation of "silent synapses": synaptic responses mediated purely by NMDA-Rs. Their increased prevalence early in development likely reflects exuberant synaptogenesis during this period. Since synaptogenesis has a large random component, it makes sense that such synapses would have only NMDA-Rs which do not transmit information if such synapses fire in isolation, but will transmit if such synapses fire in unison with other synapses.

Delivery of Glutamate Receptors to Synapses during Long-term Potentiation

Y. Hayashi

Having seen that delivery of AMPA-R occurs during development (see above), we wanted to test if long-term potentiation (LTP) can be expressed by the rapid delivery of AMPA-Rs from regions not accessible by the transmitter (either intracellular storage or extrasynaptic dendritic surfaces) to the synaptic surface (a process we term AMPA-fication). One method to test this possibility is to acutely overexpress GluR1 in hippocampal slice neruons. We find that such overexpression produces significant amounts of AMPA-Rs with only GluR1. Such AMPA-Rs have an electrophysiological fingerprint different from that of endogenous AMPA-Rs, which generally contain some GluR2; namely, homomeric GluR1 receptors show inward rectification, whereas endogenous receptors do not. Thus, we can infect neurons in hippocampal slices using a Sindbis virus that expresses GluR1-EGFP (enhanced green fluorescent protein). In infected cells, we monitor transmission onto two synaptic pathways. In one pathway, we induce LTP and are now testing if this

potentiated pathway has greater rectification than the control pathway. If this is so, it will indicate that newly synthesized recombinant receptor has been rapidly delivered to potentiated synapses.

Dendritic Trafficking, Clustering, and Synaptic Delivery of AMPA-Rs in Hippocampal Neurons

S. Shi, Y. Hayashi, J. Esteban [in collaboration with K. Svoboda, Cold Spring Harbor Laboratory, and R. Petralia and R. Wenthold, National Institutes of Health]

To monitor directly changes in AMPA-R distribution in living neurons, the AMPA-R subunit GluR1 was tagged with green fluorescent protein (GFP). This protein (GluR1-GFP) is functional and was transiently expressed in hippocampal CA1 neurons. In dendrites visualized with two-photon laser scanning microscopy or electron microscopy, most of the GluR1-GFP is intracellular, mimicking endogenous GluR1 distribution. Tetanic synaptic stimulation induces a rapid delivery of tagged receptors into dendritic spines as well as clusters in dendrites. These postsynaptic trafficking events require synaptic NMDA-R activation and may contribute to the enhanced AMPA-R-mediated transmission observed during LTP and activity-dependent synaptic maturation.

Role of CaMKII in Receptor Trafficking

A. Piccini, Y, Hayashi, S. Shi

Considerable evidence indicates that calcium-calmodulin kinase II (CaMKII) activation mediates the generation of LTP. To examine the effect of increased CaMKII activity on AMPA-R trafficking, we have generated a Sindbis expression vector with an internal ribosome entry site (IRES) sequence able to express two proteins. These proteins are GluR1-GFP and tCaMKII, the latter being the constitutively active catalytic domain of CaMKII. Extracts from neurons expressing this construct show an increase in constitutive (calcium-independent) CaMKII activity. AMPA-R-mediated synaptic transmission onto such infected cells shows an increased amount of rectification, indicating that CaMKII activity induces a delivery of recombinant GluR1-GFP to synapses. These results provide direct evidence for the view that LTP is due, at least in part, to an increased delivery of AMPA-Rs to synapses.

Role of Candidate Plasticity Genes in Synaptic Maturation

R. Malinow [in collaboration with K. Haas, E. Nedivi, and H. Cline, Cold Spring Harbor Laboratory]

cpg-15 is a gene showing increased expression following activity protocols that induce plasticity. Exogenous expression of this gene promotes growth of dendritic arbors in *Xenopus* optic tectum. We are testing the effect of this gene expression on synaptic transmission, as well as testing the view that the protein promotes maturation (AMPA-fication) of immature synapses in immature cells and promotes growth of immature (silent) synapses in mature cells. Preliminary electrophysiological results support this view.

Silent Synapses: Calcium Imaging Analysis

Z. Mainen [in collaboration with K. Svoboda, Cold Spring Harbor Laboratory]

We and others have observed electrophyisological responses mediated purely by action on NMDA-Rs. One interpretation is that there are synapses that have only NMDA-Rs (see above). Another interpretation is that a sufficient amount of transmitter can leak out of synapses to activate only NMDA-Rs on nearby synapses. The higher affinity of NMDA-Rs for glutamate, compared to that of AMPA-Rs, makes this scenario plausible. To examine the biophysical basis for pure NMDA responses, we are monitoring the stochastic responses of single synapses that are positioned next to each other. If there is transmitter leakage, nearby synapses should respond synchronously. If there is no leakage, synapses should respond independently. Our data from such cases indicate that synapses function independently, with no detectable spillover. This provides direct evidence that transmission in the central nervous system occurs as a point-to-point process and argues against transmitter spillover being responsible for pure NMDA responses.

Interactions between Short-term and Long-term Plasticity

J.-C. Poncer

If synapses are activated twice within approximately 100 msec, the second response is generally different in

amplitude from the first. At some synapses, the second response is larger (paired-pulse facilitation), whereas at other synapses, the second response is smaller (paired-pulse depression). This short-term plasticity has been studied extensively at a number of synapses and likely reflects properties of the presynaptic transmitter release and recycling machinery. There have been reports that such short-term plasticity changes during LTP. Does this indicate that LTP produces changes in presynaptic function? We find that changes in short-term plasticity during LTP can be explained by recruitment of previously silent synapses. This recruitment may occur by addition of AMPA-Rs to synapses containing only NMDA-Rs. Such functional recruitment of synapses has an impact on the information processing properties of transmission.

Plasticity of NMDA-R-mediated Synaptic Transmission

A. Barria

Tetanic stimulation of synapses leads to an increase in AMPA-R function, at least in part due to a rapid delivery of receptors to synapses. In addition, under certain conditions, an increase can be seen in NMDA-R-mediated transmission. We wish to test the hypothesis that this is due to the formation of new synapses containing only NMDA-Rs. We are testing this possibility in a number of ways, using electrophysiological imaging and molecular biological and pharmacological methods.

Electrophysiology of Transgenic Mice Carrying Mutated Presenilin-1

S. Zaman, F. Kamenetz [in collaboration with S. Sisodia, A. Parent, and D. Borchelt, Johns Hopkins University]

Mutations in presenilin-1 (PS-1) are responsible for approximately 50% of cases of familial Alzheimer's disease. Little is known regarding the cellular distribution and function of PS-1. We have recently found that calcium-triggered processes (e.g., short- and long-term potentiation) are enhanced in transgenic mice expressing mutant PS-1, consistent with the view that PS-1 controls calcium homeostasis. Furthermore, we are able to normalize this abnormality with a benzodiazepine. Of interest, these drugs have recently been associated with a decreased incidence of Alzeheimer's disease in a clinical study. We are now examining the subcellular distribution of PS-1 in neurons, measuring calcium homeostasis in neurons expressing mutant PS-1, and examining if benzodiazepines can normalize the increased secretion of Aβ seen in tissue-expressing mutant PS-1.

In conclusion, we are continuing to elucidate the basic mechanisms involved in central synaptic transmission. Such an understanding is necessary to derive a mechanistic flowchart of plastic processes. We also continue to probe the role of different molecules, including CaMKII and PS-1, in activity-induced and developmental synaptic plasticity.

PUBLICATIONS

Haas K., Cline H., and Malinow R. 1998. No change in NMDA receptor-mediated response rise-time during development: Evidence against transmitter spillover. *Neurophamacology* **37:** 1393–1398.

Hinds H., Tonegawa S., and Malinow R. 1998. CA1 long-term potentiation is diminished but present in hippocampal slices from a α-CaMKII mutant mouse. *Learn. Mem.* **5:** 344–354.

Mainen Z.F., Jia Z., Roder J., and Malinow R. 1998. Use-dependent AMPA receptor block in mice lacking GluR2 suggests postsynaptic site for LTP expression. *Nature Neurosci.* **1:** 579–586.

Maletic-Savatic M. and Malinow R. 1998. Calcium-evoked dendritic exocytosis in cultured hippocampal neurons. Part I: Trans-Golgi network-derived organelles undergo regulated exocytosis. *J. Neurosci.* **18:** 6803–6813.

Maletic-Savatic M., Koothan T., and Malinow R. 1998. Calcium-evoked dendritic exocytosis in cultured hippocampal neurons. Part II. Mediation by CaMKII. *J. Neurosci.* **18:** 6814– 6821.

Malinow R. 1998. Silent synapses in three forms of plasticity. In *Quantal transmission in the central nervous system* (ed. Faber et al.). HFSP, Strassbourg.

Malinow R. 1998. Silencing the controversy in LTP? *Neuron* **21:** 1226–1227.

Petralia R.S., Esteban J.A., Wang Y.-X., Partridge J.G., Zhao H.-M., Wenthold R.J., and Malinow R. 1998. Selective acquisition of AMPA receptors over postnatal development suggests a molecular basis for silent synapses. *Nature Neurosci.* **2:** 31–36.

In Press

Mainen Z., Malinow R., and Svoboda K. 1999. Synaptic [Ca^{2+}] transients in single spines indicate NMDA receptors are not saturated. *Nature* (in press).

Mainen Z.F., Maletic-Savatic M., Shi S.H., Hayashi Y., Malinow R., and Svoboda K. 1999. Two-photon imaging in living brain slices. *Methods* (in press).

Maletic-Savatic M., Malinow R., and Svoboda K. 1999. Rapid dendritic morphogenesis in CA1 hippocampal dendrites induced by synaptic activity. *Science* (in press).

Malinow R., Hayashi Y., Maletic-Savatic M., Zaman S., Poncer J.-C., Shi S., and Esteban J. 1999. Introduction of green fluorescent protein into hippocampal neurons through viral infection. In *Imaging living neurons* (ed. Yuste et al.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. (In press.)

THE BIOLOGY OF LEARNING AND MEMORY

A.J. Silva O. Carvalho Y. Elgersma P.W. Frankland
P. Chen N.B. Fedorov J.H. Kogan
J. Coblentz E. Friedman M. Ohno

Our laboratory is studying the biology of learning and memory. We are interested in the molecular, cellular, and circuit processes that underlie the storage and recall of information. To accomplish this, we are using a variety of techniques including transgenic manipulations, pharmacology, in vitro and in vivo electrophysiology, neuroanatomical lesions, and behavioral analysis. The focus of our studies has been on hippocampus-dependent learning and memory. Our results implicate a variety of hippocampal mechanisms in learning and memory, including long-term potentiation, short-term plasticity, and the slow after-hyperpolarization. For example, we have found mutations that affect the induction of long-term changes in synaptic function and learning, and others that affect the stability of these synaptic changes and memory (but not learning). Our laboratory is also interested in applying these findings to the development of treatments for learning and memory disorders, such as those observed with aging and in neurofibromatosis type I (NF1). Below we describe in more detail studies carried out during this last year in our laboratory.

PHARMACOLOGICAL AND GENETIC RESCUE OF THE LEARNING DEFICITS CAUSED BY THE NF1 HETEROZYGOUS MUTATION IN MICE

Specific learning disabilities are the most common neurological complication in children with neurofibromatosis type 1 (NF1). The complexity of these cognitive deficits, and the difficulty of pursuing their study in patients, motivated us to study an animal model of this disease. We have previously shown that mice with the same mutation that causes NF1 in humans (Nf1 mice) have learning deficits that resemble in important ways the deficits observed in some NF1 patients. As with NF1 patients, the learning and memory deficits of the Nf1 mice are restricted to specific types of learning, they do not affect all individuals, they can be compensated for with extra help and training, and they do not involve deficits in simple forms of learning

The advantage of having an animal model for NF1 is that these animals can be used to search for a cure for this disease. We started by testing the hypothesis that the deficits that cause tumors in NF1 patients are also responsible for their learning disabilities. Previous studies suggested that abnormally high activity of the Ras protein may result in tumor formation, since one of the functions of neurofibromin, the protein disrupted in NF1 patients, is to increase the inactivation of Ras. Our behavioral studies indicate that just as with tumors, the learning disabilities associated with NF1 may be due to increased Ras activity. Remarkably, our results also showed that treating the Nf1 mice with a drug that decreases Ras function (farnesyl transferase inhibitor or FTI) also rescues their learning deficits. We have also been able to rescue the learning deficits of these mutant mice by deleting an allele of one of the *ras* genes expressed in the brain (N-*ras*). These findings support the hypothesis that the learning deficits associated with NF1 are caused by excessive Ras activity. This is an important first step toward the development of a cure for NF1, because we have a molecular clue for the cause of the disease.

INDUCIBLE AND REGION-RESTRICTED TRANSGENIC SYSTEMS: THE LBD-ESTROGEN FUSION SYSTEM

Protein kinases and transcription factors can be regulated in a hormone-dependent manner when fused to the ligand binding-domain of steroid receptors (LBD). Proteins fused with LBD are probably kept inactive due to sterical hindrance by Hsp90 proteins bound to the LBD in the absence of hormone. Simplicity is the main advantage of this posttranscriptional approach to regulate protein activity. Only one DNA vector is required for the expression of a LBD fusion protein. In contrast to transcriptional control systems (Tet-based), this approach allows the activation of a protein rapidly by the release of preformed molecules from Hsp90 protein inhibition. Until recently, the LBD-fusion protein approach was restricted to applications in cell lines, since natural steroids, which activate the wild-type LBDs, can be excluded in vitro but not in vivo experiments. However, we have used instead a mutant LBD of the estrogen receptor (LBDm) which is not activated by endogenous estrogen. We have applied this system to

regulate the function of a mutant CREB (cAMP-responsive element binding) protein that interferes with endogenous CREB function. The CREBm/LBDm protein is expressed in the brain of the transgenic mice and activated by tamoxifen, the ligand for LBDm. Our results so far indicate that the injection of tamoxifen impairs long-term memory for contextual conditioning and for social recognition in the CREBm/LBDm transgenic mice, but not in wild-type controls.

PHARMACOLOGICAL INDUCTION OF MUTANT PHENOTYPES

To date, the genetics community has generated targeted alleles for more than 2500 genes. Even though this is a valuable resource, many of these mutations affect function both during development and in the adult. In the last year, our laboratory has developed a strategy that may allow inducible manipulation of the effects of many of these mutations. We introduced a strategy in which pharmacology is used to induce the effects of targeted mutations. For example, mice heterozygous for a null mutation of a cAMP-dependent transcription factor ($CREB^{\alpha\Delta-}$) showed normal contextual memory. However, antagonists of adenylyl cyclase-linked receptors, at concentrations that did not affect memory in wild-type littermates, revealed a specific long-term memory deficit in these mutants. Importantly, these antagonists did not disrupt memory in mice with a heterozygous point mutation (T286A) in the α calcium/calmodulin-dependent kinase II gene. Instead, a behaviorally ineffective dose of an NMDA receptor antagonist in wild type blocked short- and long-term memory in the kinase mutants. These results demonstrate that pharmacological manipulations can be used to reveal the effects of mutations in mice in a temporally controlled manner.

THE Kvβ1.1 POTASSIUM CHANNEL MUTATION RESCUES THE HIPPOCAMPAL-DEPENDENT LEARNING DEFICITS ASSOCIATED WITH AGING IN MICE

Normal aging is associated with cognitive decline unrelated to diseases such as Alzheimer's. Increases in the slow after-hyperpolarization (sAHP) of aged neurons have been proposed to contribute to this cognitive decline. In the last year, we showed that in aged mice, a targeted mutation of the modulatory potassium channel subunit Kvβ1.1 decreases the sAHP of hippocampal CA1 neurons and improves learning and memory in three hippocampus-dependent tasks: contextual conditioning, spatial learning, and the social transmission of food preferences. Importantly, young mutants do not show this improvement. Our results demonstrate that modulation of potassium channel function can improve learning and memory specifically in aged mice, and they support the hypothesis that increases in the sAHP contribute to age-related learning and memory deficits.

PUBLICATIONS

Cho Y.H., Friedman E., and Silva A.J. 1998. Ibotenate lesions of the hippocampus impair spatial learning but not contextual fear conditioning in mice. *Behav. Brain Res.* **98:** 77–87.

Cho Y.H., Giese K.P., Tanila H., Silva A.J., and Eichenbaum H. 1998. Abnormal hippocampal spatial representations in αCaMKIIT286A and CREBad- mice. *Science* **279:** 867–869.

Frankland P.W., Cestari V., Filipkowski R.K., McDonald R.J., and Silva A.J. 1998. The hippocampus is essential for context discrimination but not for contextual conditioning. *Behav. Neurosci.* **112,4:** 863–874.

Giese K.P., Fedorov N.B., Filipkowski R.R., and Silva A.J. 1998. Autophosphorylation at threonine 286 of the α calcium-calmodulin-kinase II in LTP and learning. *Science* **279:** 870–873.

Giese K.P., Storm J.F., Reuter D., Fedorov N.B., Shao L.-R., Leicher T., Pongs O., and Silva A.J. 1998. Reduced K+ channel inactivation, spike broadening, and after-hyperpolarization in Kvβ1.1-deficient mice with impaired learning. *Learn. Mem.* **5:** 257–273.

Silva A.J. and Giese K.P. 1998. Gene targeting: A novel window into the biology of learning and memory. In *Neurobiology of learning and memory* (ed. J. Martinez and R. Kesner), pp. 89–142. Academic Press.

Silva A.J., Giese P.K., and Frankland P.W. 1998. Identification of molecular and cellular mechanisms of learning and memory: The role of gene targeting. In *The aging brain* (ed. E. Wang and D.S. Snyder), pp. 68–82. Academic Press.

Silva A.J., Kogan J.H., Frankland P.W., and Kida S. 1998. CREB and memory. *Ann. Rev. Neurosci.* **21:** 127–48.

Silva A.J., Frankland P.W., Kogan J.H., Friedman E., and Fedorov N.B. 1998. Modelling the cognitive impairments associated with NF1. In *Neurofibromatosis type I: From genotype to phenotype* (ed. M. Upadhyaya and D.N Cooper). BIOS Scientific Publishers; Human Molecular Genetics Series.

Silva A.J., Giese K.P., Fedorov N.B., Frankland P.W., and Kogan J.H. 1998. Molecular, cellular, and neuroanatomical substrates of place learning. *Neurobiol. Learn Mem.* **70(1/2):** 44–61.

In Press

Elgersma Y. and Silva A.J. 1999. Molecular mechanisms of synaptic plasticity and memory. *Curr. Neurobiol.* (in press).

Narisawa-Saito M., Silva A.J., Yamaguchi T., Hayashi T. Yamamoto T., and Nawa H. 1999. Growth-factor-mediated Fyn signaling regulates α-amino-3-hydroxy-S-methyl-4-isooxazolepropionic acid (AMPA) receptor expression in rodent neocortical neurons. *Proc. Natl. Acad. Sci.* (in press).

NEOCORTICAL CIRCUITRY AND ITS PLASTICITY

K. Svoboda B. Burbach E. Nimchinsky
B. Lendvai P. O'Brien
Z. Mainen B. Sabatini
M. Maravall E. Stern

The neocortex underlies most cognitive functions in mammals. Even though these functions can be extremely diverse, the anatomy underlying these functions is relatively well conserved. Neocortical tissue is dauntingly complex: 1 μl contains nearly 1 million neurons and more than 1 billion synapses. A single neuron connects to thousands of other neurons. This awesome network underlies our perception of the world. To begin to unravel neocortical function, we are studying how the basic units of this network, namely, neurons and synapses (the connections between neurons), work within the intact network.

We use sensitive tools to monitor neuronal function in intact tissue. Two-photon laser scanning microscopy (TPLSM) allows us to keep track of neuronal excitability at the level of single synapses by measuring intracellular calcium levels at high resolution. Optical stimulation by the uncaging of neurotransmitters allows us to excite subcellular structures with great spatial and temporal control. Intracellular electrical recordings using sharp and whole-cell electrodes allow us to monitor the electrical membrane potential and synaptic currents of neurons. We have also begun to use powerful molecular tools that allow us to perturb signal transduction cascades in neurons in very specific ways. Neurons in juvenile and adult cortex can be infected with viruses or transfected with the gene gun to introduce virtually any transgene of interest.

Our efforts so far have been focused on the function and plasticity of the hardware of the neocortex. Despite the complexity of neocortical tissue, there is some reason to hope that in the foreseeable future (even perhaps our grandchildren's lifetime), it will be possible to understand how neocortical neural networks perform computations. This hope is mainly based on the realization that the neocortex is built up of repeating elemental functional modules, containing relatively few types of neurons and synapses. Thus, it could be that the neocortex is decomposable into a simpler problem, namely, understanding the function of the elemental module. Despite some growing pains, we were able to make progress on a number of fronts during the last year.

Dendritic Morphogenesis Induced by Synaptic Activity in Brain Slices

E. Nimchinsky [in collaboration with
M. Maletic-Savatic and R. Malinow,
Cold Spring Harbor Laboratory]

Coordinated patterns of activity help to organize neural circuits throughout the brain. Relatively little is known about the role of activity in the development of mammalian dendritic morphology. Dendrites, the input side of neurons, develop in a stereotypical sequence: Early after birth, the relatively smooth dendrites of neonates sprout numerous thin filopodia-like protrusions that are later replaced by dendritic spines as the brain matures. Recent studies in cultures from hippocampal area CA1 have shown that dendritic protrusions, including filopodia and spines, are structurally dynamic, perhaps powered by actin motility. These observations led to the hypothesis that dendritic filopodia are crucial in establishing synaptic connections during development, paving the way for the development of mature spines. Activity-dependent dendritic morphogenesis could therefore underlie Hebbian plasticity during development. We used vital imaging of dendrites in living brain slices to directly study dendritic morphogenesis evoked by synaptic activity.

A small number of CA1 pyramidal neurons were infected in cultured hippocampal slices with a neurotropic recombinant Sindbis virus expressing enhanced green fluorescent protein (EGFP). Visualized with a TPLSM, infected neurons showed bright homogeneous fluorescence throughout their dendritic and axonal arbors, revealing detailed morphology with numerous dendritic protrusions. Small dendritic segments labeled with EGFP could be imaged repeatedly at high resolution for hours without signs of photobleaching or phototoxicity. Time-lapse imaging of dendritic segments showed that dendritic morphology is dynamic on time scales of minutes to hours. Protrusions appeared and disappeared or changed shape.

High-frequency focal synaptic stimulation induced a period (~45 minutes) of enhanced growth of small filopodia-like protrusions (<5 μm). Synaptically evoked growth was long-lasting, localized to dendritic regions close (<50 μm) to the stimulating electrode, and was prevented by blockade of NMDA (*N*-methyl-D-aspartic acid) receptors (NMDA-R). Thus, synaptic activation can produce rapid, input-specific changes in dendritic structure. Such persistent structural changes could contribute to the development of neural circuitry. We are now attempting to show that new dendritic filopodia in fact make synapses.

Optical Studies of Single Synapses

Z. Mainen, M. Maravall, B. Sabatini [in collaboration with R. Malinow, Cold Spring Harbor Laboratory]

Does the release of a single vesicle saturate synaptic glutamate receptors? The answer to this simple question is central to an understanding of transmission at central nervous system synapses, as receptor occupancy determines how a sequence of closely spaced action potentials at the presynaptic terminal is transformed into a postsynaptic signal by the synapse: Is the process linear or nonlinear? Moreover, determining the degree of receptor occupancy will shed light on the mechanisms that determine quantal size, synaptic noise, and synaptic plasticity.

In general, the number of neurotransmitter (glutamate) molecules released from a vesicle greatly exceeds the number of postsynaptic receptors. Although experiments using a variety of electrophysiological methods have addressed the issue of receptor saturation, the results have been extremely controversial due to complexities in the interpretation of these indirect experiments. We used 2-photon imaging to measure NMDA-R-mediated [Ca^{++}] transients in single dendritic spines of CA1 pyramidal neurons in hippocampal slices. This approach can be used to discriminate failure and success of transmitter release at an *individual* synapse and to quantify the amount of NMDA-R activation following release (Fig. 1). We tested for saturation of NMDA-Rs by comparing responses evoked by single stimuli with those evoked by pairs of stimuli separated by a short interval. We find that a single release event does not saturate synaptic NMDA-Rs; we calculate that a second release occurring 10 msec later produces more than 80% of the first response. These results suggest that NMDA-Rs are essentially linear detectors of glutamate release. Synaptic NMDA-R [Ca^{++}] transients in individual spines are therefore sensitive to both the number of quanta released by a burst of action potentials and the changes in the concentration profile of glutamate in the synaptic cleft.

Spread of Excitation in Individual Axons

K. Svoboda [in collaboration with L. Cox, SUNY, Stony Brook]

Axons, the output side of neurons, are normally considered to reliably transmit action potentials throughout their arbors, delivering impulses to presynaptic terminals. However, this view was established many years ago in the reflex arc. Essentially nothing is known about the reliability of action potential propagation in the profusely branching axons of the neocortex. We used whole-cell recordings to fill individual neocortical pyramidal neurons in brain slices with the [Ca^{++}] indicator and to elicit somatic action potentials. We then measured action-potential-associated [Ca^{++}] transients in presynaptic terminals distal and proximal to the soma. Under a variety of conditions, we find that action potentials reliably invade the axonal arbors of neocortical pyramidal neurons, with no sign of branchpoint failures.

Modulation of Dendritic Excitation in Neocortex In Vivo

E. Stern, K. Svoboda

In vitro experiments, employing electrophysiological recording and optical imaging of Ca^{++} ion concentration ([Ca^{++}]), have provided increasing evidence that Na^{+} action potentials can, under appropriate conditions, propagate somatofugally into the dendritic tree of cortical pyramidal neurons. Because action potentials can open voltage-sensitive Ca^{++} channels (VSCCs) and relieve the Mg^{++} block of NMDA-Rs, they could play an important part in the control of long-term synaptic plasticity (LTP) and learning and memory. But despite the convenience of the brain slice preparation for studying dendrites, it has become clear that dendritic excitability depends on a variety of factors that are hard to reproduce in vitro. We applied in vivo TPLSM imaging together with sharp electrode intracellular recordings to study dendritic excitability and its modulation in vivo.

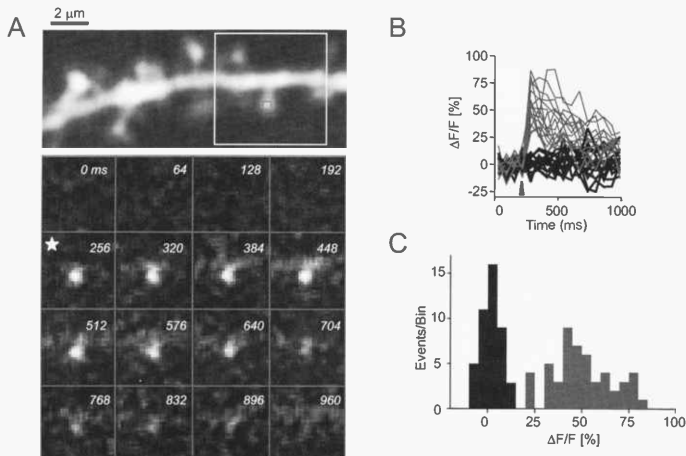


FIGURE 1 Probabilistic synaptic activation of NMDA-Rs monitored using TPLSM to image spine [Ca⁺⁺] transients. (*A, Top*) TPLSM image (single section) of a spiny tertiary apical dendritic branch of a CA1 pyramidal neuron. An active spine (*box in inset*) is indicated. (*Bottom*) Time sequence of frames showing [Ca⁺⁺]-associated fluorescence transients during synaptic activation of one synapse. The stimulus is delivered at the beginning of frame 5 (*star*). The time (in milliseconds) at the beginning of each frame is shown at its top right. (*B*) Sample fluorescence transients from the same spine. (*C*) Histogram of [Ca⁺⁺] transient amplitudes. *Black bars* are classified as release failures; *gray bars* are classified as nonfailures (Z.F. Mainen et al. 1999).

We find that in layer 2/3 pyramids in the barrel cortex in vivo, sodium action-potential-evoked [Ca^{++}] transients are limited to dendritic regions closc to the soma. By directly recording from dendrites, we show that distal dendrites of layer 2/3 cells are electrically excitable in vivo and capable of generating large [Ca^{++}] transicnts. In addition, we observe a strong attenuation and broadening of Na^+ action potentials with distance from the soma. These results demonstrate that the restricted pattern of dendritic [Ca^{++}] transients we observe is due to a suppression of Na^+ action potential propagation into dendrites. Furthermorc, we find that stimulation of subcortical activating systems by tail pinch can enhance sensory-stimulus-induced dendritic [Ca^{++}] transients, consistent with the importance of these activating systems in neocortical plasticity and learning. In the next year, we plan to investigate the effects of activity in neuromodulatory systems that are activated during attentive behaviors, such as Raphe and Nucleus Basalis, on dendritic excitability.

Molecular Basis of Neocortical Plasticity

B. Burbach, K. Svoboda [in collaboration with E. Nedivi, Massachusetts Institute of Technology]

The rodent somatosensory cortex contains a map of the facial whiskers (barrel cortex) that can be visualized using simple stains. The neuronal responses in each barrel-column are dominated by a single principal whisker (PW), with some surrounding contributions from neighboring whiskers. Whisker deprivation changes the responses in the whisker map. For example, trimming all but one whisker leads to potentiation of the spared whisker response and depression of the deprived whisker response in the deprived columns. These effects occur mostly in superficial layers over time scales of days.

Is experience-dependent plasticity in the adult neocortex associated with dendritic morphogenesis? One approach to address this question is to induce plasticity in the barrel cortex by whisker trimming and to look for

up-regulation of genes that are known to regulate dendritic morphology in other systems. One such gene, *cpg15*, has been shown to regulate dendritic growth in the developing retinotectal system in the tadpole. To induce plasticity, all but the D1 vibrissa were removed unilaterally in awake, adolescent mice (age ~4–5 weeks) for a period from 3 hours to 7 days. We subsequently measured the spatial and temporal distribution of *cpg15* expression by in situ hybridization. Induction of experience-dependent plasticity is associated with a transient change in the expression pattern of *cpg15*.

We find that (1) the barrel-column corresponding to the spared whisker (D1) shows enhanced *cpg15* expression, (2) the barrel-columns corresponding to the deprived whiskers show reduced *cpg15* expression, (3) changes in *cpg15* mRNA level first appear in layer 4, peak at about 12 hours, and then decline rapidly. In layer 2/3, changes in *cpg15* expression appear later, peak at approximately 24 hours, and persist for days. These findings suggest that experience-dependent plasticity is associated with morphological rearrangements in dendrites.

Morphological Basis of Experience-dependent Plasticity In Vivo

B. Lendvai, B. Burbach, K. Svoboda

During the last year, we have developed a method to measure the dynamics of dendrites and their spines in vivo using a combination of TPLSM and viral transfection of neurons with the gene for EGFP. This method allows us to characterize dendritic and spine morphology and dynamics with micrometer-level spatial resolution and high temporal resolution (approximately seconds). Most of our experiments were done in rats between postnatal days 8 and 27. The goal of our initial study was to characterize spine dynamics and morphology as a function of developmental age and to look at the effects of sensory experience on spine dynamics. Very little is known about dendritic dynamics in this system. In addition, these data are crucial to characterize the morphological effects of mutations (such as knockouts of the *FMR1* gene) that are thought to affect dendritic motility. Consistent with our brain slice experiments, spines and dendrites of neocortical pyramidal cells of layer 2/3 rapidly changed shape. Perhaps most exciting, we were able to modulate dendritic dynamics by changing sensory experience. For example, clipping the rat's whiskers for 2 days prior to imaging drastically reduced dendritic dynamics.

Instrumentation

K. Svoboda, P. O'Brien

Our instrumentation efforts during the last year have focused on getting several imaging/electrophysiology rigs up and running. First, we built a microscope that is optimized for TPLSM imaging together with intracellular recording in the intact brain. This microscope implements a new hanging design that allows placement of instruments and manipulators all around the preparation. Signal collection is optimized to allow imaging as deep in the brain as possible. We think that this instrument will allow in vivo cellular physiology at an unprecedented level or precision. Second, we adapted a commercial confocal microscope (generously donated by Olympus) for multiphoton multicolor imaging of GFP and its variants in brain slices. Third, we produced essentially a replica of our first instrument for brain slice physiology and imaging in brain slices. The major difference is that we developed an A/D board that allows us to acquire multiple fluorescence wavelengths simultaneously.

PUBLICATIONS

Svoboda K. 1998. Shining light on spiny matters. *Nature Neurosci.* **1:** 93–94.

In Press

Mainen Z. and Svoboda K. 1999. Synaptic [Ca^{2+}]: Intra-cellular stores spill their guts. *Neuron* (in press).

Mainen Z.F., Malinow R., and Svoboda K. 1999. Synaptic [Ca^{2+}] transients in single spines indicate NMDA receptors are not saturated. *Nature* (in press).

Mainen Z.F., Maletic-Savatic M., Shi S.H., Hayashi Y., Malinow R., and Svoboda K.. 1999. Two-photon imaging in living brain slices. *Methods* (in press).

Maletic-Savatic M., Malinow R., and Svoboda K. 1999. Rapid dendritic morphogenesis in CA1 hippocampal dendrites induced by synaptic activity. *Science* (in press).

Svoboda K., Helmchen F., Denk W., and Tank D.W. 1999. The spread of dendritic excitation in layer 2/3 pyramidal neurons in rat barrel cortex *in vivo*. *Nature Neurosci.* **2:** 65–73.

Svoboda K., Tank D.W., Stepnoski R., and Denk W. 1999. Two-photon imaging of neuronal function in neocortex in vivo. In *Imaging living cells.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. (In press.)

MOLECULAR NEUROBIOLOGY OF LEARNING AND MEMORY IN *DROSOPHILA*

T. Tully C. Alexander, T. Bridges (URP), J. Christensen, J. Connolly, J. DeZazzo, J. DiLeo, J. Dubnau, R. Gasperini, T. Gill, S. Gossweiler, L. Grady, M. Hannan, R. Jones, H. Li, J. Medalle, S. Pinto, M. Rahimzadeh, M. Regulski, M. Saitoe, J. Salvatore, P. Smith, K. Velinzon, S. Xia, L. Zhang

Two components of our efforts this year have been factory-like. With the successful implementation of RoboTrainers, our "Hartford screen" for new mutants that disrupt learning/memory would be admired by Henry Ford himself. "Hartford" now functions well as a "high-throughput" behavioral screen. With Helicon Therapeutics, we have begun to screen the Roche library of 160,000 drug compounds for their abilities to enhance long-term memory (LTM) formation. Initial experiments have yielded promising results. Together, these brute-force projects will ensure the continued contribution of *Drosophila* to our understanding of human cognitive dysfunction.

Molecular Search for CREB-dependent LTM Genes

T. Tully, J. Dubnau, J. Medalle, S. Gossweiler, J. Salvatore, T. Gill [in collaboration with Helicon Therapeutics, Inc.]

Our collaborations with Helicon Therapeutics, Inc. have afforded us access to Affymetrix DNA chip technology. Roche/Affymetrix has produced a *Drosophila* chip for our group that contains all 1500 known, cloned genes, approximately 10% of the fly genome. To these chips, we have hybridized probes derived from RNA transcripts expressed in adult fly heads 24 hours after they were subjected to one of two training protocols: spaced training (which induces CREB-dependent LTM), massed training (which produces learning and early memory levels similar to those produced by spaced training but which does not induce CREB-dependent memory). In this manner, candidate memory genes (CMGs) are sought that are differentially expressed during LTM formation. Preliminary experiments indicate that the signal to noise of such differentially expressed genes is at the limit of detection, often only a twofold effect. Hence, we have been forced to apply basic statistical methods to detect these weak signals more reliably.

By increasing the sample size of chips hybridized to particular probes and by transforming the raw data, we were able to apply parametric statistics to detect differential transcript levels. From this first time point during LTM formation, we have identified 66 CMGs, 75% of which also show signal differences in follow-up Northern blot analyses. These results demonstrate the first functional genomic response to LTM formation.

Identification of New Genes Involved with Associative Learning

T. Tully, S. Pinto, J. Christensen, T. Bridges, J. DeZazzo, K. Velinzon, M. Saitoe, P. Smith, M. Rahimzadeh, C. Alexander, M. Hannan, H. Li, L. Zhang, L. Grady [in collaboration with M. Ramaswami, University of Arizona, and with A. Dutta, Brigham and Women's Hospital and Harvard Medical School]

After cloning the *latheo* transcription unit and identifying a human homolog, we submitted the information into the public database. This contribution precipitated the identification of one function of *latheo*. A. Dutta's group at Brigham and Women's Hospital discovered that human LAT (HsLAT) appears to participate in the origin recognition complex (ORC) during DNA replication. Given this observation, they want to show that *Drosophila* LAT (DmLAT) associates with DmORC2. We showed that *latheo* null mutants die as pupae, because normal cell proliferation stops during the third instar stage and adult tissues fail to develop.

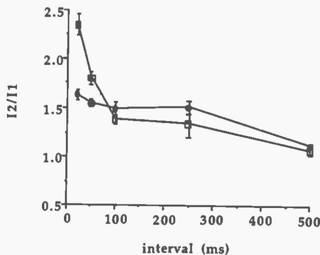


FIGURE 1 *linotte* mutants produce a defect in paired-pulse facilitation at the larval neuromuscular junction. (*Closed circles*) *lio*[1] mutants; (*open squares*) wild-type third instar larvae.

Strikingly, these phenotypic defects are similar to those produced by lethal mutations of DmORC2.

Our attempts to identify the biochemical nature of *linotte* remain elusive. Toward that end, however, we have begun to study synaptic function at the larval neuromuscular junction (lnmj) in *linotte* mutants, as we have in the past for *latheo*. Interestingly, *linotte* mutants produce functional defects in synaptic plasticity (see Fig. 1), which are rescued inducibly by an *hs-lio*$^+$ transgene.

In collaboration with M. Ramaswami's group, we also have identified a defect at the lnmj in *nalyot* mutants. This time, however, the defect does not lie in synaptic function but rather in synaptic structure. Synaptic contacts onto the muscle are modulated in direct correspondence to levels of expression of the *nalyot* gene (which itself corresponds to *Adf1*). To the extent that *Adf1*-dependent synaptogenesis has a role in adult behavioral plasticity, we presently will evaluate LTM formation in *Adf1* transgenic flies. Such experiments on *emc* transgenic flies, for instance, suggest no role for this negative regulator of transcription (which was identified as *golovan* mutants) in adult memory formation.

Finally, our new Hartford mutagenesis is progressing well. With the development and installation of the RoboTrainers, wc already have screened about 2500 P-element transposant lines for possible defects in 1-day memory retention. At this rate, we expect to identify up to a dozen new learning/memory mutants by the end of next year.

Characterization of Extant Mutants

T. Tully, M. Regulski, J. DeZazzo, S. Xia

After teaming up with U. Heberlein's group (now at University of California, San Francisco) to show that *amnesiac* mutants disrupt via cAMP signaling the sensitivity of adults to ethanol, we turned our efforts toward a rescue of *amnesiac*'s memory defect with *amn*$^+$ transgenes. In this, we succeeded. Surprisingly, however, we found that expression of *amn*$^+$ during development is sufficient to rescue the adult memory defect. This observation differs from that for alcohol sensitivity, where cxpression of *amn*$^+$ in the adult is sufficient to rescue the phenotypic defect. Given the fact that *amnesiac* is known to encode a precursor protein for one or more neuropeptides, these results suggest multiple (pleiotropic) roles for *amnesiac* in ncurodevelopment and function—a now recurring theme in *Drosophila* neurogenetics.

We refuse to give up on our attcmpts to show a role for nitric oxide (NO) in fruit fly memory formation. To date, our attempts to modulate NO synthase (NOS) function via genetic methods have failed. Hence, we turned our attention (temporarily) to more traditional pharmacology. We have developed a reliable enzymatic assay of NOS activity that can be used on adult head homogenates. With this assay, we have determined the concentrations of NOS antagonists that are sufficient, when fed to flies, to inhibit NOS activity in the brains by more than 90%. With this knowledge, we now are prepared to return to behavioral evaluations of NO during memory formation.

The Virus Hypothesis

Our entire group [in collaboration with P. Wendel in B. Stillman's group and T. Howard in the EM facility]

To a moderate degree, our behavioral experiments have been hampered by a general degradation of the health of our fly colony. This pathology first revealed itself as a significant reduction in the longevity of adult flies. Moreover, adult flies that were still alive 7 days after training (which is when we prefer to measure LTM) appeared sluggish, thereby producing a general reduction in performance in our memory

assays. More seriously, the inducible effects on learning/memory of four of our transgenic lines (hs-CREB-a, hs-CREB-b, hs-*lio*, and hs-*lat*) disappeared.

In response to this unanticipated problem, we initially spent several months systematically investigating which of dozens of variables (technical skills of new experimenters, mechanics of RoboTrainers, odor qualities, food qualities, etc.) might be responsible for this health problem. In the end, our usual culprits were not implicated, thereby forcing us to do some serious biological detective work. Antibiotics, fungicides (which normally are in our food anyway), and antiparasitics did not improve the health of our fly cultures. We noticed, however, a high incidence of dead "black pupae" in our culture bottles. Perusal of the literature suggested that this might be caused by a viral infection and that bleaching fly eggs might kill such virus. Accordingly, an initial bleaching of eggs temporarily reduced the incidence of black pupae but did not yield the return of our transgenic effects.

In the meantime, we searched outside sources to no avail for a wild-type strain that was not infected. These stocks either arrived at CSHL with a viral infection or they were infected rapidly once here. Sensitized to our dilemna, other fly behaviorists now also have noticed black pupae in their stocks, and the presence of such is coincident with reduced performance levels in their (different) learning assays.

We have focused on the behavioral evaluation of transgenes, the expression of which is driven by promoters other than heat shock. We previously had published on the abolition of olfactory learning by overexpressing a dominant-negative G protein via a yeast *GAL4*-driven promoter. In infected flies, this transgene still works to block olfactory learning. We constructed a lat^+ transgene, the expression of which was controlled by its own 5′ genomic regulatory region. In infected flies, the learning defect of *lat* mutants is rescued by this "genomic" transgene, in contrast to our recent attempts to rescue the learning defect with the inducible $hs\text{-}lat^+$ transgene. Taken together, these results suggest that the putative viral infection affects transiently expressed transgenic transcripts more than chronically expressed transgenic constructs.

Further search of the literature turned our attention to a family of RNA viruses that suppress host translation. In particular, infections by the *Drosophila* C virus (DCV) strain block translation of heat shock proteins in response to heat shock, precisely the cellular mechanism through which hs-driven transgenes are expressed. Fortunately, DCV has recently been cloned by P. Christian's group in Australia. Subsequent electron microscopic studies detected the presence of DCV in infected cultured fly cells (morphologically confirmed by P. Christian), and reverse transcriptase–polymerase chain reaction (RT-PCR) detected DCV in infected fly stocks.

Currently, we are using our RT-PCR protocol to evaluate the effectiveness of egg bleaching in eradicating DCV from infected stocks. From this, we have established that published egg-bleaching protocols are insufficient to kill all DCV, thereby leading to an eventual return of the virus in bleached stocks. Once an effective way to disinfect our stocks is worked out, we finally will be able to do the most definitive experiment: We will infect a transgenic stock (CREB, *lio* or *lat*) with DCV or not (sham-infection) and determine whether the behavioral effects of transgenic expression are blocked specifically by the presence of virus. Such a demonstration will presage the return of our beloved behavioral productivity.

PUBLICATIONS

Connolly J.B. and Tully T. 1998. Behaviour, learning and memory. In Drosophila: *A practical approach*, second edition (ed. D.B. Roberts), pp. 265–318. Oxford University Press, Oxford.

Connolly J.B. and Tully T. 1998. Integrins: A role for adhesion molecules in olfactory memory. *Curr. Biol.* **8:** R386–389.

Dubnau J. and Tully T. 1998. Gene discovery in *Drosophila:* New insights for learning and memory. *Annu. Rev. Neurosci.* **21:** 407–444.

Moore M.S., DeZazzo J., Luk A.Y., Tully T., Singh C.M., and Heberlein U. 1998. Ethanol intoxication in *Drosophila:* Genetic and pharmacological evidence for regulation by the cAMP signaling pathway. *Cell* **93:** 997–1007.

Tully T. 1998. Toward a molecular biology of memory: The light's coming on! *Nature Neurosci.* **1:** 543–545.

LONG-TERM MEMORY FORMATION IN *DROSOPHILA*

J. Yin M. Cowan, E. Drier, E. Friedman, R. Filipkowski, J. Horiuchi, W. Jiang, S. Kourpanidies, K. LaVine, Z. Lin, J. Nagel, M. Stebbins, M. Tello, P. Wu, H. Zhou

Subcellular Localization of dCREB2 Proteins

M. Tello, M. Belvin, H. Zhou, M. Cowan

When *Drosophila* head extracts are biochemically fractionated, both endogenous and transgenic dCREB2 proteins partition differentially between the nuclear and cytoplasmic fractions. The dCREB2-b isoform, whose induction totally blocks the formation of long-term memory (LTM), contains a nearly consensus nuclear localization signal (NLS) in its basic region. This isoform, whether endogenous or transgenic, always appears to be greatly enriched in the cytoplasmic fraction. In contrast, the dCREB2-a isoform, which also contains the identical NLS, is more enriched in the nuclear fraction. A similar pattern of distribution is seen when the two proteins are induced in yeast. Tissue immunocytochemistry on adult photoreceptor cells shows an enrichment for induced dCREB2-b in the cytoplasm relative to that of dCREB2-a.

We are currently trying to determine whether a mutant dCREB2-b protein that contains a mutant NLS, and is totally cytoplasmic, can still block LTM formation. Cell-based studies are under way to determine if the NLS sequence is necessary and sufficient for nuclear localization. We are also trying to determine which sequences in dCREB2-b are required for cytoplasmic partitioning, in anticipation of determining if the protein can mask its own NLS or is being tethered in the cytoplasm by another protein.

Characterization of *S162*, a Mutation in the *dCREB2* Gene

M. Belvin, H. Zhou, M. Tello

S162 is a recessive, hemizygous, larval lethal mutation that co-maps genetically and cytologically to 17A-B on the X chromosome. Mutant escaper males normally survive at less than 0.5% frequency. When the *dCREB2-b* transgene is crossed into the mutant background, rescued males survive at about 0.5% of the normal frequency, and a daily heat shock induction of the transgene during development results in about 50% survival. Western blot analysis of proteins from escaper males shows that the predominant dCREB2 doublet, which is normally approximately 40–45 kD in size, appears smaller (33–37 kD). We are currently trying to isolate and characterize the mutant proteins, as well as to locate and sequence the chromosomal mutations.

S162 escaper males have a shortened circadian locomotor period of about 22.8 hours (as compared with the normal 24 hours). When Per protein is overproduced by mutation, it results in a shorter locomotor period. Consistent with these observations, the Per protein is overproduced in the *S162* mutant background. A molecular understanding of how dCREB2 regulates *per* transcription may lead to a better functional picture of the *S162* mutation in terms of its effect on the activator/blocker ratio.

dCREB2 Is a Component of the Circadian Clock

M. Belvin, H. Zhou

Transgenic flies that contain CRE-TATA-luciferase reporters show circadian rhythmicity in reporter activity, with two peaks every 24-hour period. This activity is regulated by the *dCREB2* gene, since it is abolished in the *S162* mutant background. The reporter is also CRE-dependent, since mutations in the consensus CRE sites destroy cyclical transcription. Thus, these reporter flies reflect dCREB2 activity. Although dCREB2 activity cycles with two peaks in reporter

activity across a 24-hour period, there is no detectable cycling of any dCREB2 proteins, nor is there any cycling of (the fly equivalent to) Ser-133 phosphorylation. We also do not detect any change in the cytoplasmic localization of dCREB2-b across a circadian cycle.

The *dCREB2* gene directly affects the circadian clock. *S162* mutant flies have a shortened locomotor period (22.8 hours), which can be rescued by induction of a *dCREB2-d* transgene. dCREB2 also affects *per* expression. When *per* reporter flies are examined in the *S162* backgroud, they show diminished cycling. A *per* transcriptional fusion shows stronger effects than a *per* promoter and protein fusion, consistent with the interpretation that there are transcriptional and posttranscriptional mechanisms that contribute to Per cycling. When Per protein is examined in the *S162* background, it has an altered cycling pattern, with less degradation of Per during the trough periods (morning). Paradoxically, there is also an apparent increase in Per protein, although the *per* reporters show decreased transcription in *S162*, suggesting that a posttranscriptional compensatory mechanism exists. Since *per* also affects the dCREB2 reporters, these two genes, *per* and *dCREB2*, affect each other, forming a complex feedback loop.

scs and *scs´* Function as Insulators

M. Belvin, J. Wallach, H. Zhou

We have made a series of insulated and uninsulated CRE-TATA-*lacZ* and 3xCRE-TATA-luciferase transgenic reporter flies. Quantitatively, the insulated transgenic reporters cluster much more closely as a group with respect to their levels of luciferase activity, when compared with the uninsulated lines. In addition, on average, the uninsulated lines have lower levels of expression. Therefore, *scs* and *scs´* are capable of protecting transgenes from the effects of random insertion, which tend to decrease expression. Since the lines all show circadian rhythmicity in their transcriptional activity, insulators do not disrupt transcriptional regulation of the intervening DNA. Spatially, all of the *lacZ* lines have similar patterns of expression in the adult head. As the number of CRE sites increases, insulated lines tend to show staining in new areas of the adult head, in addition to retaining the basal pattern of staining.

Role of Phosphorylation in Controlling dCREB2 Activity

W. Jiang, J. Horiuchi, H. Zhou

CREB family molecules from the honeybee, *Aplysia*, *Drosophila*, chicken, mouse, rat, and humans show a high degree at conservation in eight putative phosphorylation sites. We are interested in the possible roles these sites have in dCREB2 function. One important role to which some of these sites contribute is DNA binding. Both in vivo and in vitro, we detect effects of phosphorylation on the binding properties of the proteins. We are currently trying to determine which physiologies affect which sites, as well as what signaling pathways are involved. Ultimately, we are interested in how phosphorylation and dephosphorylation are modulated during neuronal activity and memory formation, and how these different sites affect biochemical and cellular properties of the dCREB2 proteins. We anticipate that these posttranslational events are directly related to behavioral properties of long-term memory formation, such as the requirement for repetitive training trials and the need for rest intervals (spaced training).

Subcellular Ablation of Protein Activity

E. Drier

We are interested in further extending spatial (anatomical) and temporal inhibition of protein function by tethering dominant-negative molecules to certain parts of cells. Our approaches will be initially tested in flies using Gal4 to spatially (anatomically) limit expression of the inhibiting molecules. We will then integrate these methods with the tetracycline system, allowing temporal control of induction. These approaches will ultimately be applied to the study of molecules whose subcellular location, and movement, is postulated to be important for the processes of learning and memory formation.

LTM Formation in Flies

E. Drier, M. Cowan [in collaboration with T. Tully, Cold Spring Harbor Laboratory]

We are continuing to characterize the viral infection of our flies, which is one of the likely sources of our

behavioral problems. Reverse transcriptase–polymerase chain reaction (RT-PCR) analysis, combined with amplification of virus in cell culture, shows that we have an infection of at least the DCV virus. Western blot analysis indicates that badly infected fly stocks show dramatic decreases in heat-shock-inducible transgene expression. "Koch's" postulates type experiments show that transmission of the virus-containing supernatant is lethal and likely to cause behavioral defecits, especially for long-term memory formation. We have developed methodology for keeping viral titers very low, and these flies seem to exhibit *dCREB2* transgene-responsive effects on long-term memory formation (blocking and enhancement).

Amplifying RNA for Analysis

J. Horiuchi [in collaboration with R. Malinow, Cold Spring Harbor Laboratory]

We are investigating the feasibility of amplifying small amounts of RNA for subsequent analysis using microarray techniques. Claims have been made that various PCR- and in-vitro-transcription-based techniques can amplify with little bias. We are using RNA isolated from hippocampal slices as a test case for various approaches.

Microarray Analysis of Gene Expression during Mouse Memory Formation

R. Filipkowski, S. Kourpanidies, J. Nagel, E. Freedman [in collaboration with T. Tully, Cold Spring Harbor Laboratory]

Mice are trained in a variety of behavioral tasks (fear conditioning, the Morris Water Maze, the acoustic startle response) that produce long-lasting memory. These mice and a closely matched behavioral control (where the mice are subjected to the same stimuli, but do not produce long-lasting memory) are used as sources of RNA for complex probe preparations and microarray analysis. Much of the difficulty with this type of analysis is obtaining the proper control group.

Transgenic Reporter Mice

R. Filipkowski, J. Nagel, S. Kourpanidies, E. Friedman, J. Wallach [in collaboration with K. Svoboda, Cold Spring Harbor Laboratory], P. Wu

Transgenic CRE-TATA-*lacZ* reporter mice have been analyzed for changes in gene expression in response to a variety of behavioral tasks. Tissue histochemistry has been used to examine appropriate brain tissue sections from mice which have long-lasting memory of cued and contextual fear conditioning and conditioned taste aversion. No training-induced changes in histochemical staining have been detected. Because of the high background staining in this mouse line, we have made new transgenic reporter lines that contain a destabilized enhanced green fluorescent protein (EGFP) reporter moiety. If any of the new lines are promising, we will eventually use fiber-optic-based techniques to try to image changes in gene expression in vivo. Finally, we are beginning to generate transgenic mice for use in molecular and behavioral analysis.

Tetracycline-inducible Gene Expression

M. Stebbins, Z. Lin, W. Jiang, P. Wu

We are continuing to characterize our modified tetracycline-inducible system. In *Drosophila*, both the "tet on" (add drug to induce target gene expression) and the "tet off" (withdraw drug) systems appear to work quite well. When the *trans*-activator protein is driven by the ubiquitously expressed actin promoter, inducible gene expression can be detected on Western blots. The *trans*-activator can also be driven using the Gal4 system, allowing spatially delimited expression of the *trans*-activator. Target gene induction is very robust with the "tet off" system and quite good with the "tet on" system. We are evaluating whether the target protein is appropriately spatially limited in its expression pattern. This would mean that spatial and temporal induction of target genes can be achieved in *Drosophila*.

In mice, despite detectable "tet on" *trans*-activator expression in the hippocampus and cortex, target gene induction is poor. We are comparing the fly and mouse systems to understand what is responsible for the functional differences. Candidate factors include differences in the degradation pattern of the *trans*-activator protein and competition for target site binding by endogenous proteins.

NEURAL BASIS OF LEARNING AND MEMORY IN *DROSOPHILA*

Y. Zhong N. Wright Y. Wang J. Tong
F. Hannan J. An K. Weinreich
H.-F. Guo

We are interested in the neural basis of learning and memory, and our research is currently following two paths. First, we have developed the first preparation that enables optical recordings of neural activity from the living *Drosophila* brain, which has allowed us to investigate problems related to sensory representation, perception, and learning. Second, we are establishing *Drosophila* models for studying genes involved in human neuro-disorders that impair learning and memory. In particular, we are investigating functions of the neurofibromatosis 1 (NF1) gene and Aβ peptides. NF1 mutations cause a neurogenetic disorder identified by neurofibromas and other symptoms including learning defects. Accumulation of insoluble Aβ has been suggested as a key event in the pathogenesis of Alzheimer's disease. 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. The specific projects are described below.

Role of the NF1-regulated Rutabaga-encoded Adenylyl Cyclase Pathway in *Drosophila* Learning

H.-F. Guo, J. Tong, F. Hannan

The tumor suppressor gene Nf1 encodes a large protein containing a fragment homologous to Ras-specific GTPase-activating proteins (Ras-GAPs), which inhibit Ras activity. In addition to neurofibromas and other symptoms, about 40% of children with NF1 exhibit specific learning defects. Similarly, tumorigenesis and learning defects have also been reported in the mouse Nf1 knockout mutant. To gain insights into how NF1 affects learning in human patients and mouse mutants, we examined the performance of *Drosophila* NF1 mutants in a classical conditioning task.

Drosophila NF1 is highly conserved since 60% of its 2803 amino acids are identical to human NF1. In addition to functioning as a Ras-GAP, substantial but indirect evidence obtained from our previous studies of two *Drosophila* NF1 mutant phenotypes of a defective neuropeptide response and small body size have suggested that NF1 regulates activation of *rutabaga* (*rut*)-encoded adenylyl cyclase (AC). Because *rut* was isolated as a learning and short-term memory mutant (8,9), we were led to pursue the hypothesis that NF1 may affect learning by its control of the activation of Rut-AC/cAMP pathway.

Two mutant alleles showed learning and short-term memory defects. We demonstrated that this deficit does not arise from a developmental abnormality and that NF1 is so crucial to learning that the performance of NF1 mutants is improved in proportion to the amount of NF1 expressed by acute induction of the transgene (Fig. 1). Further behavioral analysis implies that NF1 affects the learning process via its control of the Rut-AC/cAMP signaling pathway. Direct evidence from biochemical assays shows, for the first time, that G-protein-stimulated adenylyl cyclase activity consists of two components, i.e., NF1-dependent and NF1-independent. The NF1-dependent activity is largely mediated via the *rut*-encoded adenylyl cyclase (Fig. 2).

Age-dependent Learning Defect Induced by Induced Expression of Aβ42

J. Tong, H.-F. Guo, F. Hannan, K. Weinreich

Alzheimer's disease (AD) is characterized by amyloid plaques, neurofibrillary tangles, dementia, and age-dependent memory loss. Although pathogenic mechanisms remain to be fully established, a widely supported hypothesis is that extracellular deposition of Aβ42 and Aβ43 derived from β-amyloid precursor proteins (APPs) in senile plaques may be a critical pathogenic event. Expression of Aβ42 or mutant APPs that elevate production of Aβ42 in transgenic mice has been associated with some AD neuropathogenic features (Games et al., 1995; Hisao et al., 1996). Yet events downstream from Aβ deposition that lead to neurodegeneration and impaired memory remain elusive. If flies can provide an AD model, it will greatly facilitate the testing of hypotheses, discovery of down- stream molecules, and screening for drugs because of the short life span and genetic tools available in flies. Our preliminary results indicate that induced expression of

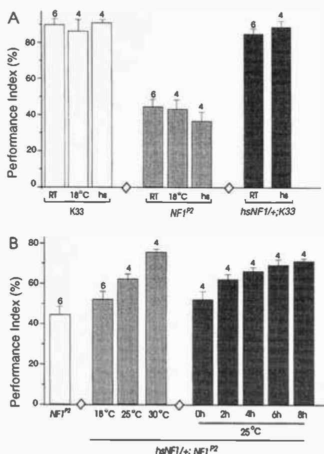


FIGURE 1 Rescue of NF1 learning defect by inducible expression of the normal NF1 gene. (*A*) Effects of heat shock on learning performance. The heatshock (hs) treatment that was used to induce expression of the NF1 transgene (see *B*) did not affect learning in the controls (K33) and mutant (*NF1*^{P2}). In addition, learning was also not affected by induced overexpression of the NF1 transgene in the control background (*hsNF1/+; K33*). (*B*) Rescue of the learning defect by induced expression of the NF1 transgene. At room temperature, a substantial improvement of learning performance was observed in *hsNF1/+; NF1*^{P2} probably due to leaky expression of hsNF1. Thus, flies were shifted from room temperature to 18°C for overnight to minimize the leaky expression of the transgene. In the first group, the flies were moved from 18°C to 25°C or 30°C for 2 hr before the learning test. The higher temperature produced a better learning score (p <0.05, Turkey Kramer HSD). In the second group, flies were shifted from 18°C to 25°C for 0, 2, 4, 6, and 8 hr, respectively. Significant differences began to show after flies were shifted to 25°C for 2 hr (p <0.05 compared to that of 18°C). The numbers of performance indices (PIs) for each group are indicated on top of the error bars.

human Aβ42 in flies caused an age-dependent decline in learning ability.

APP is a type I integral membrane glycoprotein that is subject to alternative proteolytic pathways. Although conserved structurally, the physiological function of APP remains to be identified. Aβ peptides are generated by endoproteolytic cleavage of APP by β- and γ-secretase activities. About 90% of secreted Aβ peptides are soluble Aβ40, whereas about 10% are Aβ42 and Aβ43 which are highly fibrillogenic, readily aggregated, and neurotoxic (Price and Sisodia 1998). It is the deposition of Aβ42 and Aβ43 in senile plaques that has been implicated in AD pathogenesis. Aβ42 secretion and deposition have been observed in *Caenorhabditis elegans* expressing a minigene in which an Aβ42 fragment with an artificial stop codon is linked to an artificial signal peptide. We have made transgenic flies carrying this minigene under the control of a heat shock promoter (hs-Aβ42). Induced expression of Aβ42 did not reduce learning and memory scores in young flies (2–4-day-old adult flies) (not shown), but drastically reduced learning scores in older flies (30 days old, maximum life span under these conditions is about 50–60 days) (Fig. 3). We are examining whether other AD neuropathogenic features can be observed in these flies.

Ensemble Coding and Odor Perception: Ca^{++} Imaging of Odor-evoked Neural Activity in the MB of the Living *Drosophila*

Y. Wang, N. Wright, J. An, H.-F. Guo

Drosophila has been a powerful genetic model for dissecting the molecular basis of learning and memory, but it has fallen short for studying the physiology of learning and memory because of the small size of the brain and central nervous system (CNS) neurons. It has long been our intention to find a preparation that will allow us to directly examine the *Drosophila* CNS. In this study, neural-activity-dependent changes in intracellular Ca^{++} concentrations were taken as an indicator of neural activity, which has been successfully used in studying odor-induced responses in the honeybee antennal lobe and zebrafish olfactory bulb. The recording of Ca^{++} imaging in fact turns the small size of *Drosophila* into an advantage because we can take a close look at the whole mushroom body (MB), a brain region essential for olfactory learning in flies. We show here that in combination with mutant analyses, Ca^{++} imaging of the living *Drosophila* MB, the secondary relay of the olfactory CNS, provides an ideal model for visualizing ensemble coding and revealing its biological significance because of its simple structure and because ensemble coding has a more central role at this high level of the CNS. It is demonstrated that the quality and intensity of an odor are represented not by simple amplitude of neural activity, but by a specific pattern of highly distributed neural activity. For the first time, this study demonstrates that the spatial representation of neural activity can be disrupted by mutations without affecting the overall activity level, but in close association with a behavioral defect. It is suggested that the odorant and concentration-specific activity pattern observed in the MB encodes attractiveness of an odor. On the basis of this understanding of olfactory coding in

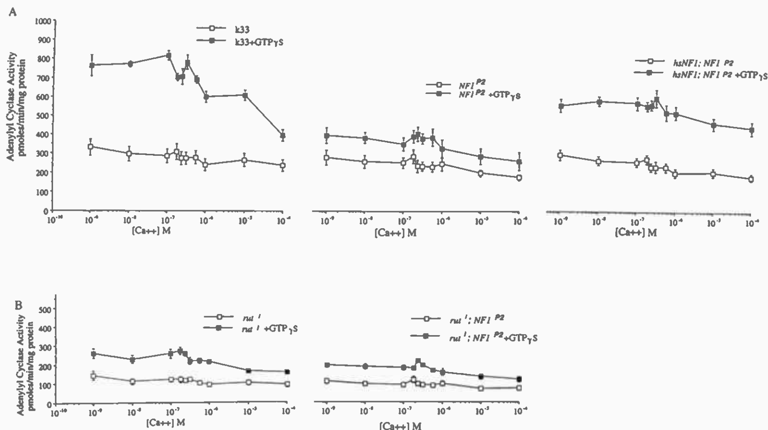


FIGURE 2 Biochemical assay of the NF1 effect on AC activity. (*A*) Reduction in GTPγS-stimulated AC activity in NF1 mutants. The procedure for the AC assay was modified to amplify the NF1 effect. To minimize variation, each experiment provided one set of results including all data points presented in Fig. 3*A*. Each data point (mean ± S.E.M.) is averaged from four independent experiments. To be comparable to the rescuing experiment, all flies were subjected to heat shock treatment (for 2 hr at 35°C and 1 hr rest at room temperature). All curves have the same scales as indicated. (*B*) Diminished NF1 effect on AC activity in the *rut* mutant background. To be comparable, flies were also subjected the same heat shock treatment as those in *A*. Each data point (mean ± S.E.M., the error bar may not be seen in some data points because it is smaller than the square symbol) is averaged from three independent experiments. The scale is the same for all curves as well as the same as that used in *A*.

MB, we are beginning to investigate how such coding is modified during olfactory learning and hope to gain insights into the neural basis of learning.

Two-hybrid Screening of NF1 Targets That Mediate the NF1-dependent cAMP Pathway

F. Hannan

Because a yeast homolog of NF1, IRA, has been proposed to bind to the yeast adenylyl cyclase and Ras to mediate Ras-dependent cAMP synthesis, as well as indications from electrophysiological data that NF1 may regulate activation of *rut*-encoded adenylyl cyclase, we examined the possibility that the NF1 protein binds to Rut-AC by yeast two-hybrid analysis. Various combinations of fragments of the coding region of the two genes were cloned into vectors to test their interactions, but none were found to give a positive result. Thus, we turned to the second scenario in which NF1 may interact with adenylyl cyclase via an intermediate. We are now preparing to use the yeast two-hybrid system for screening potential targets of NF1 from a fly cDNA library.

PUBLICATIONS

Wright N. J. D. and Zhong Y. 1998. Serotonin-sensitive channel in *Drosophila* central neurons. *J. Neurobiol.* **34:** 83–95.

In Press

Hannan F. and Zhong Y. 1999. Second messenger systems underlying plasticity at the neuromuscular junction. In *Neuromuscular junctions in* Drosophila: *An introduction* (ed. V. Budnik and S.S. Gramates). Academic Press. (In press.)

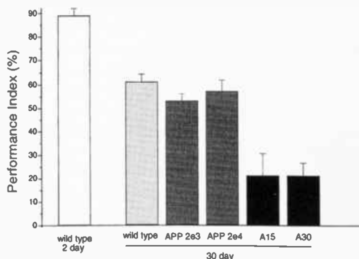


FIGURE 3 Age-dependent learning defect in Aβ42 transgenic flies. Impaired learning in old A15 and A30 flies (two independently isolated heat shock promoter-controlled Aβ42 transgenic flies). The test was taken at day 30. $n \geq 5$ for each genotype.

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 choice. Fellows are provided with a salary, research support, and technical assistance so that they can accomplish their goals free from other distractions. The interaction among research groups at the Laboratory and the program of courses and meetings on diverse topics in molecular biology contribute to a research environment that is ideal for innovative science by these Fellows.

Previous Cold Spring Harbor Fellows, Dr. Adrian Krainer (1987), Dr. Ueli Grossniklaus (1994), and Dr. Scott W. Lowe (1995), are currently members of the faculty at the Laboratory. Dr. David Barford (1991) left in 1994 to go to Oxford University (Laboratory of Molecular Biophysics) as a university lecturer. Dr. Eric Richards (1989) is currently Assistant Professor of Biology at Washington University. Dr. Carol Greider (1988) left after 9 years at the Laboratory to accept a position as Associate Professor in the Department of Molecular Biology and Genetics at Johns Hopkins University School of Medicine. Dr. Marja Timmermans joined us from Yale University in 1998.

PLANT DEVELOPMENTAL GENETICS

M. Timmermans P. Vahab

Research in our laboratory is aimed at understanding the molecular basis of axis specification and pattern formation during lateral organ development in plants. Organogenesis in higher plants continues beyond the period of embryogenesis, and lateral organs of the shoot develop progressively from small groups of stem cells named meristems. A family of homeobox genes, the *Knox* genes, is expressed in the shoot apical meristem and is required for the self-maintenance of the tissue. Lateral organs such as leaves are initiated by the recruitment of founder cells, which is facilitated by the down-regulation of *Knotted1* (*Kn1*) homeobox gene expression in a subset of cells within the meristem. During or soon after initiation, developmental axes are specified in the new primordium, which ultimately results in the differentiation of specific cell types in the appropriate positions. Signals from the meristem are thought to be required for the correct orientation of the new primordium relative to the main body axis. However, the genetic basis of initiation and patterning of lateral organs in plants is still largely unknown.

We are using transposable elements to generate mutants that affect axis specification and pattern formation during lateral organ development in maize. In particular, we are analyzing the roles of two genes: *Leafbladeless1* (*Lbl1*) and *Rough sheath2* (*Rs2*). A better understanding of the function of these and other genes involved in the early patterning steps during lateral organ formation will provide new insights into fundamental concepts of plant development. In addition, these studies may facilitate the manipulation of plant architecture, which has become increasingly more important for aspects of crop yield such as shade tolerance.

Leafbladeless1 IS REQUIRED FOR DORSOVENTRALITY OF LATERAL ORGANS

Normal maize leaves develop as flattened, dorsoventral organs with distinct cell types on the adaxial/dorsal and abaxial/ventral sides. We have shown that recessive mutations in the *lbl1* gene result in a loss of

adaxial cell types. The most extreme *lbl1* mutant phenotype is the formation of radially symmetric, abaxialized leaves. Less severe phenotypes include the formation of ectopic blade tissue at the boundaries between abaxialized sectors on the adaxial leaf surface. Leaf-like lateral organs of the inflorescences and flowers show similar phenotypes. Because *lbl1* is a recessive mutation, these observations are consistent with a role for *Lbl1* in establishing or maintaining adaxial cell identity in the leaf and other dorsoventral lateral organs. These observations also suggest that lateral growth of the organ occurs at the boundary between adaxial and abaxial domains.

Dorsoventrality of the maize leaf is in part established during leaf initiation by the lateral recruitment of founder cells. Immunolocalization using a KN1-specific antibody on transverse sections of wild-type and *lbl1* mutant apices showed that the number of founder cells incorporated into *lbl1* leaf primordia is strongly reduced. *Lbl1* and adaxial/abaxial axis specification are thus required for the lateral propagation of leaf founder cell recruitment, and *Lbl1* has a direct or indirect role in the down-regulation of the homeobox gene, *Kn1*. Our results are consistent with earlier surgical experiments which suggested that signals derived from the shoot apical meristem are needed to establish adaxial/abaxial asymmetry within lateral organs.

We are currently in the process of cloning the *Lbl1* gene and are isolating additional mutant alleles of *lbl1*. Phenotypic and restriction-fragment-length polymorphism linkage analysis placed the *lbl1* locus in close proximity to a classical leaf mutant, *ragged seedling1* (*rgd1*). Subsequent allelism tests showed that *rgd1* is a severe allele of *lbl1*. The *rgd1* mutation frequently results in embryo lethality and causes segregation distortion, suggesting that it affects gametophyte development. Several potential additional mutant *lbl1* alleles have been obtained from ethylmethanesulfonate (EMS)-mutagenized families. The availability of multiple *lbl1* alleles will be important to confirm the cloning of *Lbl1* and to fully analyze the effect of loss of adaxial cell identity on the different aspects of maize development.

Rough sheath2 REPRESSES *Knox* HOMEOBOX GENES IN LATERAL ORGANS

As mentioned above, the *Knox* homeobox gene family is normally expressed in shoot meristems and is required for their indeterminate growth. The *Knox* gene products are absent in normal leaf and floral primordia. In maize, several dominant mutants have been isolated that result in the ectopic expression of individual *Knox* genes in developing leaf primordia. These mutations interfere with cell differentiation along the proximodistal axis, resulting in the distal displacement of sheath and auricle tissues into the distal blade region of the leaf.

The recessive leaf mutant *rs2* exhibits phenotypes that resemble the phenotypes of the dominant *knox* mutants. We showed that in the *rs2* mutant, KNOX proteins are expressed not only in the meristem, but also in leaf primordia. Depending on the severity of the mutant phenotype, the number of founder cells recruited into *rs2* mutant leaves was also reduced. Both observations suggest that *Rs2* is a negative regulator of *Knox* gene expression. *Rs2* thus plays an important part in the transition from the indeterminate growth of the meristem to the determinate growth of lateral organs.

We cloned the *Rs2* gene that encodes a Myb domain protein with a novel DNA-binding motif. In wild-type apices, *Rs2* is expressed in young leaf primordia in a pattern that is consistent with the *rs2* mutant phenotype. Comparison of the *Rs2* and *Kn1* expression patterns in wild-type vegetative and floral apices showed that these two genes are expressed in mutually exclusive domains. However, the down-regulation of *Kn1* during leaf initiation preceded the onset of *Rs2* expression, suggesting that *Rs2* may not act to down-regulate *Knox* gene expression, but rather to maintain the *Knox* genes in an "off" state. The pattern of ectopic KNOX accumulation in *rs2* mutants is consistent with this interpretation. Ectopic KNOX protein accumulation in *rs2* mutant leaves occurs in a subset of the normal *Rs2*-expressing cells. Furthermore, KNOX proteins accumulated in patches with sharp lateral boundaries, suggesting that *rs2* leaves are clonal mosaics of *Knox*$^+$ and *Knox* sectors. This variegated accumulation of KNOX proteins in *rs2* mutants suggests the *Rs2* represses *Knox* expression through epigenetic means.

Rs2 is the maize homolog of the *Antirrhinum Phantastica* (*Phan*) gene (Waites et al., *Cell 93*: 779 [1998]). We showed that a *Knox*-like homeobox gene from *Antirrhinum* is similarly misregulated in *phan* mutants, suggesting that *Phan* and *Rs2* have orthologous functions. Like *rs2* mutants, mutations in the *Phan* gene cause patterning defects along the proximodistal axis in leaves and other lateral organs (Fig. 1). However, unlike *rs2, phan* mutants exhibit adaxial/abaxial polarity defects. KNOX proteins delay the transition from cell proliferation to differentiation.

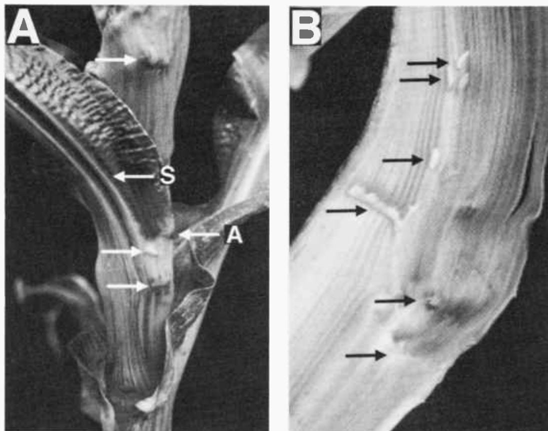


FIGURE 1 Mutations in the maize *Rs2* gene alter the pattern of cell differentiation along the proximodistal axis. The maize leaf consists of proximal sheath and distal blade tissue, which are separated by the auricle and ligule. The ligule is an epidermal fringe that develops on the adaxial leaf surface at the sharp boundary between sheath and blade. In the *rs2* mutant, sectors of sheath and auricle tissue are displaced into the distal blade region (*A*) and ectopic ligular fringes develop along the sectors of displaced sheath tissue (*B*). (S) Sheath; (A) auricle.

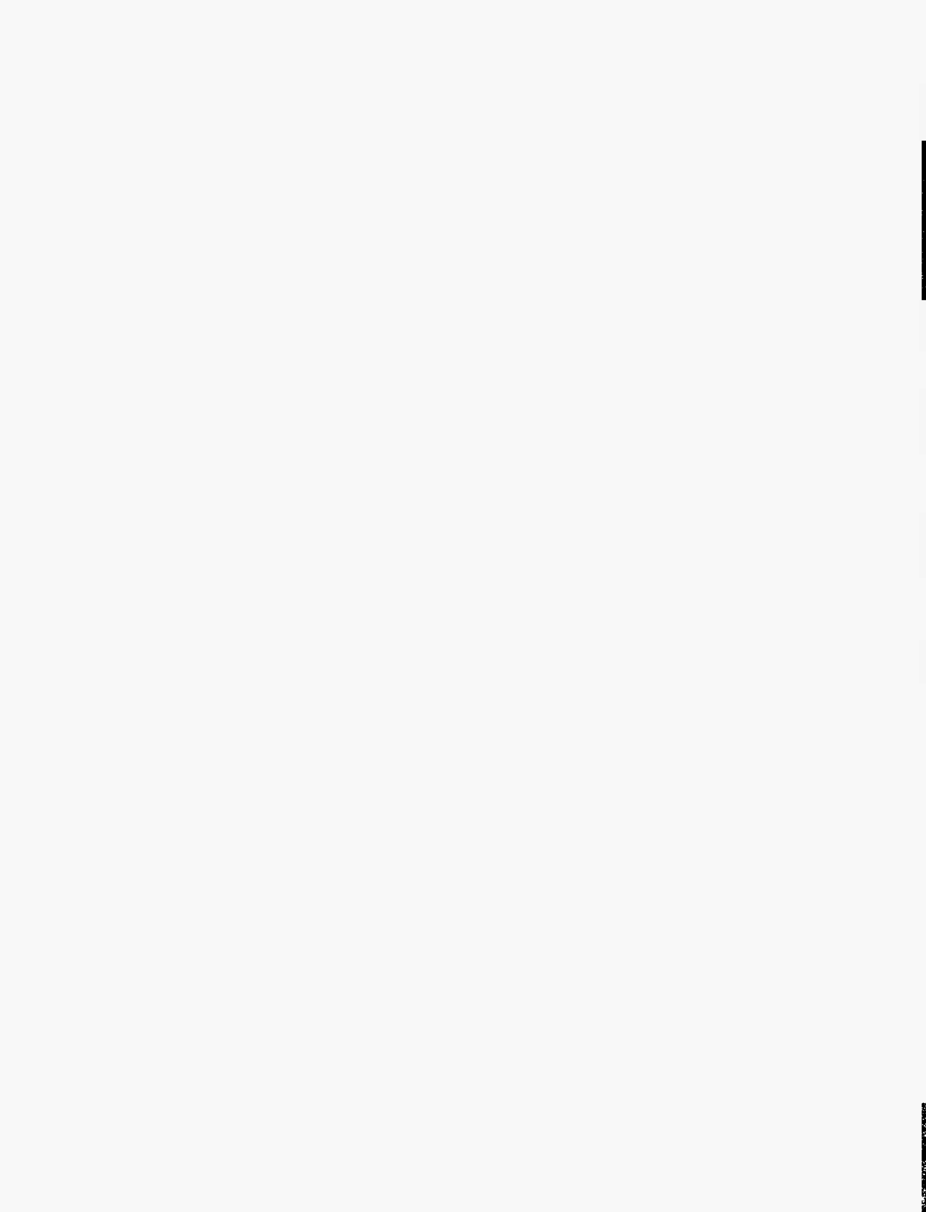
Our analysis suggests that the spatial pattern of this transition in leaves differs among plant species resulting in different leaf morphologies. In maize, this pattern occurs along the proximodistal axis, such that ectopic *Knox* expression results in a distal displacement of proximal features. The development of abaxial features on the adaxial leaf surface of *phan* mutant leaves suggests that this transition in *Antirrhinum* is deferred along both the proximodistal and adaxial/abaxial axes.

PUBLICATIONS

Timmermans M.C.P., Schultes N.P., Jankovsky J.P., and Nelson T. 1998. *Leafbladeless1* is required for dorsoventrality of lateral organs in maize. *Development* **125:** 2813–2823.

In Press

Timmermans M.C.P., Hudson A., Becaft P.W., and Nelson T. 1999. ROUGHSHEATH2: A Myb protein that represses knox homeobox genes in maize lateral organ primordia. *Science* (in press).



AUTHOR INDEX

Alexander, C., 168
Almanzar, D., 101
An, J., 174
Arroyo, J.M., 91, 97
Aufiero, D., 47
Azumi, Y., 94

Babb, R., 47
Bari, R., 147
Barria, A., 159
Baskar, R., 79
Beach, D., 72
Bennett, A.M., 120
Berthier, C., 109
Birkenmeier, G., 91
Boettner, B., 124
Braun, E.L., 83
Bridges, T., 168
Brodsky, J., 101
Bronson, K., 152
Buglino, A., 101
Burbach, B., 164
Byrne, M., 97

Cantallops, I., 152
Cartegni, L., 50
Carvalho, O., 162
Chan, B., 147
Chang, Y.-N., 60
Chen, C., 109
Chen, G., 63
Chen, J., 72
Chen, P., 162
Chew, S., 50
Chong, C., 128
Chong, J., 66
Christensen, J., 168
Cline, H.T., 152
Coblentz, J., 162
Cohen, I., 152
Coluccio, A., 79
Conklin, D., 72
Connolly, J., 168
Cowan, M., 171
Cronin, K., 66
Cui, W., 137
Curtis, M., 97

Dawkins, N., 159
de la Bastide, M., 133
de Stanchina, E., 56
Debrowski, A., 94
Dedhia, N., 133
Del Vecchio, R.L., 120
Desnoyers, S., 88
DeZazzo, J., 168
DiLeo, J., 168
Dodes, R., 88
Dong, D., 101
Dong, P., 86
Donovan, J., 75
Douglas, J., 101
Drier, E., 171
Du, Y.-C. (Nancy), 66
Dubnau, J., 168
Dunham, M., 97

Edgington, N., 75
Elgersma, M., 54
Elgersma, Y., 162
Ellison, V., 66
Ellstein, J., 94
Enikolopov, G., 156
Esposito, D., 101
Esteban, J., 159

Faleiro, L., 54
Fearnhead, H., 54
Fedorov, N.B., 162
Feng, J., 147
Ferbeyre, G., 56
Ferrezuelo, F., 75
Filipkowski, R., 171
Foa, L., 152
Ford, E., 112
Frangou, C., 86
Frankland, P.W., 162
Fraser, A., 88
Friedman, E., 162
Friedman, E., 171
Futcher, B., 75

Gartner, A., 88
Garton, A.J., 120
Gasperini, R., 168
Gill, T., 168
Gillitzer, E., 63
Gnoj, L., 133
Gossweiler, S., 168
Govek, E.-E., 124
Grady, L., 168
Greenberg, M.E., 60
Gremski, K., 79
Grewal, S., 106
Groover, A., 97
Grossman, J., 109
Grossniklaus, U., 79
Grotewold, E., 83
Gumienny, T., 88
Guo, H.-F., 174
Gutch, M.J., 120

Haas, K., 152
Habermann, K., 133
Hallock, P., 79
Hamaguchi, M., 84, 101
Hannan, F., 174
Hannan, M., 168
Hannon, G., 86
Hastings, M., 50
Hatchwell, E., 101
Hayashi, M., 143
Hayashi, Y., 159
Helfman, D.M., 109
Hemish, J., 156
Hendren, K., 143
Hengartner, M., 88
Henry, R.W., 112
Herbst, A., 69
Hernandez, M.J., 83
Hernandez, N., 112
Herr, W., 47
Hill, B., 60
Hinkley, C., 47
Hirano, M., 116
Hirano, T., 116
Hoeppner, D., 88
Holden, R., 47
Honey, S., 75
Horiuchi, J., 171
Horwitz, B., 94
Howard, T., 118
Hu, Y., 94

Ioschikhes, I., 147
Ioschikhes,.Z, 147

Jackson, D., 91
Jafrate, A.J., 60
Janardhan, A., 60
Javaherian, A., 152
Jay, J., 152
Jiang, J., 143
Jiang, W., 171
John, S., 156
Jones, R., 168
Joshua-Tor, L.,128

Kamenetz, F., 159
Kashige, N., 131
Katari, M., 101
Katz, E., 94
Keller, J., 88
Khalfan, Z., 91
Kidner, C., 97
Kim, S.Y., 69
Kimura, K., 116
Kobayashi, R., 131
Kogan, J.H., 162
Kourpanidies, S., 171
Krainer, A.R., 50
Krasnov, P., 156
Krimper, R., 106
Kuhlman, T.L., 112

LaMontagne, K.R., 120
Laulicht, B., 94
LaVine, K., 171
Lazebnik, Y., 54
Lee, A., 63
Lee, S., 47
Lendvai, B., 164
Li, H., 168
Li, Z., 152
Liang, C., 66
Lin, A., 56
Lin, Z., 171
Liu, D., 94
Liu, H.-X., 50
Liu, J.P., 120
Liu, Q., 88
Liu, R., 137
Liu, Y., 47
Lock, M., 60
Losada, A., 116
Lowe, S., 56
Lucito, R., 101
Luo, S., 94

Ma, B., 112
Ma, H., 94
Mahapatra, I., 152
Mainen, Z., 159, 164
Maletic-Savatic, M., 159
Malinow, R., 159
Manche, L., 50
Maravall, M., 164
Marin, M., 124
Martienssen, R., 97
Matero, A., 133
May, B., 97
Mayeda, A., 50
McCombie, W.R., 133
McCurrach, M., 56
McDonough, M., 124
Medalle, J., 168
Mendez, J., 66
Meschan, V., 47
Messick, T., 128
Michurina, T., 156
Mignone, J., 156
Milstein, S., 88
Mintz, P., 118
Misteli, T., 118
Mittal, V., 112
Moore, J., 79
Moran, S., 94
Morrison, D.J., 112
Muratani, M., 69

Murray, M., 50
Myers, M.P., 120

Nagel, J., 171
Nakamura, M., 101
Nakaya, N., 156
Nedivi, E., 152
Nestler, H.P., 137
Neuwald, A.F., 140
Newman, J., 152
Nimchinsky, E., 164
Ning Huang, E., 133

O'Brien, P., 164
O'Connor, B., 84, 101
O'Farrell, P., 128
Ohno, M., 162
Onaka, H., 94
O'Shaughnessy, A., 133

Packer, M., 156
Packer, R., 124
Palka, H.L., 120
Parkey, J., 79
Parnell, L., 133
Pendergrast, P.S., 112
Pennino, K.A., 120
Peunova, N., 156
Piccini, A., 159
Pinto, S., 168
Pizzirusso, M., 83
Poleksic, A., 140
Polyakova, J., 56
Poncer, J.-C., 159
Poppito, N., 131
Preston, R., 133
Pultorak, P., 79

Rabinowicz, P., 97
Rabinowicz, P.D., 83
Rahimzadeh, M., 168
Rai, A.J., 109
Rajagopal, R., 156
Rajan, I., 152
Reader, J., 47
Redler, Y., 79
Regulski, M., 168
Reilly, P., 47
Riggs, M., 101
Rodgers, L., 101
Rodriguez, J., 54
Rodriguez, M., 133
Rosa, D., 152
Rosenbaum, J., 128
Rubinelli, P., 94
Ruthazer, E., 152

Sabatini, B., 164
Sabino, G., 91
Saitoe, M., 168
Salghetti, S., 69
Salvatore, J., 168
Samatar, A.A., 120
Samuelson, A., 56
Sanders, C., 63
Scheinker, V., 156
Schmitt, C., 56
Schmitz, A., 124
Schneider, B., 75
Schramm, L.M., 112
Schutz, K., 133
Selvakumar, M., 109
Sepehri, S., 112
Serina, L., 101
Settles, A.M., 97
Shah, R., 133
Shekher, M., 133
Shen, R., 97
Shen, Y., 120
Sherlock, G., 75
Sherlock, R., 137
Shi, H., 143
Shi, S., 159
Shibahara, K., 66
Shohdy, N., 60
Siddiqi, I., 79
Silva, A.J., 162
Simorowski, J., 97
Simpson, K., 66
Sin, W.C., 152
Skowronski, J., 60
Smith, P., 168
Soengas, M., 56
Sosa, G.M., 83
Spector, D.L., 118
Spector, M., 88
Spiegel, L., 133
Spillane, C., 79
St. Fleu, D., 91
Stasiv, Y., 156
Stebbins, M., 171
Stein, L.D., 145
Stenlund, A., 63
Stern, E., 164
Stillman, B., 66
Stolarov, J., 101
Sun, P., 72
Sun, Y., 112
Svoboda, K., 164
Swaby, I., 133
Swigut, T., 60

Tabaska, J., 147
Tansey, W., 69
Tello, M., 171
Tharin, S., 88
Thomas, J., 79
Timmermans, M., 177
Tong, J., 174
Tonks, N.K., 120
Troge, J., 84, 101
Tseng, T.-L., 50
Tu, H., 101
Tu, V., 101
Tubon, T., 47
Tully, T., 168
Tworkowski, K., 69

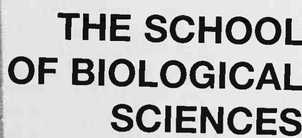
Vaahtokari, A., 109
Vahab, P., 177
van Aalten, D., 128
Van Aelst, L., 124
Vaughn, D., 128
Velinzon, K., 168
Verreault, A., 66
Vielle-Calzada, J.-P., 79
Vil, M.D., 133
Virani, S., 94
Vollbrecht, E., 97
Volpe, T., 75
Waga, M., 66
Waga, S., 66
Wanat, K., 131
Wang, J., 72
Wang, Y., 174
Wang, Y., 94
Wang, Y.-C., 109
Weinreich, K., 174
Weinreich, M., 66
Wendel, P., 66
West, J., 101
Wigler, M., 101
Wijnen, H., 75
Wright, N., 174
Wu, G.-Y., 152
Wu., K., 47
Wu, P., 171
Wu, Q., 50
Wysocka, J., 47

Xia, S., 168
Xu, R.-M., 143

Yamashita, N., 159
Yang, M., 94
Yen, C., 101
Yin, J., 171
Yokochi, T., 116
Yordan, C., 97
Yuan, Z., 91

Zaman, S., 159
Zhang, L., 120, 168
Zhang, M.Q., 147
Zhang, S.H., 120
Zhang, Y., 143
Zhang, Z., 66
Zhao, M., 124
Zhao, X., 47, 112
Zhong, Y., 174
Zhou, H., 171
Zhou, J., 75
Zhu, J., 50
Zhu, J., 147
Zou, D.-J., 152
Zou, L., 66
Zou-Yang, X.H., 66

THE SCHOOL OF BIOLOGICAL SCIENCES



THE SCHOOL OF BIOLOGICAL SCIENCES

On September 18, 1998, Cold Spring Harbor Laboratory became a degree-granting institution for the first time in its history. On that day, the Laboratory received authority from the Board of Regents of the New York State Education Department to grant the Doctor of Philosophy (Ph.D.) and Master of Science (M.S.) degrees in the biological sciences.

It is an exciting time to establish a new doctoral program in the biological sciences. The pace of discovery in the biological sciences is breathtaking. It is hard to imagine that less than 50 years ago, the structure of DNA was unknown and that within the next decade, we will know the complete sequence of the human genome. Almost daily, scientists report new discoveries and developments in the biological sciences that have a profound influence on society. The Laboratory's new doctoral program will train biologists for this new era in biology.

Since its inception in 1890, Cold Spring Harbor Laboratory has been an institution of higher learning and for more than 25 years has been involved in graduate education leading to the Ph.D. degree. Graduate students from institutions with Ph.D. degree-granting authority, most notably the State University of New York (SUNY) at Stony Brook, perform their doctoral research at Cold Spring Harbor Laboratory. Until this year, however, the Laboratory has had no graduate degree-granting program of its own.

The Laboratory is an exciting place for a doctoral program in the biological sciences. Its special environment provides a unique venue for an innovative and stimulating program. Its international faculty provides a global view of graduate education in the biological sciences. And its maverick tradition inspires a new type of doctoral program.

Establishing a Graduate School

The establishment of a graduate school at the Laboratory was the culmination of nearly three years of effort, which began in November 1995 when the Board of Trustees initiated a study into the desirability and feasibility of a graduate school at the Laboratory. During an exploratory period, there were numerous discussions among the Laboratory trustees and staff and with other institutions to fully explore the concept of establishing a graduate school at the Laboratory. Encouraged by the discussions, in June 1997 the Board of Trustees authorized the Laboratory to apply to the New York State Education Department for the authority to grant the Ph.D. degree in the biological sciences.

A coordinated effort to prepare for a Ph.D. program at the Laboratory began. Working in concert with the New York State Education Department, a timetable was established by which the Laboratory could begin accepting students for the fall 1999. During the fall 1997, a curriculum was developed through weekly meetings of interested staff members, and the goals of the Ph.D. curriculum were established. We set out six ambitious goals.

- to offer a doctoral education that takes approximately four years. In the context of a well-designed program, we believe that students need no longer than four years to develop the skills necessary to become successful biologists.
- to provide extensive guidance, which we will provide in part through a "two-tier" mentoring program of research and academic mentors.
- to focus course instruction on teaching students how to think about biology independently and critically.
- to provide a broad education in the biological sciences and to demonstrate to students how discoveries in seemingly unrelated fields influence one another.
- to offer students an outstanding research experience while emphasizing that learning is a lifelong process that goes hand in hand with research.
- to educate biologists who can communicate effectively with nonscientists.

The doctoral program that we developed to meet these goals is intense and is designed for students with exceptional ability and a deep commitment to their graduate education.

In April 1998, our proposed program was evaluated by a New York State-endorsed review committee. The committee was chaired by Dr. Keith Yamamoto (University of California, San Francisco) and included Mr. William T. Golden (Chairman Emeritus of the American Museum of Natural History in New York City), Dr. Irwin B. Levitan (Brandeis University), Dr. Joachim Messing (Rutgers University), Dr. Barbara J. Meyer (University of California, Berkeley), and Dr. Frank Solomon (Massachusetts Institute of Technology). The reviewers were enthusiastic about the proposed program but cautioned the Laboratory that it would have to take great care in implementing the new innovative and intense program to ensure that the students were not overwhelmed.

During preparation of the Ph.D. program, we paid special attention to ensure that there would be no adverse impact on SUNY Stony Brook or our current shared Ph.D. programs with SUNY Stony Brook. Currently, 90% of the graduate students at Cold Spring Harbor Laboratory belong to a graduate program at SUNY Stony Brook, and these SUNY Stony Brook students form an important element of the educational and research environment at the Laboratory. Furthermore, by establishing a graduate school, the Laboratory is eager to enhance—not detract from—the environment for research in the biological sciences on Long Island. As a result of discussions with SUNY Stony Brook, a mechanism for coordinated recruitment of students to both institutions was established.

In June 1998, the State Education Department distributed an abstract of the Laboratory's proposal for degree-granting status to all higher education institutions on Long Island and all Ph.D. degree-granting institutions in the biological sciences in New York State. The State invited representatives of these institutions to comment on the plan. The responses to the proposal reflected strong support, particularly from our two neighboring institutions: SUNY Stony Brook and the Picower Institute. The response helped ensure approval of the proposal by the Board of Regents in September.

While the proposal was in the final stages of approval, the Laboratory initiated a capital campaign to raise an endowment for the School. Encouraged by the response of benefactors, it was agreed at the Laboratory's Board of Trustees meeting on November 7 to matriculate a first class of five students in the fall 1999, and to begin recruitment of that first class.

An Innovative Ph.D. Program

Advances in biology depend on multidisciplinary approaches, in which knowledge and technology from diverse areas intersect to inspire new discoveries. Today, however, the breadth of accumulated knowledge about biology is immense—far more extensive than any individual can assimilate. Thus, the Laboratory's doctoral curriculum has been designed to train self-reliant students who, under their own guidance, can acquire and assimilate the knowledge their research or career demands require.

The curriculum takes advantage of the unique and flexible environment of Cold Spring Harbor Laboratory. The first year of the curriculum assumes an innovative format, in which students progress rapidly from course instruction to doctoral research. The year begins with a 14-week fall course term. The curriculum used in the fall course term provides intense instruction in a series of integrated courses. Students participate in three core courses—Scientific Reasoning and Logic, Scientific Exposition and Ethics, and Research Topics—which span the length of the fall term. In parallel, students participate in three, tandem four-week lecture courses in specialized disciplines. The courses will be taught by a teaching faculty, consisting of self-selected research and nonresearch faculty.

During the fall term, students are free of research responsibilities, which allows them to devote their full attention to intensive course instruction and seminars. During the subsequent winter and spring, students participate in three six-week-long laboratory rotations, a week-long Topics in Biology course, and teaching high school students at the DNA Learning Center. In May, students select a research mentor and prepare for the Ph.D. qualifying exam that takes place in June. After the requirements of the qualifying exam have been satisfied, students focus on their doctoral research.

The Laboratory is committed to the success of its graduate students. To promote a high level of student achievement, the faculty and administration will take an active role in mentoring and supervising the students. A special feature of the curriculum is the "two-tier" mentoring program, which involves an academic mentor and a research mentor for each student. At matriculation, each student will be assigned a faculty member as an academic mentor. The academic mentor follows the student's academic and research progress and provides advice for the duration of the student's tenure in the graduate program. After the laboratory rotations, each student chooses a research mentor. The research mentor is the doctoral thesis research advisor, who supervises the student's independent laboratory research. By providing both academic and research mentors, the School of Biological Sciences provides each student with advice from faculty who hold different views and can offer unique and in-depth evaluations of the student.

After the first year, students focus on laboratory research. Nevertheless, course instruction, in the form of the annual Topics in Biology course, and annual participation in the Laboratory's postgraduate courses (of the student's choosing) continues. Approximately six to nine months before defending the research thesis, students will prepare and defend a postdoctoral research proposal. For those students who plan to continue in research, this proposal will outline a postdoctoral research program, which will aid students in the preparation of postdoctoral fellowship applications. For students who do not plan to continue in scientific research, the proposal will describe their future plans and how they plan to use their doctoral education after receiving the Ph.D. degree.

An Exciting and Intensive Graduate Program

The Cold Spring Harbor Laboratory School of Biological Sciences is designed to provide an exciting and intensive educational experience. Course work is concentrated in, but not limited to, the fall term of the first year. Students are expected to begin full-time laboratory research within ten months and to complete the doctoral degree within approximately four years. The program has been designed for outstanding, self-motivated students. To provide each student with the individual attention necessary to ensure success in the program, we intend to keep class sizes small. In April of this year, we learned that six outstanding students from across the country and around the world—the founding class—will be joining us this fall to begin this exciting new adventure. We look forward to the beginning of the School with great excitement...and a proper touch of apprehension!

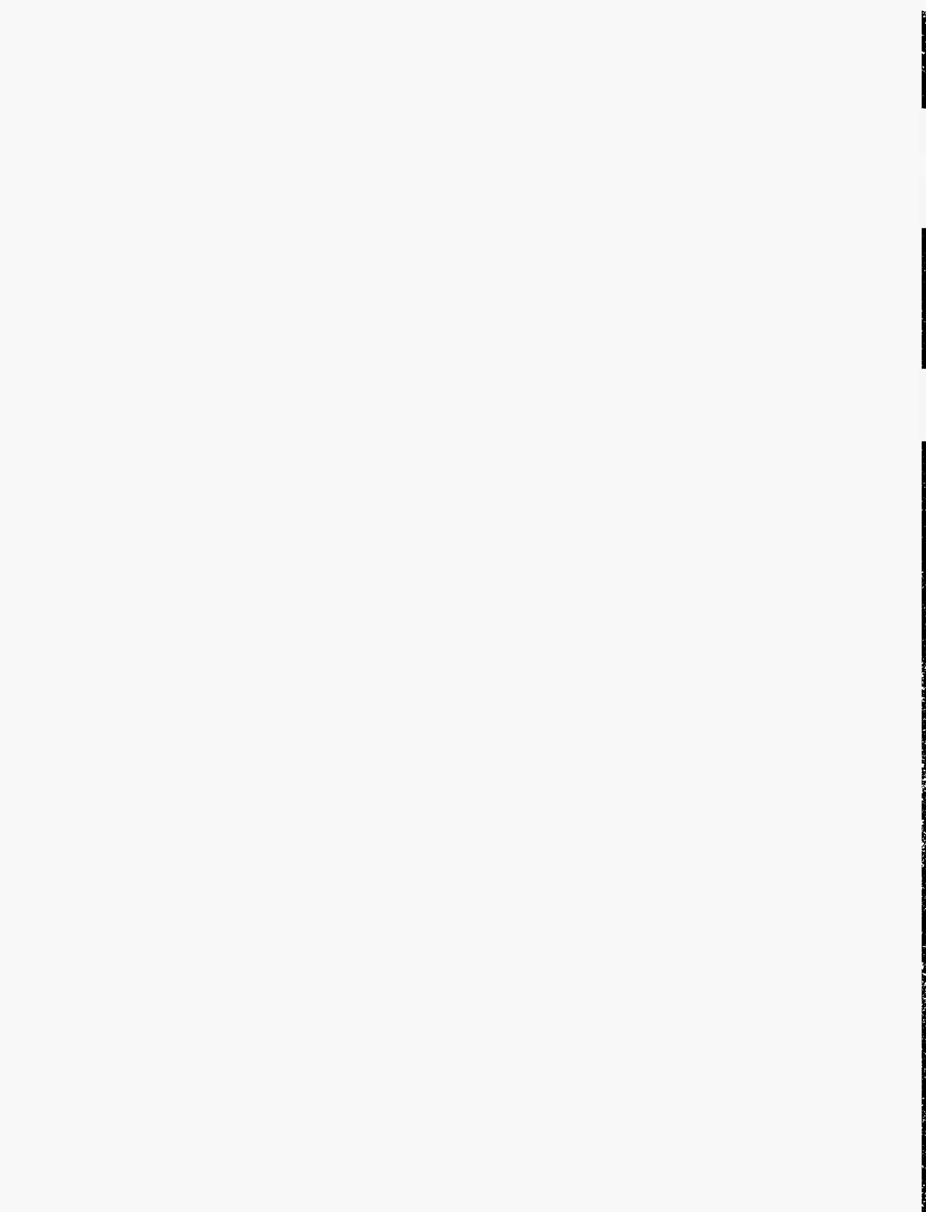
A New Name

As the initiative to establish a graduate school came to fruition, the Laboratory's administration and Board of Trustees strongly agreed that it would be appropriate to name the unique, new educational program for long-term Laboratory director and now president Jim Watson. Jim was asked, and reluctantly approved, and an application for the formal name change was submitted to New York State. On February 3, 1999, the New York State Board of Regents granted Cold Spring Harbor Laboratory authority to change the name of the graduate program to Cold Spring Harbor Laboratory Watson School of Biological Sciences in recognition of its president James D. Watson. It is fitting that, after all that Jim Watson has done for the biological sciences, for education, and for Cold Spring Harbor Laboratory, the new school is named in his honor.

May 1999

Winship Herr
Dean

COLD SPRING HARBOR LABORATORY MEETINGS AND COURSES



ACADEMIC AFFAIRS

The academic program at Cold Spring Harbor serves to communicate new discoveries, concepts, and methodologies to an international community of scientists from universities, medical schools, research institutes, and industry. A broad-ranging series of postgraduate laboratory and lecture courses, workshops, and large meetings now extend almost year round. In addition, a summer research program for undergraduates enables college students to work in the laboratories of Laboratory scientists.

The courses and workshops serve a unique function, emphasizing the state-of-the art methodologies that may be applied immediately to the students' own research. Lectures and discussions serve to set the experiments in the context of the field and its most recent scientific advances. The courses are of relatively short duration so that senior scientists as well as junior faculty, postdoctoral fellows, and graduate students can attend. All of the courses are oversubscribed, which is a tribute to the skills and talents of the instructors as well as the timeliness of the courses. The meetings held this year enabled scientists to present their latest results in a broad range of areas in molecular biology, genomics, and neurobiology. A key feature of most meetings is that talks are selected by the organizers from submitted abstracts, allowing junior as well as senior scientists to present innovative work. This year, more than 7000 scientists came to the Laboratory, including instructors, lecturers, and students in the courses as well as meeting participants.

This year, 20 molecular genetics, genomics, structure, and neurobiology laboratory courses were held. These were taught in the Howard Hughes Medical Institute (HHMI) teaching laboratories in the Beckman Neuroscience Center and the Delbruck Laboratory. In addition, five neurobiology lecture and workshop courses were held at the Banbury Center. Course instructors, assistants, lecturers, and students are listed in the following pages. The instructors, who come from universities and research institutes around the world to teach at Cold Spring Harbor, are really the key to the high quality and success of the courses. It is their creativity and skill that make the program so effective.

Each year, some scientists who have taught in the courses for several years retire. We are sorry to see them go, but we know that we can count on them to return to give advice and lectures in future years. This year, they include Michael Carey, an instructor in the Eukaryotic Gene Expression course; Eric Lam and Jane Glazebrook who taught the *Arabidopsis* Molecular Genetics course; Dan Gottschling, a Yeast Genetics course instructor; Tito Serafini who taught the course on Molecular Cloning of Neural Genes; Nipam Patel and Michael Dickenson, instructors in the Neurobiology of *Drosophila* course; and instructors John Caldwell, Rock Levinson, and Robert Maue, who taught the course on Integrated Approaches to Ion Channel Biology.

The courses are supported by, and would not be possible without, a series of grants from federal and private sources. The summer molecular genetics courses have been supported for many years by grants from the National Institutes of Health (NIH) and the National Science Foundation (NSF). A grant from the National Institute of Mental Health supports several of the neurobiology courses. A large and most valuable education grant from HHMI has provided stable support for the neurobiology program and has allowed the Laboratory to begin and support a variety of new courses. We are also pleased to acknowledge an award from the Esther and Joseph A. Klingenstein Fund for the support of neurobiology courses and funds from the Grass Foundation for scholarships for students in neurobiology courses. In addition, the Laboratory receives valuable support from many companies that donate supplies and lend equipment for the courses.

Eighteen meetings were held at the Laboratory this year. Several new meetings took place as well as many old favorites that recur on a biannual basis. Organizers and meeting chairs are listed on the following pages, and we are, as always, most grateful for their efforts. New meetings this year included: The Genetics of Aging organized by Judith Campisi, Lenny Guarente, and Cal Harley; Axon Guidance and Neural Plasticity, organized by Corey Goodman, Carla Schatz, and Marc Tessier-Levigne;

Gametogenesis, organized by Brigid Hogan and Allan Spradling; and Dynamic Organization of Nuclear Function, organized by Robert Goldman, John Newport, Pam Silver, and David Spector. A special winter meeting on Pathways to Cancer, organized by Ed Harlow, Bruce Stillman, and Jan Witkowski, took place in March. Several returning meetings continue to serve the scientific community and were, in fact, oversubscribed. These included Zebrafish Development and Genetics; Genome Mapping, Sequencing and Biology; Retroviruses; Cancer Genetics and Tumor Suppressor Genes; Mouse Molecular Genetics; and Translational Control. We expect these meetings to be held again in 2 years.

The Symposium continues to be the centerpiece of the Laboratory's meeting program. The meeting this year on Mechanisms of Transcription was organized and is discussed below by Bruce Stillman. It brought together a large and animated crowd of scientists to discuss their latest research.

This year, two technology conferences, The *Arabidopsis* Genome: From Sequence to Function and Conditional Genetics Technologies in the Mouse, were held outside of the regular meetings schedule. These biotechnology conferences, now in their third successful year, will continue in the future. All organizers and contributors to the meetings are listed on the following pages. The sources of support for these meetings come from the Laboratory's Corporate Sponsor program, NIH, NSF, DOE, and various corporations and foundations. Grants helped scientists at all stages of their careers to attend the conferences.

While graduate students, postdoctoral fellows, and faculty participate in the courses and meetings, the Undergraduate Research Program (URP) provides an opportunity for college undergraduates to spend 10 weeks at the Laboratory during the summer. The program, headed by Michael Hengartner, allows students to do research in the laboratories of staff scientists.

The large numbers of courses and meetings proceed with skill and efficiency, thanks to the collaborative efforts of a large number of people at the Laboratory. The staff of the Meetings and Courses Office coordinates the arrangements for all of the visiting scientists. This enormous job, which seems to grow every year, is carried out not only with extreme efficiency, but with patience and tact as well. The staff includes Micki McBride (the Course Registrar), Nancy Weeks (Head Concierge), Diane Tighe, Marge Stellabotte, Andrea Stephenson, Drew Mendelson, Mary Smith, and Michael Glaessgen, Donna Dykeman, Andrea Newell, as well as Herb Parsons, Ed Campodonico, Bill Dickerson, and the part-time audiovisual staff. Staff from several other departments are crucial to the success of the meetings and courses including Cliff Sutkevich and his staff who set up and maintain course equipment; and Wendy Crowley, the Education Grants Manager. We especially want to acknowledge the splendid work of Edie Kappenberg, the course coordinator, who left the Laboratory this year. We are most pleased that Barbara Zane, a former buyer in the Purchasing Office, has returned to the Laboratory to take over the position of course coordinator.

Terri Grodzicker
Assistant Director for Academic Affairs

David Stewart
Director of Meetings and Courses

63rd COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

Mechanisms of Transcription

June 3–8 437 participants

ARRANGED BY **Bruce Stillman,** Cold Spring Harbor Laboratory

The field of gene transcription intertwines with many other areas of modern biological research because of its fundamental significance. It has been some time since a Cold Spring Harbor Laboratory Symposium focused solely on this important topic, in part because transcription is usually included in all Symposia in one form or another. But because of the significant advances that have occurred during the last few years in our understanding of how genes are transcribed, it seemed appropriate to hold a meeting on this topic again. The dramatic advances made in this field over the last 20 years have led to the identification of a vast set of basal transcription factors in eukaryotes. Moreover, this field has encompassed the exciting progress in related areas such as structural biology and chromatin structure and function.

Nearly 40 years have passed since the discovery of the first RNA polymerase by Sam Weiss. Many previous Cold Spring Harbor Laboratory Symposia have incorporated aspects of gene transcription and gene regulation into the program, as this topic in many ways represents the core of biology. Most noteworthy here was the famous 1977 Symposium on Chromatin, where the fundamental understanding of gene structure in eukaryotes was overturned. Today, we are in the midst of a very stimulating era that is focused on understanding the mechanisms of gene transcription and, at this Symposium, we listened to 67 talks, and studied posters, that reflected this excitement.

It is also most fitting that a Symposium on gene transcription be held this year as we celebrated the 30th year that Jim Watson has worked at the Laboratory, first as Director for 25 years and now as President. At the beginning of this meeting, I presented to Jim, on behalf of the Laboratory, a gold model of the DNA double helix. Five years ago, Jim presented the twin of this golden helix to Francis

A. Krainer, J.D. Watson, L. Miller, T. Maniatis, B. Stillman

Crick at a Cold Spring Harbor meeting on the occasion of the 40th anniversary of their discovery of the structure of DNA. What better occasion to make this presentation to Jim than when many of his former students and colleagues were present? Prior to his coming to Cold Spring Harbor, Jim's laboratory at Harvard had made some of the key contributions to the early work on the mechanisms of gene transcription, including the discovery of σ factor.

One of the many responsibilities of the Director is to organize the annual Symposium and this Jim did for many years. The first Symposium he organized, in 1970, was appropriately enough on "Transcription of Genetic Material." It celebrated, among other things, the discovery 2 years before of the σ factor for RNA polymerase in bacteria. At that meeting, David Baltimore also presented the exciting news of reverse transcriptase.

Jim noted in his foreword to the Symposium volume of that year: "The final result was a compromise between a desire to hear everyone with relevant data and the need to restrict the talks to a number ingestible within a week's time." In this respect, nothing has changed in the last 28 years. It was necessary to make the usual hard choices in selecting speakers. For the organization of this meeting, my colleagues Winship Herr, Nouria Hernandez, Bob Tjian, Carol Gross, Rich Losick, and a number of others provided valuable advice.

The Symposium started with a fascinating first night of introductory talks from Carol Gross, Robert Tjian, Tom Maniatis, and David Allis. Bob Roeder, who has made many seminal contributions to the biochemistry of transcription, presented this year's Reginald Harris Lecture. The Dorcas Cummings Lecture for our friends and neighbors was this year given by Ronald Evans, who gave a spectacular and fascinating assessment of obesity, "The Molecular Biology of Fat: Weighing the Risks." The formal scientific program consisted of 67 oral presentations and a record 155 poster presentations, and the meeting attracted 437 participants. I thank Rich Losick for agreeing to summarize the meeting and writing such a marvelous and thoughtful summary, matching the great summaries of previous Symposia.

We were particularly fortunate this year to have Lewis Miller as an artist in residence during the meeting. Lewis, who hails form Melbourne, Australia, won the prestigious Archibald Portrait Prize in 1998. He sketched many of the Symposium participants, and Blackford Hall is now graced with some of these marvelous portraits.

Essential funds to run this meeting were obtained from the National Cancer Institute, a branch of the National Institutes of Health. In addition, financial help from the Corporate Sponsors of our meetings program is essential for these Symposia to remain a success and we are most grateful for their continued support. These sponsors are Amgen Inc., BASF Bioresearch Corporation, Bayer Corporation, Bristol-Myers Squibb Company, Chiron Corporation, Chugai Research Institute for Molecular Medicine, Inc., Diagnostic Products Corporation, Forest Laboratories, Inc., Genentech, Inc., Genetics Institute, Hoechst Marion

K. Yamamoto

J. Roberts, T. Steitz

T. Grodzicker, R. Losick, S. Gottesman

R. Evans, R. Roeder

Roussel, Hoffmann-La Roche Inc., Johnson & Johnson, Kyowa Hakko Kogyo Co., Ltd., Eli Lilly and Company, Merck Genome Research Institute, Novartis Pharma Research, Novo Nordisk Biotech, Inc./ZymoGenetics, Inc., OSI Pharmaceuticals, Inc., Pall Corporation, Parke-Davis Pharmaceutical Research, The Perkin-Elmer Corporation, Applied Biosystems Division, Pfizer Inc., Pharmacia & Upjohn, Inc., Research Genetics, Inc., Schering-Plough Corporation, SmithKline Beecham Pharmaceuticals, Wyeth-Ayerst Research, and the Zeneca Group PLC. *Plant Corporate Associates* include American Cyanamid Company, Monsanto Company, Pioneer Hi-Bred International, Inc., and Westvaco Corporation. We also thank our *Foundation Associate*, The Albert B. Sabin Vaccine Institute at Georgetown University, and our *Corporate Contributors*, Genome Systems, Lexicon Genetics, and Qiagen, Inc.

PROGRAM

Welcoming Remarks: Bruce Stillman

Introduction
Chairperson: W. Herr, *Cold Spring Harbor Laboratory*

Activation
Chairperson: P. Sharp, *Massachusetts Institute of Technology, Cambridge*

Promoter Recognition
Chairperson: L. Rothman-Denes, *University of Chicago, Illinois*

Repression Mechanisms
Chairperson: T. Maniatis, *Harvard University, Cambridge, Massachusetts*

Reginald G. Harris Lecture: "Transcriptional Regulation through General and Gene-specific Coactivators"
Speaker: R.G. Roeder, *Rockefeller University, New York*

Core Factors
Chairperson: R. Burgess, *University of Wisconsin, Madison*

Polymerase and Promoter Clearance
Chairperson: J. Kadonaga, *University of California, San Diego*

Chromosome Structure and Transcription
Chairperson: C. Wu, *National Cancer Institute, Bethesda, Maryland*

Remodeling Chromatin
Chairperson: R. Tjian, *University of California, Berkeley*

Regulation
Chairperson: G. Felsenfeld, *NIDDK, National Institutes of Health, Bethesda, Maryland*

Dorcas Cummings Lecture: "The Molecular Biology of Fat: Weighing the Risks"
Speaker: R. Evans, *Howard Hughes Medical Institute, The Salk Institute, San Diego, California*

More Regulation, Initiation, and Elongation
Chairperson: K. Yamamoto, *University of California, San Francisco*

Starting and Stopping
Chairperson: J. Conaway, *Oklahoma Medical Research Foundation, Oklahoma City*

Summary: R. Losick, *Harvard University, Cambridge, Massachusetts*

SPECIAL CONFERENCES

Breakthrough! How News Influences Health Perception and Behavior

February 27–March 1 133 participants

ARRANGED BY **Susan Cooper,** Trudeau Institute, New York
Eric Rosenthal, Fox Chase Cancer Center
Dianne Shaw, University of North Carolina
Jan Witkowski, Cold Spring Harbor Laboratory
Laurie Young, University of Arizona

This unique weekend workshop examined what shapes the presentation and dissemination of research findings and how this affects public expectations about health and science. The workshop aimed to generate a dialogue between scientists, journalists, and communicators and to explore how scientific news is communicated and reported. With more than 130 participants, and 35 oral presentations, the workshop provided a thought-provoking opportunity to address these issues.

Speakers included editors (Richard Horton/*The Lancet*, Marcia Angell/*New England Journal of Medicine*, Ellis Rubinstein/*Science*), journalists (Bob Bazell/*NBC*, Joe Palca/*NPR*, Victor Cohn/*Washington Post*, Gina Kolata/*New York Times*, Richard Saltus/*Boston Globe*) and scientists (Richard Klausner/*National Cancer Institute*, Frank Rauscher III/*The Wistar Institute*, Carol Greider/*Johns Hopkins School of Medicine*).

In particular, the organizers chose to focus primarily on cancer, with sessions devoted to the National Cancer Act 25 years on, mammography screening, genetic testing (using BRCA-1 as an example), clinical trials, and complementary/ alternative therapies, although a fascinating session was included to examine how the cloning of Dolly was reported and perceived. All in all, this workshop was considered to be an outstanding success in many ways and should lay the foundation for special workshops of this type in the future.

This meeting was supported in part by the National Cancer Institute, a branch of the National Institutes of Health, and Cold Spring Harbor Laboratory.

S. Cooper, J. Witkowski

PROGRAM

National Cancer Act
Moderator: V. DeVita, *Yale Cancer Center*

Mammography Screening
Moderator: R. Horton, *The Lancet*

Genetic Testing: BRCA-1 as a Case Study
Moderator: E. Rubinstein, Editor, *Science*

The Cloning Story
Moderator: R. Cook-Deegan, *National Academy of Sciences*

Clinical Trials
Moderator: M. Angell, *New England Journal of Medicine*

Complementary Therapies
Moderator: Z. Hall, *University of California, San Francisco*

Summary and Conclusion
Moderator: S. Cooper, *Trudeau Institute*

E. Rosenthal, D. Shaw, L. Young

Pathways to Cancer

March 11–14 154 participants

ARRANGED BY **Ed Harlow,** Massachusetts General Hospital
Bruce Stillman, Cold Spring Harbor Laboratory
Jan Witkowski, Cold Spring Harbor Laboratory

The year 1998 marked three important anniversaries: 50 years ago Jim Watson came to Cold Spring Harbor Laboratory; 30 years ago he became Director; and 30 years ago was the beginning of the DNA Tumor Virus Program. A special meeting—appropriately on cancer research—was held in Grace Auditorium to celebrate these anniversaries. The Pathways to Cancer meeting examined both the molecular mechanisms that lead to cancers and the molecular events underlying particular types of cancer. Many of the world's leading cancer researchers took part, and Ed Scolnick, President of Merck Research Laboratories, gave the keynote address on "Cancer through a Therapeutic Looking Glass."

PROGRAM

Introduction: A Brief Description of CSHL and Cancer Research
Chairperson: B. Stillman, *Cold Spring Harbor Laboratory*

Control of Cancer Cell Proliferation
Chairperson: E. Harlow, *Massachusetts General Hospital, Charlestown*

Cancer Genetic Pathways
Chairperson: G. Rubin, *University of California, Berkeley*

Signaling Pathways in Cancer
Chairperson: J. Witkowski, *Cold Spring Harbor Laboratory*

Cancer Cell Growth Controls
Chairperson: S. Courtneidge, *Sugen, Inc., Redwood City, California*

Cancer Pathways from Cell to Clinic
Chairperson: B. Stillman, *Cold Spring Harbor Laboratory*

Concluding Remarks: James D. Watson, *Cold Spring Harbor Laboratory*

H. Varmus, B. Stillman, G. Rubin

E. Harlow, R. Jain

R. Nusse, B. Ponder, A. Pawson

SPRING MEETINGS

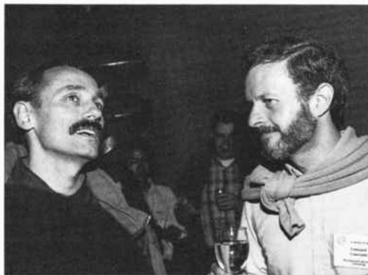
Genetics of Aging

April 2–5 179 participants

ARRANGED BY **Judith Campisi,** University of California, Berkeley
Leonard Guarente, Massachusetts Institute of Technology
Calvin Harley, Geron Corporation

This was the first meeting anywhere devoted solely to the genetics and molecular biology of aging. Approximately 180 participants heard plenary presentations on topics including aging in model systems, DNA helicases and aging, senescence of cultured mammalian cells, telomere shortening in mammalian cells, and genomics of aging. The talks centered on new molecular and genetic approaches to studying aging that promise to elucidate basic underlying mechanisms in model systems and, ultimately, in humans. Notable advances were reported in studies of yeast, *C. elegans*, and cultured fibroblasts. The meeting led to the view that the pace of aging research is accelerating and will provide exciting new insights in the near term.

This meeting was funded in part by the National Institute of Aging, a branch of the National Institutes of Health. Contributions from the Corporate Sponsors, Plant Corporate Associates, and Foundations provided core support for this meeting.



W. Herr, L. Guarente

C. Dennis, C. Harley

PROGRAM

Model Systems
Chairperson: C. Kenyon, *University of California, San Francisco*

Wrn-like Genes
Chairperson: L. Guarente, *Massachusetts Institute of Technology, Cambridge*

Short Talks from Abstracts
Chairpersons: J. Campisi, *Lawrence Berkeley National Laboratory, California;* W. Funk, *Geron Corporation, Menlo Park, California;* L. Guarente, *Massachusetts Institute of Technology, Cambridge;* C. Harley, *Geron Corporation, Menlo Park, California*

Telomeres and Telomerase
Chairperson: T. de Lange, *Rockefeller University, New York, New York*

Cell Senescence and Telomeres
Chairperson: C. Harley, *Geron Corporation, Menlo Park, California*

Cell Senescence and Gene Expression
Chairperson: J. Campisi, *Lawrence Berkeley National Laboratory, California*

Impact of Genomics and Human Genetics on Aging Research
Chairperson: M. Rose, *University of California, Irvine*

Zebrafish Development and Genetics

April 29–May 3 438 participants

ARRANGED BY **Marie-Andree Akimenko,** University of Ottawa
Jose Antonio Campos-Ortega, University of Koln
John Postlethwait, University of Oregon
Eric Weinberg, University of Pennsylvania
Stephen Wilson, Kings College London

This third Cold Spring Harbor Laboratory meeting featured more than 300 platform talks and poster presentations, which covered many of the most interesting areas of current zebrafish research. Exciting sessions were held on Early Development and Dorsoventral Patterning (including maternal control), Organizing the Axis (including reports of cloning of a number of key genes identified in chemical mutagenesis screens), Mesoderm and Endoderm Patterning, Neural Patterning, Axonal Pathfinding, Organogenesis and Histogenesis, Genomics, and Innovative Methods. 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

W. Driever, N. Hopkins

L. Zon, T. Zhong

C. Nislein-Vollard

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.

This meeting was funded in part by the National Institute of Child Health and Human Sciences; National Institute of Environmental Health Sciences; National Institute on Deafness and Other Communication Disorders; National Institute of Neurological Disorders and Stroke; National Institute of Diabetes and Digestive and Kidney Diseases; National Human Genome Research Institute; and the National Center for Research Resources (all branches of the National Institutes of Health); and the National Science Foundation. Contributions from the Corporate Sponsors, Plant Corporate Associates, Foundations, and Technical Contributors provided core support for this meeting.

PROGRAM

Early Development and Dorsoventral Patterning
Chairperson: F. Rosa, *Ecole Normale Supérieure, Paris, France*

Organizing the Axis
Chairperson: L. Solnica-Krezel, *Vanderbilt University, Nashville, Tennessee*

Mesoderm and Endoderm Patterning
Chairperson: M. Halpern, *Carnegie Institution of Washington, Baltimore, Maryland*

Neural Patterning I
Chairperson: C. Beatty, *Ohio State University, Columbus*

Neural Patterning II
Chairperson: M. Cooper, *University of Washington, Seattle*

Axonal Pathfinding
Chairperson: F. Bonhoeffer, *Max-Planck-Institut für Entwicklungsbiologie, Tübingen, Germany*

Sensory Systems, Neural Crest and Head
Chairpersons: T. Whitfield, *University of Sheffield, United Kingdom;* D. Raible, *University of Washington, Seattle*

Organogenesis and Histogenesis I
Chairperson: D. Stainier, *University of California, San Francisco*

Organogenesis and Histogenesis II
Chairperson: A. Shima, *University of Tokyo, Japan*

Keynote Speaker
Chairperson: J. Rossant, *Samuel Lunenfeld Research Institute*

Genomics
Chairperson: P. Hafter, *Max-Planck-Institut für Entwicklungsbiologie, Tübingen, Germany*

Innovative Methods
Chairperson: P. Hackett, *University of Minnesota, St. Paul*

Molecular Chaperones and the Heat Shock Response

May 6–10 400 participants

ARRANGED BY **Carol Gross,** University of California, San Francisco
Arthur Horwich, HHMI/Yale School of Medicine
Susan Lindquist, University of Chicago

The meeting featured advances in three major areas: 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 in understanding the mechanism of action of Hsp20, Hsp60, Hsp70, Hsp90, and Hsp100 class chaperones were presented. A highlight in this area was a first look at high resolution at a small heat shock protein, a hollow "soccer ball" with windows, that likely captures non-native proteins at its surface during heat shock. In the area of stress response, further advances in understanding the heat shock transcription factor were reported. Evidence for a role of Hsp90 as a thermometer sensing cytosolic stress was presented. There were also several exciting talks summarizing the rapid progress in understanding the unfolded response of the ER, by which mis-

C. Gross, P. Walter

M.-J. Gething, E. Craig

I. Yahara, S. Lindquist

folded proteins in this compartment signal to the nucleus to induce a collective of ER chaperones and other components that likely serve a protection function. The topic of protein misfolding in disease was inaugurated with two dedicated sessions, including reports on amyloid formation in Alzheimer's disease, Lowey body formation in Parkinson's disease, nuclear aggregate formation in polyglutamine repeat disease (Huntington), and amyloid formation in prion disease and in transthyretin polyneuropathy. The interactions of chaperones both with amyloidogenic protein in prion disease in yeast and with mutant CFTR in the ER and cytoplasm were also discussed.

This meeting was funded in part by the National Institute of Neurological Disorders and Stroke, a branch of the National Institutes of Health; Affinity Bioreagents, Inc.; Alzheimer's Association; Bristol-Myers Squibb Company; Cystic Fibrosis Foundation; Medical and Biological Laboratories Company, Ltd.; Merck Research Laboratories; and StressGen Biotechnologies Corporation. Contributions from the Corporate Sponsors, Plant Corporate Associates, and Foundations provided core support for this meeting.

PROGRAM

Diseases of Protein Misfolding
Chairperson: S. Radford, *University of Leeds, United Kingdom*

Regulation of the Heat Shock Response
Chairperson: C. Wu, *NCI, National Institutes of Health, Bethesda, Maryland*

Chaperone Structure and Function I
Chairperson: L. Gierasch, *University of Massachusetts, Amherst*

Cell Biology and Proteolysis
Chairperson: E. Vierling, *University of Arizona, Tuscon*

Cell Biology
Chairperson: I. Yahara, *The Tokyo Metropolitan Institute of Medical Science, Japan*

Chaperones in Disease
Chairperson: R. Kaufman, *University of Michigan Medical Center, Ann Arbor*

Chaperone Structure and Function II
Chairperson: B. Bukau, *Albert-Ludwigs Universität Freiburg, Germany*

Genome Mapping, Sequencing, and Biology

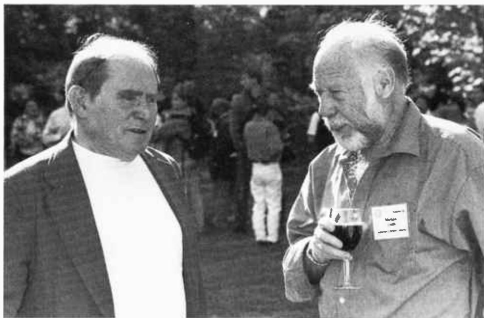
May 13–17 453 participants

ARRANGED BY **Mark Boguski,** National Center for Biotechnology Information
Richard Gibbs, Baylor College of Medicine
Stephen Brown, MRC Mouse Genome Centre

This meeting marked the 11th annual gathering of genome scientists in this setting. The past 10 years have seen remarkable progress in the mapping and sequencing of the genomes of many "model organisms," and there is now strong confidence that the human genome sequence is an attainable goal. Phrases such as "functional genomics" and "post-genome biology" have become common terms. In the light of this emerging shift of emphasis, the new organizers chose to modify the name of the annual meeting to reflect these changes, by the inclusion of the term "biology." More than 450 people from around the world attended the meeting, with 254 abstracts presented describing a broad array of topics relating to the analysis of genomes from a number of different organisms.

E. Landes, R. Gibbs

S. Brown, M. Bucán



S. Brenner, M. Smith

The session topics includes areas such as biological insights from phylo-genomics, mapping methods and technologies, functional genomics, computational genomics, and 21st century genetics. This year's poster session featured progress on sequencing the human genome. 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 a panel discussion on the genome ELSI (Ethical, Legal, and Social Implications) program, chaired by Francis Collins, Director of the National Human Genome Research Institute, which included a timely presentation on the ethical and scientific issues relating to the application of single nucleotide polymorphisms (SNPs).

The major themes of the meeting related to the gathering pace of human genomic sequencing and the increasing number of approaches being developed for using sequence data to perform important biologic studies. Specific presentations continued to report major achievements in the sequencing of microbial genomes, the development of new sequencing technologies, and the use of microarrays for performing genome analysis. The now traditional Saturday afternoon keynote talk was delivered by Harold Varmus, Nobel Laureate and Director of the National Institutes of Health.

With the anticipated achievements in the next decade, future genome meetings at Cold Spring Harbor should continue to serve as the premiere showcase for the Human Genome Project and genome research.

This meeting was funded in part by the National Human Genome Research Institute, a branch of the National Institutes of Health. Contributions from the Corporate Sponsors, Plant Corporate Associates, and Foundations provided core support for this meeting.

PROGRAM

Phylo-Genomics
Chairpersons: C. Fraser, *The Institute for Genomic Research, Rockville, Maryland;* H. Jacob, *Medical College of Wisconsin, Milwaukee*

Computational Genomics
Chairpersons: D. Lipman, *NCBI, National Institutes of Health, Bethesda, Maryland;* G.Duyk, *Millenium Pharmaceuticals, Cambridge, Massachusetts*

Computer Demonstrations I
Chairperson: O. Lichtarge, *Baylor College of Medicine, Houston, Texas*

Mapping
Chairpersons: J. MacPherson, *Washington University School of Medicine, St. Louis, Missouri;* L. Stubbs, *Lawrence Livermore National Laboratory, California*

Large-scale Sequencing Poster Symposium
Chairperson: E. Green, *NHGRI, National Institutes of Health, Bethesda, Maryland*

Computer Demonstrations II
Chairperson: J. Zhang, *NCBI, National Institutes of Health, Bethesda, Maryland*

ELSI Panel Discussion
Chairperson: F. Collins, *NHGRI, National Institutes of Health, Bethesda, Maryland*

Functional Genomics I
Chairpersons: V. Cheung, *University of Pennsylvania, Philadelphia;* L. Stadt, *NCI, National Institutes of Health, Bethesda, Maryland*

Functional Genomics II
Chairpersons: M. Bucan, *University of Pennsylvania, Philadelphia;* E. Rubin, *Lawrence Berkeley National Laboratory, California*

Computer Demonstrations III
Chairperson: R. McCombie, *Cold Spring Harbor Laboratory*

Keynote Speaker
Chairperson: H. Varmus, *National Institutes of Health*

21st Century Genetics
Chairpersons: A. Chakravarti, *Case Western Reserve University, Cleveland, Ohio;* M. Meisler, *University of Michigan, Ann Arbor*

The Cell Cycle

May 20–24 342 participants

ARRANGED BY **Fred Cross,** Rockefeller University
Jim Roberts, Fred Hutchinson Cancer Research Center

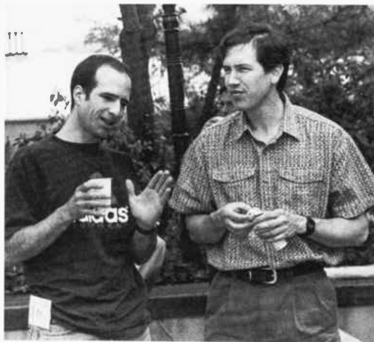
The fifth biannual Cell Cycle meeting was held this year at Cold Spring Harbor. This conference is internationally recognized for its ability to bring together scientists who study cell cycle regulation in eukaryotes ranging from yeast to humans. Diverse themes from previous years received continued attention: the interaction of cell cycle control with developmental and cancer biology; mechanisms of CDK activation by cyclins and by activating phosphorylation; the key role of proteolysis in regulating cell cycle transitions; 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. There was a high level of new interest in the long-standing problem of regulation of DNA replication in the cell cycle, its onset, and its restriction to once per cell cycle. This problem was examined from the perspective of regulation of nuclear transport and binding to origins of key activators of DNA replication in yeast, flies, and mammalian cells in culture. 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*, and mammals were well represented. Once again the striking phylogenetic conservation of cell cycle regulatory mechanisms was readily evident.

The cell cycle conference brought together 342 scientists for talks and the presentation of 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, National Institute of Aging, and the National Institute of Child Health and Human Development, all branches of the National Institutes of Health, and the National Science Foundation. Contributions from the Corporate Sponsors, Plant Corporate Associates, and Foundations provided core support for this meeting.

J. Roberts, C. Sherr


P. Oliver, M. Fero

F. Cross, T. Grodzicker

PROGRAM

Keynote Address
Speaker: B. Stillman, *Cold Spring Harbor Laboratory*

Replication I
Chairperson: J. Diffley, *ICRF Clare Hall Laboratories, South Mimms, United Kingdom*

CDK Regulation
Chairperson: J. Ruderman, *Harvard Medical School, Boston, Massachusetts*

Cell Cycle Control I
Chairperson: C. Sherr, *Howard Hughes Medical Institute, St. Jude Children's Research Hospital, Memphis, Tennessee*

Cell Cycle Control II
Chairperson: L.-H. Tsai, *Howard Hughes Medical Institute, Harvard Medical Institute, Boston, Massachusetts*

Replication II
Chairperson: T. Orr-Weaver, *Whitehead Institute/MIT, Cambridge, Massachusetts*

Development
Chairperson: B. Edgar, *Fred Hutchinson Cancer Research Center, Seattle, Washington*

Mitosis
Chairperson: B. Dunphy, *Howard Hughes Medical Institute, California Institute of Technology, Pasadena*

Telomeres/Senescence/Cancer/Checkpoints
Chairperson: T. de Lange, *Rockefeller University, New York*

Retroviruses

May 26–31 439 participants

ARRANGED BY **Paul Jolicoeur,** Clinical Research Institute of Montreal
Michael Emerman, Fred Hutchinson Cancer Research Center

The annual Retrovirus meeting is the major international meeting to discuss fundamental aspects of retrovirus biology. It has become a comprehensive meeting in which the replication and pathogenesis of many different viruses (including HIV) can be compared. New information was forthcoming from many different levels. For example, a session on viral assembly presented progress on determination of the structure of these viruses and on the pathways in which the structures are assembled. New data were also presented on the means by which viruses enter cells and what steps are limiting in this process. Two new sessions were also added this year to discuss the burgeoning amount of information on how the virus usurps cellular processes to transport viral RNA and proteins and how the virus redirects trafficking of cellular proteins. Participants also learned how cellular proteins may affect integration of the viral DNA and about determinants of pathogenesis and viral transcription.

Contributions from the Corporate Sponsors, Plant Corporate Associates, and Foundations provided core support for this meeting.

PROGRAM

Early Events
Chairpersons: J. Cunningham, *Harvard Medical School, Boston, Massachusetts*; M. Roth, *UMDNJ-Robert Wood Johnson Medical School, Piscataway*

Integration
Chairpersons: A. Skalka, *Fox Chase Cancer Center, Philadelphia, Pennsylvania;* R. Bushman, *Salk Institute, La Jolla, California*

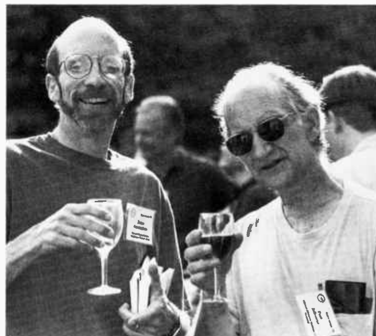
Evolution/Recombination/RT
Chairpersons: J. Levin, *National Institutes of Health, Bethesda, Maryland;* S. Hughes, *National Cancer Institute, Frederick, Maryland*

Pathogenesis
Chairpersons: S. Ross, *University of Pennsylvania, Philadelphia;* H. Fan, *University of California, Irvine*

Transcriptional and Posttranscriptional Regulation
Chairpersons: A. Rice, *Baylor College of Medicine, Houston, Texas;* K.-T. Jeang, *National Institutes of Health, Bethesda, Maryland*

Host-Cell Interactions and New Viruses
Chairpersons: S. Marriot, *Baylor College of Medicine, Houston, Texas;* Y. Ikawa, *Tokyo Medical and Dental University, Japan*

Assembly
Chairpersons: J. Wills, *Pennsylvania State University, Hershey;* P. Boulanger, *Montpellier School of Medicine, France*



J. Cunningham, P. Jolicoeur

Trafficking I
Chairpersons: M. Malim, *University of Pennsylvania, Philadelphia;* E. Hunter, *University of Alabama, Birmingham*

Trafficking II
Chairperson: R. Swanstrom, *University of North Carolina, Chapel Hill*

Packaging and Anti-Virals
Chairpersons: W.-S. Hu, *West Virginia University, Morgantown;* V. Vogt, *Cornell University, Ithaca, New York*

FALL MEETINGS

Cancer Genetics and Tumor Suppressor Genes

August 19–23 431 participants

ARRANGED BY **Terri Grodzicker,** Cold Spring Harbor Laboratory
Doug Hanahan, University of California, San Francisco
Ed Harlow, Massachusetts General Hospital
David Livingston, Dana Farber Cancer Institute
Carol Prives, Columbia University
Bert Vogelstein, Johns Hopkins University

This second 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 oversubscribed, with more than 450 scientists meeting to present more than 320 talks and posters. Launched by a keynote address by Harold Varmus, Director of the National Institutes of Health, the meeting presented an extensive range of work: from location and nature of mutations in different tumors to properties of the p53 tumor

B. Stillman, P. Howley, C. Thompson

T. Jacks, H. Varmus

S. Lowe, P. Branton

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 knockout and transgenic mouse models. The wide range of studies in this area was emphasized by presentations that dealt with tumor progression, angiogenesis, and metastasis. This meeting continued to be enthusiastically supported and all sessions found the lecture and poster halls packed with extensive discussions and exchanges of information. A new feature this year was a session called "NCI Listens," chaired by David Livingston. NCI staff and scientists who served on institute panels answered questions from the audience regarding NCI grants, policy, and future directions.

This meeting was funded in part by the National Cancer Institute, a branch of the National Institutes of Health. Contributions from the Corporate Sponsors, Plant Corporate Associates, Foundations, and Corporate Contributors provided core support for this meeting.

PROGRAM

Keynote Address
Speaker: H. Varmus, *National Institutes of Health, Bethesda, Maryland*

Mouse Models I
Chairperson: D. Hanahan, *University of California, San Francisco*

Signal Transduction
Chairpersons: A. Levine, *Princeton University, New Jersey;* G. Vande Woude, *NCI-Frederick Cancer Research and Development Center, Maryland*

Apoptosis
Chairpersons: M. Oren, *Weizmann Institute of Science, Rehovot, Israel;* C. Thompson, *University of Chicago, Illinois*

Genetics/Genomics
Chairpersons: L. Strong, *University of Texas, Houston;* B. Vogelstein, *Johns Hopkins University Oncology Center, Baltimore, Maryland*

National Cancer Institute Panel
"NCI Listens"

Introduction
Speaker: D. Livingston, *Dana-Farber Cancer Institute, Boston, Massachusetts*

Transcription Regulation
Chairpersons: T. Tlsty, *University of California, San Francisco;* P. Howley, *Harvard Medical School, Boston, Massachusetts*

Repair/Cell Cycle
Chairpersons: M. Kastan, *Johns Hopkins University School of Medicine, Baltimore, Maryland;* C. Harris, *NCI, National Institutes of Health, Bethesda, Maryland*

Mouse Models II
Chairpersons: A. Berns, *The Netherlands Cancer Institute, Amsterdam;* T. Jacks, *Massachusetts Institute of Technology, Cambridge*

Molecular Genetics of Bacteria and Phages

August 25–30 212 participants

ARRANGED BY: **Richard Gourse,** University of Wisconsin, Madison
Allan Grossman, Massachusetts Institute of Technology
Marjorie Russel, Rockfeller University

The Molecular Genetics of Bacteria and Phages meeting continued to re-invent itself this year, keeping up with changing times. 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 topology and dynamic properties of bacterial chromosomes—experiments assessing the frequency with which distant sites on the chromosome "touch" one another led to the conclusion that knots and tangles in DNA restrict the movement of supercoil domains; spectacular images of living *Bacillus subtilis* cells suggest that DNA polymerase is fixed (to the membrane?) and replicating DNA threads its way through this key enzyme. A refined picture of the geometry of the transcription complex emerged from cleverly conceived fusion proteins, sophisticated imaging techniques, and novel chemical cleavage tags. New ways of thinking about populations were discussed—how bacterial pathogens (and the phage of pathogens!) interact with and compromise eukaryotic cells, what genes determine biofilm formation, how bacteria respond to and evolve in unusual environments, and the beginnings of a holistic description of how phage λ makes an appropriate binary "decision."

This meeting was funded in part by the National Science Foundation. Contributions from the Corporate Sponsors, Plant Corporate Associates, Foundations, and Corporate Contributors provided core support for this meeting.

B. Landrick, P. Model, M. Russel

A. Grossman, P. Higgens

PROGRAM

Bacterial Genome I
Chairperson: J. Roth, *University of Utah, Salt Lake City*

Bacterial Genome II
Chairperson: P. Model, *Rockefeller University, New York*

Structure of the Transcription Complex
Chairperson: S. Darst, *Rockefeller University, New York*

Transcriptional Control
Chairperson: L. Rothman-Denes, *University of Chicago, Illinois*

Posttranscriptional Control
Speaker: S. Gottesman, *National Cancer Institute, Bethesda, Maryland*

Signaling and Global Circuits
Speaker: D. Dubnau, *New York Public Health Research Institute, New York*

Heat Shock/Stress Response
Speaker: T. Yura, *HSP Research Institute, Kyoto, Japan*

Cell Cycle and Cell Division
Speaker: A. Wright, *Tufts University School of Medicine, Boston, Massachusetts*

Pathogens and Bacteria-Host Interactions
Speaker: H. Shuman, *Columbia University, New York*

Cell Surfaces, Import, and Export
Speaker: R. Young, *Texas A&M University, College Station*

Mouse Molecular Genetics

September 2–6 441 participants

ARRANGED BY **Allan Bradley,** Baylor College of Medicine/HHMI
Rosa Beddington, National Institute for Medical Research, United Kingdom
Maja Bucan, University of Pennsylvania
Richard Harvey, Walter & Eliza Hall Institute of Medical Research, Australia

This meeting held every other year at Cold Spring Harbor attracted the usual large group of researchers from across the globe. A significant fraction of scientists who apparently had not yet reached saturation from the conditional knockout workshop (which was scheduled immediately before) stayed on for the second meeting refreshed by a free afternoon with BBB (Beach, Barbecue, and Beer).

The Mouse Molecular Genetics 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), 8–9 speakers per session were selected from the abstracts for more concise presentations. The meeting also included the presentation of several

R. Beddington, M. Shen

A. Bradley

R. Behringer

hundred posters in two afternoon sessions. In an attempt to highlight some of the contemporary approaches, an "ENU workshop" was arranged where several groups who are doing mutagenesis in the mouse came and presented their approaches. Although concurrent with one of the poster sessions, this workshop was 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, and a very entertaining introduction on how to analyze mouse behavior.

The meeting exemplified some of the special characteristics of the mouse genetics community—sharing of unpublished data and reagents (including mice). Inevitably, this meeting is also a mouse dating service! Mice described in the meeting have found their way to new homes to mate with other mutants to prove (or disprove) hypotheses developed during interactions at Cold Spring Harbor.

This meeting was funded in part by the National Institute of Child Health and Development, the National Human Genome Research Institute, the National Institute of Mental Health, and the National Cancer Institute, all branches of the National Institutes of Health; and the National Science Foundation. Contributions from the Corporate Sponsors, Plant Corporate Associates, Foundations, and Corporate Contributors provided core support for this meeting.

PROGRAM

Genetics I
Chairperson: L. Silver, *Princeton University, New Jersey*

Genomics
Chairperson: K. Moore, *Millennium Pharmaceuticals, Cambridge, Massachusetts*

Signals and Receptors
Chairperson: P. Beachy, *Johns Hopkins University School of Medicine, Baltimore, Maryland*

Organogenesis
Chairperson: R. Lovell-Badge, *MRC National Institute for Medical Research, London, United Kingdom*

ENU Workshop
Chairperson: S. Brown, *Medical Research Council, Harwell, United Kingdom*

Tumorigenesis
Chairperson: T. Van Dyke, *University of North Carolina, Chapel Hill*

Genetics II
Chairperson: T. Magnuson, *Case Western Reserve University, Cleveland, Ohio*

Patterning
Chairperson: R. Beddington, *National Institute for Medical Research, London, United Kingdom*

Neural Development
Chairperson: A. Joyner, *New York University Medical Center*

Translational Control

September 9–13 445 participants

ARRANGED BY **Lynne Maquat,** Roswell Park Cancer Institute
Michael Mathews, University of Medicine and Dentistry of New Jersey
Peter Sarnow, Stanford University School of Medicine

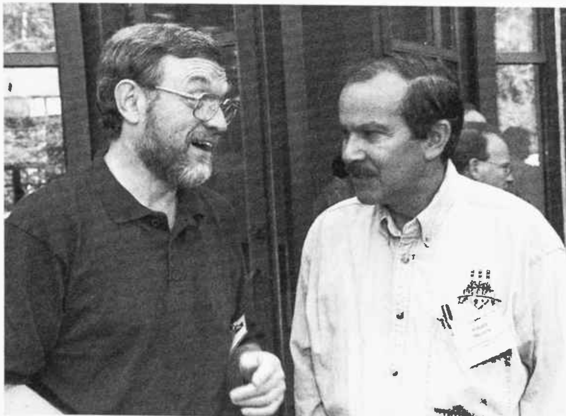
The 1998 Translational Control meeting featured eight platform and three poster sessions that covered 337 published abstracts.

Highlights of the session on Factors and Mechanisms included the macromolecular organization of the yeast and mammalian translation initiation factor eIF3 complex (Hershey and Hinnebusch labs), interactions of eIF5 with eIF2 and its role in formation of 40S preinitiation complexes (Maitra and Hinnebusch labs), and the surprising role of factors eIF1 and eIF1A in the positioning of 48S complexes at the initiator AUG codon (Pestova and Hellen).

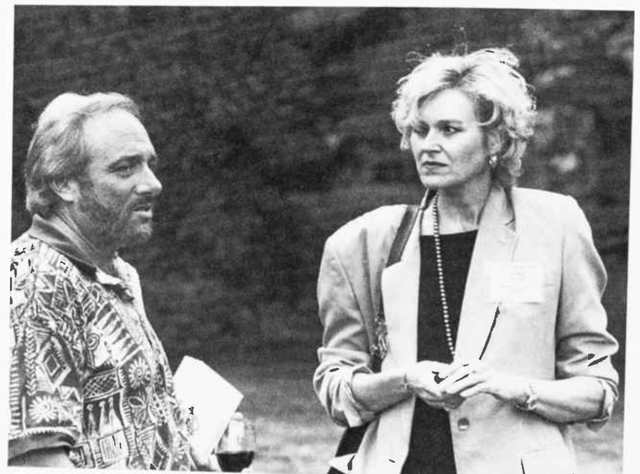
The highlight of the Development session was a talk by Joel Richter demonstrating that visual experience (i.e., light-induced synaptic activation) is accompanied by the polyadenylation of α-Ca^{++}-calmodulin-dependent protein kinase Ii α mRNA in rodent brain. Polyadenylation was shown to be mediated by the binding of cytoplasmic polyadenylation element binding protein (CPEB) to the two CPEs within the mRNA 3′ UTR. Other talks focused on how 3′ UTR-binding proteins, including Bicoid in *Drosophila* (Niessing lab), CPEB and the p82 masking factor in *Xenopus* oocytes (Wormington lab), and Pumilio in *Drosophila* (Williamson and Lehmann labs), repress or activate mRNA translation. Protein binding to 3′ UTR sequences can also affect mRNA stability (Goodwin lab), and protein binding to either 5′ or 3′ UTR RNA was shown to regulate RNA localization and, in some cases, translation (Standart lab, Nielsen lab). Notably, a report from the Wolffe lab demonstrated that the presence of an intron near the 5′ end of H1 transcripts relieves translational silencing of the resulting mRNA in *Xenopus* oocytes.

The UTR Functions session featured talks from the Hohn and Schneider labs that pointed to a role of RNA hairpin structures and base pair complementarity to 18S rRNA in the translational initiation mechanism of ribosome shunting. Most of the remaining talks further substantiated the finding that the 5′ and 3′ ends in mRNA can interact to regulate translation initiation (Millard, Tahara, Benchimol, Allen, and Browing labs).

The RNA Turnover session consisted of four talks on nonsense-mediated mRNA decay (NMD) in either *S. cerevisiae* (Peltz lab, Jacobson lab) or mammalian cells (Maquat lab and Kulozik lab), five talks



M. Mathews, W. Merrick



R. Kaufman, L. Maquat

that described AU-rich element (ARE)-directed effects on mRNA half-life (Brewer lab, Shyu lab, Schneider lab, Wilusz lab, and Kiledjian lab), and presentations on proteins that affect parathyroid mRNA stability (Haveh-Many lab), decapping (Parker lab), or mRNA degradation via poly(A) binding (Sachs lab). The session included evidence for both yeast and mammalian cells that NMD, which appears to involve cytoplasmic translation, requires earlier nuclear processes (Peltz, Maquat, and Kulozik labs). Also noteworthy was the first report of ARE-mediated mRNA decay in vitro and use of in vitro decay to demonstrate that ELAV proteins stabilize ARE-containing mRNAs (Wilusz lab).

The IRES Elements session reported on factors that regulate viral (Hellen, Siddiqui, Jackson labs) and cellular (Sarnow, Elroy-Stein labs) IRESs. Microarray display techniques were employed to demonstrate that cap-independent translation is more prevalent than previously anticipated (Sarnow lab). An interesting story was presented by Andino, who reported that the switch from translation to replication in the polioviral genome is regulated by viral factors that displace IRES-interacting factors, resulting in a stop of translation and commencement of viral replication.

Highlights of the Ends and Means session included the description of structures of eIF4E (Burley lab) and eIF4E binding proteins (Fletcher lab). Regulation of eIF4E by phosphorylation (Sonenberg lab), and sequestration of eIF4G by HSP70 during heat shock (Schneider lab) were additional exciting topics in this session.

The Kinases session concentrated primarily on PKR, the double-stranded RNA-dependent protein kinase. Talks on PKR dealt with a characterization of negative sequences within the amino-terminal region of the protein (Wek lab), associations of PKR with nuclear factor 90 and RNA helicase A (Mathews lab), the role of PKR in apoptosis (Kaufman lab), and the inhibition of PKR by either the E2 envelope protein (Hinnebusch and Lai labs) or the nonstructural 5A protein (Katze lab) of interferon-resistant hepatitis C viruses. Among other presentations was an impressive description of how phosphorylated eIF2a binds the regulatory subcomplex of eIF2B and inhibits a productive interaction with the catalytic subcomplex, thereby inhibiting translation by inhibiting guanine nucleotide exchange (Hinnebusch lab).

The last session, Elongation and Termination, highlighted the regulation of translation by upstream open reading frames (Morris, Geballe, and Sachs labs). In addition, new insights into the mechanism of translational frameshifting were provided (Farabaugh and Atkins labs).

This meeting was funded in part by RiboGene Inc.; Irvine Scientific; and Promega Corporation. Contributions from the Corporate Sponsors, Plant Corporate Associates, Foundations, and Corporate Contributors provided core support for this meeting.

PROGRAM

Factors and Mechanisms
Chairperson: T. Dever, *NICHD, National Institutes of Health, Bethesda, Maryland*

Development
Chairperson: B. Goodwin, *Northwestern University Medical School, Chicago, Illinois*

UTR Functions
Chairperson: D. Morris, *University of Washington, Seattle*

RNA Turnover
Chairperson: S. Peltz, *University of Medicine and Dentistry of New Jersey, Piscataway*

IRES Elements
Chairperson: A.-C. Prats, *INSERM, Toulouse, France*

Ends and Means
Chairperson: A. Sachs, *University of California, Berkeley*

Kinases
Chairperson: S. Morley, *University of Sussex, United Kingdom*

Elongation and Termination
Chairperson: E. Goldman, *UMDNJ-New Jersey Medical School, Newark*

Axon Guidance and Neural Plasticity

September 16–20 365 participants

ARRANGED BY **Corey Goodman,** University of California, Berkeley
Carla Shatz, University of California, Berkeley
Marc Tessier-Lavigne, University of California/HHMI

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

B. Stillman, J. Witkowski, C. Goodman

T. Grodzicker, C. Bargmann

J. Kupa, M. Emerson, J. Flanagan

more difficult for scientists to keep abreast of new developments. It therefore seemed desirable to start a new biennial CSH conference series on "Axon Guidance and Developmental Plasticity of the Nervous System", modeled after the very successful CSH conferences on Drosophila and Zebrafish Developmental Biology. The meeting had an open registration. It was divided into 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.

The response of the field was one of overwhelming enthusiasm. Despite a late start in organization and advertising, and the fact that many scientists in the field already had plans to attend the annual Society for Neuroscience meeting in Los Angeles just a few weeks later, 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, a keynote address was given by Dr. Gerry Fischbach, the new director of the National Institutes of Neurological Diseases and Stroke, who outlined a vision for the field of Neuroscience, and of Developmental Neurobiology in general. 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. It was therefore decided therefore to hold the meeting every two years, alternating every other year with an EMBO meeting in Europe on the same topic.

Contributions from the Corporate Sponsors, Plant Corporate Associates, Foundations, and Corporate Contributors provided core support for this meeting.

PROGRAM

Migration and Guidance I
Chairpersons: L. Landmesser, *Case Western Reserve University, Cleveland, Ohio;* J. Kuwada, *University of Michigan, Ann Arbor*

Migration and Guidance II
Chairpersons: C. Bargmann, *HHMI, University of California, San Francisco;* M.B. Hatten, *Rockefeller University, New York*

Migration and Guidance III
Chairpersons: J. Raper, *University of Pennsylvania School of Medicine, Philadelphia;* J. Culotti, *Samuel Lunenfeld Research Institute, Toronto, Canada*

Signal Transduction
Chairpersons: L. Zipursky, *HHMI, University of California, Los Angeles;* M.-M. Poo, *University of California, San Diego*

Topographic Mapping
Chairpersons: J. Flanagan, *Harvard Medical School, Boston, Massachusetts;* F. Bonhoeffer, *Max-Planck-Institut für Entwicklungsbiologie, Tübingen, Germany*

Targeting and Synaptogenesis
Chairpersons: D. O'Leary, *Salk Institute, La Jolla, California;* S. Burden, *New York University Medical Center*

Developmental Plasticity I
Chairpersons: J. Lichtman, *Washington University School of Medicine, St. Louis, Missouri;* H. Cline, *Cold Spring Harbor Laboratory*

Keynote Address: Growth and regeneration and NINDS
Speaker: G. Fischbach, *Harvard Medical School*

Developmental Plasticity II
Chairpersons: L. Katz, *HHMI, Duke University Medical Center, Durham, North Carolina;* T. Bonhoeffer, *Max-Planck Institute of Neurobiology, München, Germany*

Gene Therapy

September 23–27 385 Participants

ARRANGED BY

Theodore Friedmann, University of California, San Diego
Margaret Liu, Chiron Corporation
Richard Mulligan, HHMI/Children's Hospital/Harvard University
Gary Nabel, University of Michigan

This was the fourth in the Laboratory's series of bi-yearly meetings on Gene Therapy. 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, this 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, including adenoviral, adeno-associated viral, retroviral, and nonviral vectors, whereas other sessions examined emerging

G. Brownlee, T. Friedmann

J. Wilson, J. Leiden

P. Johnson, G. Nabel

technologies, the immune system, and disease models including cancer, cardiovascular diseases, and AIDS. While results and progress in a number of ongoing clinical trials were presented at the latest meeting, the program of the meeting emphasized the molecular genetics and cell biology issues underlying approaches to human gene therapy. The organizers are convinced that interest and progress in human gene therapy will grow enormously in the very near future and that further meetings at Cold Spring Harbor will continue to satisfy an important need in the scientific and medical communities.

This meeting was funded in part by the National Institute of Child Health and Human Development, a branch of the National Institutes of Health. Contributions from the Corporate Sponsors, Plant Corporate Associates, Foundations, and Corporate Contributors provided core support for this meeting.

PROGRAM

Viral Vectors I: Adenovirus
Chairpersons: R. Mulligan, *HHMI, Children's Hospital, Harvard Medical School, Boston, Massachusetts;* J. Leiden, *University of Chicago, Illinois*

Viral Vectors II: AAV; Nonviral Vectors
Chairpersons: J. Samulski, *University of North Carolina, Chapel Hill;* M. Cotten, *Research Institute of Molecular Pathology, Vienna, Austria*

Retrovirus and Lentivirus Vectors
Chairpersons: T. Friedmann, *University of California, San Diego;* L. Leinwand, *University of Colorado, Boulder*

The Immune System
Chairpersons: J. Wilson, *University of Pennsylvania, Philadelphia;* M. Liu, *Chiron Corporation, Emeryville, California*

Emerging Technologies
Chairpersons: I. Verma, *Salk Institute, La Jolla, California;* P. Davis, *Case Western Reserve University, Cleveland, Ohio*

Disease Models I: Cancer, Cardiovascular System
Chairpersons: J. Folkman, *Children's Hospital, Boston, Massachusetts;* S. Woo, *Mt. Sinai School of Medicine, New York*

Disease Models II: Infectious Disease (AIDS), Hematopoietic System
Chairpersons: G. Nabel, *HHMI, University of Michigan, Ann Arbor;* R.M. Blaese, *NHGRI, National Institutes of Health, Bethesda, Maryland*

Other Disease Models
Chairpersons: D. Curiel, *University of Alabama, Birmingham;* G. Brownlee, *University of Oxford, United Kingdom*

Gametogenesis

October 1–4 151 participants

ARRANGED BY **Brigid Hogan,** Vanderbilt University Medical School/HHMI
Allan Spradling, Carnegie Institution of Washington

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 Gametogenesis 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, investigators using *Drosophila* and *C. elegans* reported the identification of new genes required for germ cell specification, meiosis, and germ cell migration. At least one of these, a mouse homolog of the *Drosophila vasa* gene, was shown to be required for spermatogenesis in mice. In addition, germ cells in the mouse ovary appear to be arranged into cysts resembling those in the *Drosophila* ovary. The highlight of the meeting was a talk given by R. Yanagimachi, who discussed initiation of mouse embryonic development in unorthodox ways, including nuclear transfer in eggs (cloning). Following Dr. Yanagimachi's talk, a lively public forum, chaired by A. McLaren, was held on the ethics and potential medical benefits of cloning mammals. The impact of basic research on human reproduction was also discussed in relation to the genetics of human infertility. The next Gametogenesis meeting will be held in the year 2000 with G. Seydoux and C. Wylie serving as the organizers.

This meeting was funded in part by the National Institute of Child Health and Human Development, a branch of the National Institutes of Health; the National Science Foundation; the Lalor Foundation; and the March of Dimes. Contributions from the Corporate Sponsors, Plant Corporate Associates, Foundations, and Corporate Contributors provided core support for this meeting.

PROGRAM

Germ Cell Specification
Chairperson: A. Mahowald, *University of Chicago, Illinois*

Germ Cell Proliferation and Migration
Chairperson: A. McLaren, *University of Cambridge, United Kingdom*

Meiosis I
Chairperson: J. Kimble, *University of Wisconsin, Madison*

Germ-line–Soma Interactions I
Chairperson: B. Hogan, *Vanderbilt Medical Center, Nashville, Tennessee*

Germ-line–Soma Interactions II
Chairperson: M. Matzuk, *Baylor College of Medicine, Houston, Texas*

Gamete Development
Chairperson: L. Cooley, *Yale University School of Medicine, New Haven, Connecticut*

PUBLIC FORUM—CLONING MAMMALS

Meiosis II
Chairperson: S. Roeder, *Yale University School of Medicine, New Haven, Connecticut*

A. McLaren, M. Renfree

S. Wasserman, T. Schupback

Dynamic Organization of Nuclear Function

October 7–11 386 Participants

ARRANGED BY **Robert Goldman,** Northwestern University
John Newport, University of California, San Diego
Pamela Silver, Dana Farber Cancer Institute/Harvard University
David Spector, Cold Spring Harbor Laboratory

The Dynamic Organization of Nuclear Function meeting was a successful gathering of researchers from disparate fields to discuss nuclear structure, its functions, and how both change over time. The opening session focused on the nuclear envelope and lamina, and Jan Ellenberg showed a movie revealing the dynamics of the nuclear envelope during the cell cycle by following the movement of GFP-tagged components of the nucleus. The Nuclear Import/Export session illustrated the complexity of transport of macromolecules across the nuclear envelope, which involves many different receptors, cargoes, and pathways. Elena Conti from the Kuriyan lab presented the X-ray crystal structure of the import receptor, Karyopherin α, both free and bound to an NLS peptide. The interactions between the α receptor and its peptide cargo as seen in the crystal structure suggest a mode for recognition of any NLS bearing import substrate. The idea that the GTPase Ran may not provide the energy for transport but

M. Paddy, A. Belmont

H. Herrmann, R. Goldman

instead may function to load and unload cargo was presented by Karsten Weis in a poster. In the DNA Replication session, John Newport presented evidence that DNA replication does not require the nuclear envelope or nuclear lamina and that initiation of ORC-dependent replication does not appear to require a unique origin sequence. The RNA Processing and Dynamics session focused on the interplay between the events leading up to the production of mature RNA and its subsequent movement out of the nucleus. Joan Politz of the Singer lab presented a new method for following RNA movement within the nucleus of living cells. Melissa Moore discussed preliminary results which suggest that decay of mRNAs containing premature stop codons may occur in the nucleus rather than in the cytoplasm. The meeting closed with a session that focused on connections between nuclear structure and disease. Norma Neff presented data that correlated the activity of the ssDNA helicase BLM, which is absent from cells of Bloom syndrome patients, with its nuclear localization. The variety of subjects and detailed discussions at this meeting offered a comprehensive view of the importance of the dynamic organization of nuclear function.

Contributions from the Corporate Sponsors, Plant Corporate Associates, Foundations, and Corporate Contributers provided core support for this meeting.

PROGRAM

Nuclear Envelope/Lamina
Chairperson: U. Aebi, *University of Basel, Switzerland*

Nuclear Import/Export
Chairperson: D. Forbes, *University of California, San Diego*

Nucleoli and Other Inclusions
Chairperson: J. Gall, *Carnegie Institution of Washington, Baltimore, Maryland*

Genome Organization
Chairperson: A. Belmont, *University of Illinois, Urbana*

DNA Replication and Repair
Chairperson: B. Stillman, *Cold Spring Harbor Laboratory*

Transcription
Chairperson: A. Greenleaf, *Duke University Medical Center, Durham, North Carolina*

RNA Processing and Dynamics
Chairperson: C. Guthrie, *University of California, San Francisco*

Nuclear Structure and Disease
Chairperson: G. Dreyfuss, *University of Pennsylvania School of Medicine, Philadelphia*

SPECIAL WORKSHOP ON EMERGING TECHNOLOGIES

Conditional Genetic Technologies in the Mouse

August 31–September 2 362 participants

ARRANGED BY **Allan Bradley,** HHMI/Baylor College of Medicine, Houston
Klaus Rajewsky, Institute for Genetics, Weyertal, Germany
Janet Rossant, Mt. Sinai Hospital, Toronto, Canada

This new workshop represents the first in a series to address emerging technologies in cancer research. The timing of the workshop was arranged to allow interested participants to attend both workshop and meeting, with a picnic on the beach to wind down between events.

The primary goal of this workshop was to bring together a diverse group of scientists interested in new genome alteration approaches in the mouse, including the key developers of these emerging technologies, researchers who have begun to apply and assess these new approaches, and parties interested in using and extending the technologies. The workshop encouraged an in-depth and unvarnished explanation of technologies such as Cre-Lox, tetracycline-based strategies, hormone-modulated systems, ligand-induced dimerization strategies, insect hormone-based systems, adenovirus-mediated gene transfer, and other *trans*-activating systems, including discussion of real-world experiences in diverse systems and second-generation developments. The schedule included 34 oral presentations and 66 posters focused on all the latest technology and methodology available for controlling gene activation and inactivation in transgenic mice.

In an interesting experiment, the talks (but not the discussions that followed) were recorded and made available on a subscription basis as slide-and-audio presentations over the World Wide Web to anyone with an Internet connection and reasonably up-to-date computer. The experimental Web site (www.leadingstrand.org) has netted more than 175 subscribers and will remain available until September 1999. It is hoped that this workshop on conditional genetic technologies will become a model for future workshops intended to focus on the enabling technologies underlying new trends in cancer research.

G. Schoetz, M. Mayford

K. Rajewsky, L. Federov

J. Witkowski, A. McManon

This meeting was funded in part by the National Cancer Institute, a branch of the National Institutes of Health. Contributions from the Corporate Sponsors, Plant Corporate Associates, Foundations, and Corporate Contributors provided core support for this meeting.

PROGRAM

Site-specific Recombination: Tools of the Trade
Chairperson: K. Rajewsky, *University of Cologne, Germany*

Inducible Systems
Chairperson: H. Bujard, *University of Heidelberg, Germany*

Inducible Recombination
Chairperson: B. Sauer, *NIDDKD, National Institutes of Health, Bethesda, Maryland*

Genomic Engineering: New Developments
Chairperson: A. Bradley, *Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas*

Final Discussion
Chairperson: J. Rossant, *Samuel Lunenfeld Research Institute, Toronto, Canada*

WINTER BIOTECHNOLOGY CONFERENCE

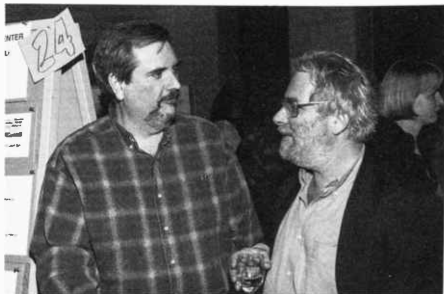
The *Arabidopsis* Genome: A Model for Crop Plants

December 3–6 122 participants

ARRANGED BY **R. Martienssen**, Cold Spring Harbor Laboratory
M. Bevan, John Innes Center, United Kingdom

This meeting was the second in a series of biotechnology symposia aimed at bringing together those groups sequencing and interpreting the sequence of *Arabidopsis thaliana* with those attempting to determine the biological function of plant genes and other chromosomal sequences. Steven Tanksley opened the meeting with a fascinating overview of the impact plant genomics is beginning to have upon the identification and use of economically valuable traits for crop improvement. This was immediately followed by a session on crop plant genomics, illustrating how comparative approaches are yielding information on traits in crops such as maize, sorghum, rice, and legumes. The following two sessions revealed the current status of *Arabidopsis* sequencing, the development of accessible databases, the progress being made in mapping (including the first *Arabidopsis* SNP map), and the analysis of chromosome structure in plants. Further sessions on functional genomics highlighted approaches to the

S. Rounsley, M. Anderson, J. Jones



D. McCombie, M. Ashburner

identification of gene function, including multiple insertional mutagenesis strategies, and gave a foretaste of how these strategies will be used to identify biological function on a large scale. A final session on gene expression and bioinformatics highlighted novel means to catalog, assemble, interpret, and disseminate this vast flood of new data. The need to simultaneously examine gene function and expression in a systematic way was emphasized in a large number of the presentations. In all, the meeting included 39 oral presentations, 13 posters, and additionally, a number of posters were presented by the sequencing consortium on current progress in *Arabidopsis* genome sequencing, which is likely to be completed within the next 2 years. In light of the probable completion of the genome, the third conference on this topic is planned to be held at the end of the year 2000.

This meeting was funded in part by Cereon Genomics Inc. (a division of Monsanto); DeKalb Genetics Corporation, Keygene N.V.; Novartis Crop Protection, Inc., and Pioneer Hi-Bred International, Inc. Contributions from the Corporate Sponsors, Plant Corporate Associates, Foundations, and Corporate Contributors provided core support for this meeting.

PROGRAM

Keynote Address
Chairperson: S.D. Tanksley, *Cornell University, Ithaca, New York*

Crop Plant Genomics
Chairperson: R. Michelmore, *University of California, Davis*

Progress in *Arabidopsis* Genome Sequencing and Database Development
Chairperson: J. Ecker, *University of Pennsylvania, Philadelphia*

Mapping and Chromosome Structure
Chairperson: D. Preuss, *University of Chicago, Illinois*

Functional Genomics I
Chairperson: J. Jones, *John Innes Centre, Norwich, United Kingdom*

Functional Genomics II: Applications
Chairperson: M. Sussman, *University of Wisconsin, Madison*

Gene Expression and Informatics
Chairperson: M. Bevan, *John Innes Centre, Norwich, United Kingdom*

POSTGRADUATE COURSES

The summer program of Postgraduate Courses 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 a workshop staff from many laboratories around the world and supplement this staff with a series of seminar speakers.

Advanced Molecular Cytogenetics

March 4–10

INSTRUCTORS **Ried, Thomas,** M.D., National Institutes of Health
Schrock, Evelin, M.D., National Institutes of Health

ASSISTANTS **Janisch, Catherine,** Applied Spectral Imaging
Padilla-Nash, Hesed, National Institutes of Health

This course focused on advanced molecular cytogenetic methodologies for the visualization of chromosome aberrations. Emphasis was placed on applications of spectral karyotyping (SKY) for the refinement of clinical and tumorcytogenetic diagnostics. In addition, the students covered comparative genomic hybridization (CGH) and novel sensitive methods for visualization of FISH experiments (tyra-

mides). Methodologies, technologies, and scientific expertise for the microscopic visualization of chromosomal alterations were provided. The course was designed for cytogeneticists who use chromosome analytical methods in diagnostics and basic research and for the molecular biologists who intend to employ advanced molecular cytogenetic methods in mammalian chromosome analysis. Among the methods presented were the preparation of tagged DNA probes, FISH and multicolor detection methods, quantitative digital image analysis, spectral imaging, and data interpretation. Students were encouraged to bring their own samples of human or mouse origin for SKY and CGH analysis. State-of-the-art microscopic and imaging equipment was available to gain hands-on experience. The laboratory portion of the course was supplemented by invited lectures by Drs. David H. Ledbetter, Janet D. Rowley, Jeffrey M. Trent, and David C. Ward, who presented updates in the field of molecular cytogenetic research and diagnostics.

PARTICIPANTS

Adeyinka, A., B.S., Lund University Hospital, Sweden
Barch, M., B.S., M.S., University of Louisville, Kentucky
Burki, N., B.S., M.D., Yale University, New Haven, Connecticut
Capua, E., B.S., North York General Hospital, Canada
Davidson, J., B.S., University of Cambridge, United Kingdom
Exeler, J.R., B.S., Ph.D., University of Munster, Germany
Forus, A., B.S., M.S., Ph.D., Norwegian Radium Hospital, Norway
Kahkonen, M., Ph.D., Tampere University Hospital, Finland
Lau, C., Ph.D., M.D., Baylor College of Medicine
Lytle, C., B.S., Genzyme Genetics, New Mexico
Montgomery, K., B.S, Ph.D., University of New Mexico
Nordgren, A., M.D., Karolinska Institute, Sweden
Rao, V.K., B.S., M.D., National Cancer Institute
Roque, L, B.S., Portuguese Cancer Institute, Portugal
Wong, N., B.S., M.S., Ph.D., Chinese University of Hong Kong, China
Zou, Y, B.S, M.D., Ph.D., Medical College of Virginia

SEMINARS

Garini, Y., Applied Spectral Imaging, Carlsbad, California. Spectral imaging.
Hohman, B., Carl Zeiss, Inc., Thornwood, New York. Fluorescence microscopy.
Ledbetter, D., University of Chicago, Chicago, Illinois. Current status and future prospect of FISH in the clinical laboratory.
Moens, P., NEN Life Science Products, Inc., Boston, Massachusetts. Tyramide signal amplification.
Piper, J. VYSIS, Inc., Downers Grove, Illinois. Software for comparative genomic hybridization.
Ried, T., National Institutes of Health, Bethesda, Maryland. Cancer cytogenetics revisited: Comparative genomic hybridization and spectral karyotyping.
Rowley, J., University of Chicago Medical Center, Chicago, Illinois. Chromosome translocations: Dangerous liasions.
Trent, J., National Institutes of Health, Bethesda, Maryland. Applications of new genomic technologies in cancer research: Microarrays and microdissection.
Ward, D., Yale University School of Medicine, New Haven, Connecticut. Padlock probes and rolling-circle amplification.

Advanced Genome Sequence Analysis

March 18–31

INSTRUCTORS

Chen, Ellson, Ph.D., PE Applied Biosystems, Inc.
Gibbs, Richard, Ph.D., Baylor College of Medicine
McCombie, W. Richard, Ph.D., Cold Spring Harbor Laboratory
Mardis, Elaine, Ph.D., Washington University School of Medicine
Muzny, Donna, Ph.D., Baylor College of Medicine
Wilson, Richard, Ph.D., Washington University School of Medicine
Zuo, Lin, M.D., AxyS Pharmaceuticals Inc.

ASSISTANTS

Bentley, Kimberly, Case Western Reserve University
Dedhia, Neilay, Cold Spring Harbor Laboratory
De la Bastide, Melissa, Cold Spring Harbor Laboratory
Fitzgerald, Michael, Genome Therapeutics Corporation
Galvin, Margaret, AxyS Pharmaceuticals Inc.
Gnoj, Lidia, Cold Spring Harbor Laboratory
Greco, Tracie, Washington University School of Medicine
Habermann, Kristina, Cold Spring Harbor Laboratory
Huang, Emily, Cold Spring Harbor Laboratory
Johnson, Doug, Washington University School of Medicine
Ma, Peter, PE Applied Biosystems, Inc.
Parnell, Larry, Cold Spring Harbor Laboratory
Schutz, Kristin, Cold Spring Harbor Laboratory

The purpose of this course was to teach students the range of techniques and project management skills required to carry out a large-scale sequencing project. The course provided a 2-week, extensive laboratory experience in which the students worked as a team to carry out a large-scale sequencing

project. Recent advances in the automation of DNA sequencing have opened new possibilities for the analysis of complex genomes at the DNA sequence level. This course provided intensive training in this rapidly evolving field. The course emphasized techniques and strategies for using automated sequences to sequence large, contiguous genomic regions. Students carried out all of the steps in the sequencing process from preparing cosmid DNA to computer analysis of the finished sequence. Topics included subclone library generation, large-scale template purification, sequencing reactions, gel analysis on automated sequencers, sequence assembly, gap filling, and conflict resolution. Students worked in groups of four to sequence and analyze a large region of genomic DNA. A 143-kb human BAC clone containing two chemokine receptor genes was sequenced and analyzed. Through this process, the students were trained in crucial project and data management techniques. A series of lecturers discussed their applications of these techniques as well as alternate strategies for high-speed automated DNA sequencing and analysis. This year, the course introduced new material in the analysis phase. Students had an opportunity to use laboratory methods to confirm findings predicted from the genomic sequence data. The analysis phase also included the use of DNA sequencing technology for polymorphism and mutation detection.

PARTICIPANTS

Bashkin, J., B.S. Ph.D., Molecular Dynamics, Sunnyvale, California
Battistutti, W., C.V.M., D.V.M., National Institute of Sera and Vaccines, Vienna, Austria
Blair, M., B.S., Ph.D., Cornell University, Ithaca, New York
Bruce, D., B.S., Los Alamos National Laboratory, New Mexico
Eley, G., B.S., Mayo Foundation, Rochester, Minnesota
Frazer, K., B.A., Ph.D., Lawrence Berkeley National Laboratory, Berkeley, California
Gardmo, C., M.S., Karolinska Institute, Huddinge, Sweden
Hinkle, G., B.A. Ph.D., Marine Biological Laboratory/University of Massachusetts, Woods Hole
Lee, R., B.S., M.S., Hoechst Marion Roussel, Inc., Bridgewater, New Jersey
Li, L., B.S. Ph.D., University of Texas, Houston
Lovitt, C., B.S., SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania
Oklu, R., B.S., University of Cambridge, Cambridge, United Kingdom
Penuela, S., B.S., University of Minnesota, St. Paul
Rath, P.C., B.S., Ph.D., Jawaharlal Nehru University, New Delhi, India
Rode, C., B.A., M.S., University of Michigan, Ann Arbor
Shan, Y., B.S., Hua Zhong Agricultural University, WuHan, China

SEMINARS

Chen, E., Applied Biosystems, Inc., Foster City, California. Ordered-shotgun sequencing.
Fraser, C., National Institutes of Health, Bethesda, Maryland. Microbial genome sequencing.
Kwok, P.-Y., Washington University School of Medicine, St. Louis, Missouri. Single nucleotide polymorphisms.
Mardis, E., Washington University School of Medicine, St. Louis, Missouri. Technology development at large scale sequencing centers.
McPherson, J., Washington University School of Medicine, St. Louis, Missouri. Physical mapping of the human genome.
Meldrum, D., University of Washington, Seattle. New sequencing technologies.
Miller, W., Pennsylvania State University, University Park. Comparative genomics of the human and mouse.
Schwartz, D., New York University, New York. Optical mapping.
Stein, L., Cold Spring Harbor Laboratory. Genome informatics resources on the Internet.
Watson, J., Cold Spring Harbor Laboratory. History of the Human Genome Project.
Wilson, R., Washington University School of Medicine, St. Louis, Missouri. The *C. elegans* genome project.

Protein Purification and Characterization

April 15–28

INSTRUCTORS	**Burgess, Richard,** Ph.D., University of Wisconsin, Madison **Courey, Albert,** Ph.D., University of California, Los Angeles **Lin, Sue-Hwa,** Ph.D., University of Texas/MD Anderson Cancer Center, Houston **Mische, Sheenah,** Ph.D, Rockefeller University
ASSISTANTS	**Arthur, Terry,** University of Wisconsin, Madison **Chen, Guoqing,** University of California, Los Angeles **Earley, Karen,** University of Texas/MD Anderson Cancer Center **Thompson, Nancy,** University of Wisconsin, Madison **Fernandez, Joseph,** Rockefeller University **Tantingco, Vicky,** University of Texas/MD Anderson Cancer Center **Weinberg, Catherine,** Rockefeller University

This course was intended for scientists who are not familiar with techniques of protein isolation and characterization. It was a rigorous program that included laboratory work all day and a lecture with discussion session every evening. Each student became familiar with each of the major techniques in protein purification by actually performing four separate isolations including (1) a regulatory protein from muscle tissue; (2) a sequence-specific, DNA-binding protein; (3) a recombinant protein overexpressed in *E. coli*; and (4) a membrane-bound receptor. A variety of bulk fractionation, electrophoretic, and chromatographic techniques were employed including precipitation by salts, pH, and ionic polymers; ion exchange, gel filtration, hydrophobic interaction, and reverse-phase chromatography; lectin affinity,

ligand affinity, oligonucleotide affinity, and immunoaffinity chromatography; polyacrylamide gel electrophoresis and electroblotting; and high-performance liquid chromatography. Procedures were presented for solubilizing proteins from inclusion bodies and refolding them into active monomeric forms. Methods of protein characterization were utilized including immunological and biochemical assays, peptide mapping, amino acid analysis, protein sequencing, and mass spectrometry. Emphasis was placed on strategies of protein purification and characterization using both classical and modern techniques and equipment. Guest lecturers discussed protein structure and function, methodologies for protein purification and characterization, and applications of protein biochemistry to cell molecular and developmental biology.

PARTICIPANTS

Bartsch, S., Ph.D., Columbia University, New York
Belostotsky, D., Ph.D., State University of New York, Albany
Byrne, M., Ph.D., Cold Spring Harbor Laboratory
Cinar, B., M.S., D.V.M., University of Virginia, Charlottesville
De Las Penas, A., Ph.D., University of Virginia, Charlottesville
Ehsani, N., Ph.D., University of Helsinki, Finland
Ferreira, J., M.D., University of Lisbon, Portugal
Hua, V., Ph.D., Massachusetts Institute of Technology/HHMI, Cambridge
Lieberthal, W., M.D., Boston University Medical Center, Massachusetts
Maiorella, B., Ph.D., Chiron Corporation, Emeryville, California
McDermott, B., M.A., Columbia University, New York
Merchant, J., M.D., Ph.D., University of Michigan, Ann Arbor
Merrick, A., Ph.D., National Institutes of Health, Research Triangle Park, North Carolina
Stuckey, J., Ph.D, University of Michigan, Ann Arbor
Szigyarto, C., B.S., Stockholm University, Sweden
Teixeira, M.T., B.S., Institut Pasteur, Paris, France

SEMINARS

Burgess, R., University of Wisconsin, Madison. Overview of protein purification, immunoaffinity purification. Biochemical studies of RNA polymerase/sigma factor interactions.
Collier, J., Harvard Medical School, Boston, Massachusetts. Membrane translocation by diphtheria and anthrax toxins.
Courey, A., University of California, Los Angeles. Protein-protein interactions in dorsal/ventral pattern formation in *Drosophila*.
Eisenberg, D., University of California, Los Angeles. Domain swapping and protein-protein interactions.
Graves, B., University of Utah, Salt Lake City. Structural analysis of autoinhibition of transcription factor ETS1.
Guidotti, G., Harvard University, Boston, Massachusetts. Membrane proteins and extracellular ATP.
Lin, S.-H., University of Texas/M.D. Anderson Cancer Center, Houston. Role of cell adhesion molecule (C-CAM) in prostate cancer.
Mische, S., Rockefeller University, New York. Microanalytical protein preparation and characterization.
Stillman, B., Cold Spring Harbor Laboratory. Biochemical approach to understanding replication of the eukaryotic cell.

Early Development of *Xenopus laevis*

April 19–28

INSTRUCTORS
Krieg, Paul, Ph.D., University of Texas, Austin
Moody, Sally, Ph.D, George Washington University Medical Center

ASSISTANTS
Cleaver, Ondine, University of Texas, Austin
Kenyon, Kristy, George Washington University Medical Center
Kroll, Kristen, Harvard University, Boston
Zorn, Aaron, Wellcome/CRC Institute, Cambridge, United Kingdom

This course provided extensive laboratory exposure to the biology, manipulation, and use of embryos from the frog, *Xenopus laevis*. This course was suited both for investigators who had no experience with *Xenopus* and for those who worked with *Xenopus* and wished to learn new techniques. All students had current training in molecular biology and some knowledge of developmental biology. The course consisted of intensive laboratory sessions, supplemented by daily lectures and demonstrations from experts in both experimental and molecular embryology. Areas covered included (1) care of adults and embryo isolation; (2) stages of embryonic development and anatomy; (3) whole-mount in situ hybridization and immunocytochemistry; (4) microinjection of eggs and oocytes, including lineage tracers, DNA constructs, mRNA, and antisense oligonucleotides; (5) micromanipulation of embryos, including explant and transplantation assays; (6) preparation of transgenic embryos; and (7) use of *Xenopus tropicalis* as a genetic model.

PARTICIPANTS

Bogdan, S., B.S., University of Essen Medical School, Germany
Frommer, G., B.S. Ph.D., University of Tübingen, Germany
Gross, S., B.A., Ph.D., University of Colorado School of Medicine/HHMI, Denver
Henrich, T., B.S., Max-Planck Institute, Goettingen, Germany
Hutcheson, D., B.A., University of Utah, Salt Lake City
Meijer, H., M.S., Utrecht University, The Netherlands
Ohan, N., B.S., Ph.D., Ottawa Civic Hospital/Loeb Research Institute, Ontario, Canada
Poage, R., B.S. Ph.D., University of Pittsburgh, Pennsylvania
Radcliffe, M., B.S., Ph.D., University College London, United Kingdom
Rollins-Smith, L., B.A., Ph.D., Vanderbilt University, Tennessee
Saulnier, D., B.S., Swiss Federal Institute of Technology, Zurich, Switzerland
Singhatat, V., B.S., Ohio State University College of Pharmacy, Columbus
Stancheva, I., M.S., Ph.D., University of Edinburgh, Scotland, United Kingdom
Wheeler, G., B.S., Ph.D., University of Dundee, United Kingdom
Woda, J., B.A., Harvard Medical School, Cambridge, Massachusetts

SEMINARS

Christian, J., Oregon Health Sciences University, Portland. Posttranscriptional regulation of BMP activity during vertebrate embryonic patterning.
Dawid, I., National Institutes of Health, Bethesda, Maryland. Some aspects of organizer function in *Xenopus*.
Grainger, R., University of Virginia, Charlottesville. Lens determination.
Heasman, J., University of Minnesota School of Medicine, Minneapolis. Maternal control of cell fates in the blastula.

Keller, R., University of Virginia, Charlottesville. *Xenopus* gastrulation.

King, M.L., University of Miami Medical School, Florida. RNA localization in the *Xenopus* oocyte.

Klymkowsky, M., University of Colorado, Boulder. Plakoglobin, the other ARM of vertebrates: Its role in cell adhesion and signaling.

Kroll, K., Harvard University, Boston, Massachusetts. An introduction to transgenesis in *Xenopus*.

Krieg, P., University of Texas, Austin. Development of the cardiovascular system.

Moody, S., George Washington University Medical Center, Washington, D.C. Cell-cell interactions and ancestry influence the fate to become retina.

Arabidopsis Molecular Genetics

June 3–23

INSTRUCTORS	**Barton, Katherine,** Ph.D., University of Wisconsin, Madison **Glazebrook, Jane,** Ph.D., University of Maryland, College Park **Lam, Eric,** Ph.D., Rutgers University, New Brunswick, New Jersey
ASSISTANTS	**Long, Jeff,** University of Wisconsin, Madison **Pontier, Dominique,** Rutgers University, New Brunswick, New Jersey **Zhou, Nan,** University of Maryland, College Park

This course provided an intensive overview of topics in plant growth and development, focusing on molecular genetic approaches to understanding plant biology. It emphasized recent results from *Arabidopsis thaliana* and other model plants and provided an introduction to current methods used in *Arabidopsis* research. It was designed for scientists with experience in molecular techniques or in plant biology who wish to work with *Arabidopsis*. The course consisted of a vigorous lecture series, a hands-on laboratory, and informal discussions. Discussions of important topics in plant research were presented by the instructors and by invited speakers. These seminars included plant anatomy; plant development (including development of flowers, roots, meristems, embryos and the epidermis); perception of light and photomorphogenesis; responses to pathogens and to other environmental stresses; synthesis and function of secondary metabolites and hormones; macromolecular transport; and the role of ion channels in plant cell physiology. Lectures describing bioinformatics tools available to the

Arabidopsis community and the potential of the *Arabidopsis* genome project for accelerating *Arabidopsis* research were also included. Speakers provided overviews of their fields, followed by in-depth discussions of their own work. The laboratory sessions provided an introduction to important techniques currently used in *Arabidopsis* research. These included studies of *Arabidopsis* development, mutant analysis, studies of epidermal features, in situ detection of RNA, histochemical staining, transient gene expression, detection and analysis of plant pathogens, applications of green fluorescent protein fusions, techniques commonly used in genetic and physical mapping, and map-based cloning.

PARTICIPANTS

Alvarez, M., M.S., Ph.D., National University Cordoba, Argentina
Boch, J., B.S., Ph.D., Washington University, St. Louis, Missouri
Chalivendra, S., M.S., Ph.D., University of Illinois, Urbana
Destefano, L., B.S., Ph.D., Institute of Paper Science and Technology, Atlanta, Georgia
Hagenblad, J., M.S., Lund University, Sweden
Harmer, S., B.A., University of California, San Francisco
Hay, A., B.S., University of California, Berkeley
Hillebrand, H., M.S., Ph.D., Ruhr-Universitat Bochum, Germany
Kadalayil, L., M.S., Ph.D., Stockholm University, Sweden
Leader, D., B.S., Ph.D., Zeneca Plant Science, Berkshire, United Kingdom
Maloof, J., B.A., University of California, San Francisco
Matteucci, A., B.S., Ph.D, National Institute of Nutrition, Rome, Italy
Rajasekhar, V., B.S., Ph.D., Samuel Roberts Nobel Foundation, Ardmore, Oklahoma
Reynaga-Pena, C., B.S., Ph.D., University of California, Riverside
Shimizu, K., B.S., Kyoto University, Japan
Xiao, Y., B.S., M.S., Iowa State University, Ames

SEMINARS

Assmann, S., Pennsylvania State University, University Park. Patch-clamping ion channels and transport.
Barton, K., University of Wisconsin, Madison. Development of leaf or embryo.
Bleecker, T., University of Wisconsin, Madison. Ethylene/auxin signaling.
Briggs, W., Carnegie Institution of Washington, Stanford, California. Light perception.
Britt, A., University of California, Davis. DNA repair.
Chapple, C., Purdue University, West Lafayette, Indiana. Secondary metabolism.
Cherry, M., Stanford University, California. *Arabidopsis* resources AtDB.
Citovsky, V., State University of New York, Stony Brook. Macromolecular trafficking.
Clark, S., University of Michigan, Ann Arbor. Organ formation.
Crosby, B., National Research Council of Canada, Saskatoon. Protein interaction and floral morphogenesis.
Dengler, N., University of Toronto, Canada. Plant anatomy.
Glazebrook, J., University of Maryland, College Park. Map-based cloning strategies.
Haughn, G., University of British Columbia, Vancouver, Canada. Seed and ovule development.
Last, R., Boyce Thompson Institute for Plant Research/Cornell University, Ithaca, New York. Plant stress responses.
Ma, H., Cold Spring Harbor Laboratory. Molecular analysis of plant development.
Martienssen, R., Cold Spring Harbor Laboratory. Enhancer traps in *Arabidopsis*.
Preuss, D., University of Chicago, Illinois. Male gametophyte development and fertilization.
Richards, E., Washington University, St. Louis, Missouri. Genome organization and epigenetics. Programmed cell death.
Rounsley, S., The Institute for Genomic Research, Rockville, Maryland. Computer lab.
Schifelbein, J., University of Michigan, Ann Arbor. Root development.
Sederoff, R., North Carolina State University, Raleigh. Tree genetics.
Theologis, A., Plant Gene Expression Center, Albany, California. *Arabidopsis* genome project.
Weigel, D., Salk Institute, La Jolla, California. Floral development: Evolution meets function.
Wessler, S., University of Georgia, Athens. Transposons and genome evolution.

Genetic Epidemiological Studies of Complex Diseases

June 10–16

INSTRUCTORS **Risch, Neil,** Ph.D., Stanford University
Squires-Wheeler, Elizabeth, Ph.D., Columbia University College of Physicians & Surgeons

This lecture course considered the difficulties in studying the genetic basis of complex disorders, such as diabetes, Alzheimer's disease, schizophrenia, and epilepsy, with a particular emphasis on neuropsychiatric conditions. Discussed were genetic-epidemiologic study designs, including family, twin, and adoption studies and the 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 analysis. The efficiency and robustness of different designs for such analysis were discussed, and how evidence from genetic-epidemiologic studies informs both the design and interpretation of molecular genetic studies was considered. Recent discoveries of genes for both Mendelian and non-Mendelian diseases guided the discussion of the various methodologic issues.

PARTICIPANTS

Andersson, C., B.M., M.D., Karolinska Hospital, Stockholm, Sweden
Bergen, A., B.A., Ph.D., National Institutes of Health, Rockville, Maryland
Bradley, M., M.D., Karolinska Institute, Stockholm, Sweden
Burk, M., M.D., Albert Einstein College of Medicine, Bronx, New York
Dahlman, Ingrid, M.D., Karolinska Institute, Stockholm, Sweden
Ferrence, B., M.S, M.D., Yale University School of Medicine, New Haven, Connecticut
Gaffney, P., B.S., M.D., University of Minnesota, Minneapolis
Honer, W., M.D., University of British Columbia/Jack Bell Research Center, Vancouver, Canada

Iacoviello, L., M.D., Consorzio Mario Negri Sud, Santa Maria Imbaro, Italy
Kanes, S., M.D., Ph.D., Yale University, New Haven, Connecticut
Lee, Y.-A., B.S., M.D., Max Delbrück Center for Molecular Medicine, Berlin, Germany
Lin, J.P., M.D., Ph.D., National Institutes of Health, Bethesda, Maryland
Neul, J., M.D., Ph.D., University of Chicago, Illinois
Post, W., B.A., M.D., Johns Hopkins University Hospital, Baltimore, Maryland
Ritter, D., M.D., D.M.S., St.Louis University Medical Center, St. Louis, Missouri
Rossing, M.A., B.S., D.V.M., Ph.D., Fred Hutchinson Cancer Research Center, Seattle, Washington
Silver, J., B.S., Ph.D., New York University Medical School/North Shore Hospital, Manhasset, New York
Tseng, T.-L., B.S., M.S., State University of New York, Stony Brook
Verheyen, G., B.S., Ph.D., University of Antwerp, Wilrijk, Belgium
Wijemga, C., B.S., Ph.D., Utrecht University, The Netherlands
Willour, V., B.S., Ph.D., Johns Hopkins University, Baltimore, Maryland
Wong, G., B.S., Ph.D., University of Washington, Seattle
Zwaigenbaum, L., M.D., McGill University, Montreal, Canada

SEMINARS

Bressman, S., Beth Israel Medical Center, New York. Dystonia.
Keats, B., Louisiana State University Medical Center, New Orleans. Linkage analysis I.
Merikangas, K., Yale University, New Haven, Connecticut. Further issues in genetic epidemiology.
Ott, J., Rockefeller University, New York. Linkage analysis II.
Ottman, R., Columbia University, New York. First principles of genetic epidemiology.
Risch, N., Stanford University, Stanford, California. Biometrical genetics: Twin and adoption designs.
Sandkuijl, L., Leiden University, The Netherlands. Linkage disequilibrium in founder populations. Bipolar disorder.
Spielman, R., University of Pennsylvania School of Medicine, Philadelphia. Association studies: Candidate genes, transmission disequilibrium test, genome mismatch scanning. Diabetes.
Risch N., Stanford University, Stanford, California. Multiple sclerosis.

Advanced Bacterial Genetics

June 10–30

INSTRUCTORS	**Bassler, Bonnie,** Ph.D., Princeton University **Manoil, Colin,** Ph.D., University of Washington, Seattle **Slauch, James,** Ph.D., University of Illinois, Champaign-Urbana
ASSISTANTS	**Cosma, Christine,** University of Washington, Seattle **Freeman, Jeremy,** Princeton University **Janakiraman, Anuradha,** University of Illinois, Champaign-Urbana

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; PCR; 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

Agrawal, R., M.S., Ph.D., New York State Department of Health, Albany
Boehm, A., B.S., University of Konstanz, Germany
Ciampi, S., B.S., Ph.D., University of Bari, Italy
Dixon, T., B.S., Duke University Medical Center, Durham, North Carolina
Folkesson, A., M.S., Karolinska Institute, Stockholm, Sweden
Fox, J., B.A. Ph.D., Tufts University School of Medicine, Boston, Massachusetts
Hochkoeppler, A., B.S., Ph.D., University of Bologna, Italy
Karkhoff-Schweizer, R.A., B.S.,B.A., Ph.D., Colorado State University, Fort Collins
Membrillo-Hernandez, J., B.S., Ph.D., Harvard Medical School, Boston, Massachusetts
Nechaev, S., M.S., State University of New Jersey/Rutgers State University, Piscataway
Rabus, R., B.S. Ph.D., University of California, San Diego
Tearle, R., B.S., Ph.D., BresaGen Ltd., Thebarton SA, Australia
Tegmark-Wisell, K., M.D., Karolinska Institute, Stockholm, Sweden
Tersteegen, A., B.S., M.S., Max-Planck Institute, Marburg, Germany
Viboud, G., B.S., Ph.D., National Institute of Infectious Diseases, Buenos Aires, Argentina
Winrow, M.A., B.S., M.S., Ph.D., Elizabeth City State University, North Carolina

SEMINARS

Brown, S., Copenhagen University, Denmark. Genetic analysis of the biological-inorganic interface.
Gottesman, S., National Cancer Institute, Bethesda, Maryland. Two levels of posttranscriptional control of the stationary phase σ factor, RpoS.
Hultgren, S., Washington University School of Medicine, St. Louis, Missouri. The link between pilis biogenesis and pathogenesis.
Kaplan, H., University of Texas, Houston. Sensing and integration of multiple signals during *Myxococcus* multicellular development.
Maloy, S., University of Illinois, Urbana. Regulation of gene expression by membrane sequestration.
Silhavy, T., Princeton University, New Jersey. Parallel pathways perceive periplasmic problems.
Stewart, V., Cornell University, Ithaca, New York. Indirect inference.
Storz, G., National Insitutes of Health, Bethesda, Maryland. Regulation of the *E. coli* response to oxidative stress.
Taylor, R., Dartmouth University, Hanover, New Hampshire. Genetic approaches to understanding the virulence mechanisms of *Vibrio cholerae*.

Integrated Approaches to Ion Channel Biology

June 10–30

INSTRUCTORS **Coldwell, John,** Ph.D., University of Colorado Health Sciences Center, Denver
Levinson, Rock, Ph.D., University of Colorado Health Sciences Center, Denver
Maue, Robert, Ph.D., Dartmouth Medical School, Hanover

ASSISTANT **Whisenand, Teri,** University of Colorado Health Sciences Center, Denver

This intensive laboratory/lecture course introduced students to the multidisciplinary use of molecular biological, biochemical, immunological, and electrophysiological approaches to the study of ion channels. The laboratory focused on the cellular regulation of channel expression and function. Hands-on exercises included characterization of regulatory elements that control transcription of channel genes, identification of ion channel isoform transcripts and proteins, visualization of channel distributions, and

the biophysical analysis of channel isoform function in excitable cells and tissues and in exogenous expression systems. Specific techniques employed included PCR, Western blot, transient transfection and expression, immunocytochemistry, and patch-clamp analysis of ionic currents. Lectures covered the techniques employed while providing broad exposure to current issues surrounding ion channel mechanisms and cellular expression. This course was intended for advanced students with specific plans to apply the techniques taught to a defined problem, and students were encouraged to bring their preparation to the course for preliminary studies.

PARTICIPANTS

Baruscott, M., B.S., Universita Degli Studi Di Milano, Milano, Italy
Decker, K., B.S., Justus Liebig University, Giessen, Germany
Donohoe, P., M.S., Ph.D., University of California, San Francisco
Fraser, H., B.S., University of Alberta, Edmonton, Canada
Kuusinen, A., M.S., University of Helsinki, Finland
Sadreyev, R., B.S., Moscow State University, Russia
Spura, A., B.S., Brown University, Providence, Rhode Island
Tonini, R., B.S., M.S., University of Milan, Italy
Townsend, T.M., B.S., Ph.D., Yale University, New Haven, Connecticut
Vernon, J., B.S., University of Cambridge, United Kindgom
Xu, X.Z.S., B.S., M.S., Johns Hopkins University School of Medicine, Baltimore, Maryland
Yellowley, C., B.S., Ph.D., Pennsylvania State University College of Medicine, Hershey

SEMINARS

Caldwell, J., University of Colorado Health Sciences Center, Denver. NaCh6: An abundant CNS and PNS voltage-gated sodium channel.
Froehner, S., University of North Carolina, Chapel Hill. Localization of ion channels and receptors at synapses.
Gardner, P., University of Texas Health Science Center, San Antonio. Regulation of neuronal nicotinic acetylcholine receptor gene expression.
Gilly, W., Hopkins Marine Station, Stanford University, Pacific Grove, California. Expression of sodium and potassium channel isoforms in squid neurons.
Henderson, L., Dartmouth Medical School, Hanover, New Hampshire. Pumping ions: Steroids, sex, and the $GABA_A$ receptor.
Isom, L., University of Michigan School of Medicine, Ann Arbor. Regulation of sodium channel density and localization by auxiliary β subunits.
Levinson, R., University of Colorado Health Sciences Center, Denver. Selective expression and localization of sodium channel isoforms in the peripheral nervous system.
Lewis, R., Stanford University School of Medicine, California. Local calcium domains and the regulation of store-operated calcium channels.
Mandel, G., State University of New York, Stony Brook. Regulation of sodium channels: How to quiet your nerves.
Maue, R., Dartmouth Medical School, Hanover, New Hampshire. Molecular analysis of neuronal sodium channel expression.
O'Dowd, D., University of California, Irvine. Analysis of ion channel gene expression by single-cell RT-PCR.
Ribera, A., University of Colorado Health Science Center, Denver. Study of development of electrical excitability using both forward and reverse genetics.
Salkoff, L., Washington University School of Medicine, St. Louis, Missouri. Impact of genome sequencing projects on potassium channel biology.
Sargent, P., University of California, San Francisco. Location and functional role of nicotinic receptors in a purportedly simple neuronal system: The vertebrate autonomic ganglion.
Snutch, T., University of British Columbia, Vancouver, Canada. Molecular determinants of calcium channel modulation: From worms to mammals.

Computational Neuroscience: Vision

June 18–July 1

INSTRUCTORS	**Heeger, David,** Ph.D., Stanford University **Shadlen, Michael,** Ph.D.,University of Washington, Seattle **Simoncelli, Eero,** Ph.D., New York University
ASSISTANTS	**Horwitz, Gregory,** Stanford University **Wichmann, Felix,** Oxford University, Oxford, United Kingdom

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 approaches can interact. Through a combination of lectures and hands-on experience with a computer laboratory, this intensive course examined several areas, including feature extraction, motion analysis, binocular stereopsis, color vision, higher-level visual processing, visual neural networks, and oculomotor function. The theme 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. Students had experience in neurobiological or computational approaches to visual processing. Some background in mathematics was beneficial.

PARTICPANTS

Alliston, E., B.S., New York University, New York
Bailey, D., B.S., M.S., University of Washington, Seattle
Barberini, C., B.S., Stanford University, California
Bridge, H., University of Oxford, United Kingdom
Brown, M., B.A., New York University, New York
Cai, R., B.S., Ph.D., University of California, Los Angeles
Chukoskie, L., B.A., New York University, New York
Cook, E., B.S., Ph.D., Baylor College of Medicine, Houston, Texas
Gardner, J., B.S., University of California, Berkeley
Hol, K., M.S., University of Tübingen, Germany
Loeffler, G., B.S., M.S., Glasgow Caledonian University, United Kingdom
McMahon, M., B.S., M.A., University of California, San Diego
Oomes, A., M.S. University of Nijmegen, The Netherlands
Prince, S., B.S., University of Oxford, United Kingdom
Rees, G., B.A., University College London, United Kingdom
Rieger, J., B.A., Max-Planck Institute, Tübingen, Germany
Roitman, J., B.S., University of Washington, Seattle
Rossi, A., B.A., Ph.D., National Institutes of Health, Bethesda, Maryland
Stanley, G., B.E., Ph.D., University of California, Berkeley
Sun, H., B.S., M.S., University of Chicago, Illinois
Tsai, J., B.S., M.S., Cornell University Medical College, Ithaca, New York
Turner, J., B.A., Ph.D., Rutgers University, Newark, New Jersey
Wainwright, M., B.M., Harvard University, Cambridge, Massachusetts
Walker, G., B.S., University of California, Berkeley

SEMINARS

Adelson, T., Massachusetts Institute of Technology, Cambridge. Elements of early vision.
Brainard, D., University of California, Santa Barbara. Color, trichromacy, and color spaces. Color appearance and color constancy.
Chichilnisky, E.J., The Salk Institute, San Diego, California. Color appearance and color constancy. White noise analysis of retinal ganglion cell receptive.
Dacey, D., University of Washington, Seattle. Retina: Anatomy and physiology of various types of bipolar, horizontal, amacrine, and ganglion cells.
Glimcher, P., New York University, New York. Models of VOR and OKN eye movements.
Graham, N., Columbia University, New York. Pattern detection and texture discrimination.
Groh, J., Dartmouth College, Hanover, New Hampshire. MT and pursuit eye movements/Models of saccadic eye movements.
Heeger, D., Stanford University, California. Linear systems, convolution, and Fourier transform. Signal detection theory. Models of V1 physiology, orientation, direction, and disparity selectivity, contrast normalization control. Multiscale/pyramid/wavelet image transforms as models of neural image representations. Neural codes, noise, and the statistics of cortical spike trains. Linking brain and behavior with fMRI.
Maunsell, J., Baylor College of Medicine, Houston, Texas. Attention physiology.
Movshon, T., New York University, New York. LGN-V1 physiology. Extrastriate and MT physiology.
Palmer, J., University of Washington, Seattle. Attention psychophysics.
Schall, J., Vanderbilt University, Nashville, Tennessee. Frontal eye fields and the neural basis of saccade target selection.
Shadlen, M., University of Washington, Seattle. MT and the neural basis of visual motion discrimination. Decisions, LIP, and frontal cortex.
Simoncelli, E., New York University, New York. Linear systems, convolution, and Fourier transform. Statistics of natural images. Model of MT physiology.
Welch, L., Brown University, Providence, Rhode Island. Visual motion perception and psychophysics.

Molecular Cloning of Neural Genes

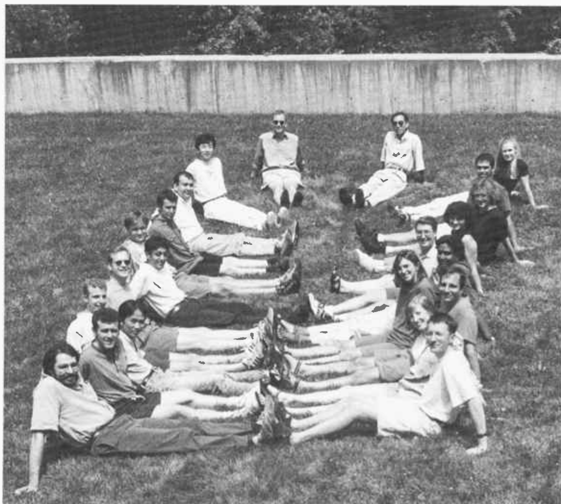
July 3–23

INSTRUCTORS
Darnell, Robert, M.D., Ph.D., Rockefeller University
Dulac, Catherine, Ph.D., Harvard University
Lai, Cary, Ph.D., Scripps Research Institute
Rupp, Fabio, Ph.D., John Hopkins University
Serafini, Tito, Ph.D., University of California

CO-INSTRUCTORS
Amrein, Hubi, Columbia University
Lavery, Daniel, Glaxo Wellcome Experimental Research, Lausanne, Switzerland

ASSISTANTS
Dredge, Kate, Rockefeller University
Herrada, Gilles, Harvard University
Jensen, Kirk, Rockefeller University
Okano, James, Rockefeller University

This intensive laboratory and lecture course teaches neuroscientists current approaches to molecular neurobiology. The course consisted of daily laboratory exercises, detailed discussions on the practice of molecular neurobiology, with an emphasis on modern approaches to cloning and analyzing the expression of neural genes, and a series of evening research seminars by invited speakers. This lecture series examined the ways in which molecular techniques studied in the laboratory have been suc-



cessfully applied to the study of neural genes, including expression cloning, single-cell cloning, subtractive cDNA cloning strategies, and genetic and mechanistic studies of neurologic disease, acquisition of cell identity, and axon guidance in the developing nervous system.

The focus of the laboratory portion of the course was to introduce different approaches to generating cDNA libraries and analyzing gene expression that are of particular interest to neurobiologists. Students learned to generate and examine cDNA libraries from large regions of the nervous system (e.g., mouse cerebellum and forebrain), from several hundreds to thousands of cells (embryonic tissue, specific nuclei) and from single neurons. Specific methods taught included the isolation and characterization of poly(A)$^+$ RNA; construction and screening of cDNA libraries; expression cloning; analysis of cDNA clones, including phagemid rescue, restriction analysis, Southern blotting, ligations, transformation, and subcloning; PCR analysis including oligonucleotide design and PCR cloning; and an exploration of advanced cloning techniques including single-cell library synthesis and PCR-based subtraction cloning technique, SABRE. Gene expression studies included Northern blot analysis and mammalian cell transfection, including use of a biolistic gene gun.

PARTICIPANTS

Altemus, M., B.A., M.D., Cornell University, New York
Anton, E., B.A., Ph.D., Yale University, New Haven, Connecticut
Barnes, G., B.S., M.D., Ph.D., Duke University Medical Center, Durham, North Carolina
Cohen, E., B.A., Ph.D., Yale University, New Haven, Connecticut
Couillard-Despres, S., B.S., Montreal General Hospital, Montreal, Canada
Dunaevsky, A., B.A., M.A., Ph.D., Columbia University, New York
Ebersole, T., B.S., University of Notre Dame, Indiana
Grabham, P., B.S., Ph.D., Columbia University, New York
Kavalali, E., B.S., Ph.D., Stanford University School of Medicine, California
Khakh, B., B.S, Ph.D., California Institute of Technology, Pasadena
Kossel, A., B.S., Ph.D., Max-Planck Institute, Munich, Germany
Svoboda, K., B.A., Ph.D., Cold Spring Harbor Laboratory
Thallmair, M., B.S., M.S., University of Zurich, Switzerland
Wenner, P., B.S., Ph.D., National Institutes of Health, Bethesda, Maryland
Williams, J., B.A., Ph.D., Massachusetts General Hospital, Boston
Zhong, H., B.S., Johns Hopkins University, Baltimore, Maryland

SEMINARS

Darnell, R., Rockefeller University, New York. Cloning neuron-specific genes with neurologic disease antisera.
Dulac, C., Harvard University, Cambridge, Massachusetts. Molecular biology of pheromone perception in mammals.
Edwards, R., University of California, San Francisco. Neurotransmitter transporters and the regulation of neurotransmitter release.
Hemmati-Brivanlou, A., Rockefeller University, New York. In vivo systems for expression cloning in vertebrates.
Huganir, R., Johns Hopkins University, Baltimore, Maryland. Molecular studies of synaptic transmission.
Lavery, D., Glaxo Wellcome, Lausanne, Switzerland. Approaches to cloning of differentially expressed genes.
Lemke, G., Salk Institute, La Jolla, California. Neuregulins in development.
Serafini, T., University of California, Berkeley. In vitro analysis of axon guidance and targeting.
Stein, L., Cold Spring Harbor Laboratory. Sequence analysis on the Web.
Streittmatter, S., Yale University, New Haven, Connecticut. Transduction of inhibitory guidance signals by the axonal growth cone.
Walsh, C., Harvard University/Beth Israel Hospital, Boston, Massachusetts. Genes that regulate cortical control mechanisms.
Worley, P., Johns Hopkins University, Baltimore, Maryland. Novel brain IEGs: Insights into synaptic plasticity.

Neurobiology of *Drosophila*

July 3–23

INSTRUCTORS **Dickinson, Michael,** Ph.D., University of California, Berkeley
Patel, Nipam, Ph.D., University of Chicago
Taylor, Barbara, Ph.D., Oregon State University, Corvallis

ASSISTANTS **Davis, Gregory,** University of Chicago
Song, Ho Juhn, Oregon State University, Corvallis

This laboratory/lecture course was intended for researchers at all levels who want to use *Drosophila* as an experimental system for studying behavior, physiology, and development. Daily seminars introduced students to a variety of research topics and developed those topics by including recent experimental contributions and outstanding questions in the field. Guest lecturers brought original preparations for

viewing and discussion and/or direct laboratory exercises and experiments in their area of interest. The course provided students with hands-on experience using a variety of experimental preparations employed 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 included neurogenesis, axon pathfinding, synaptogenesis, membrane excitability, learning and memory, biological rhythms, courtship, neural circuits, and walking and flight behaviors.

PARTICIPANTS

Beckstead, R., B.S., Baylor College of Medicine, Houston, Texas
Butler, A., B.A., Michigan State University, East Lansing
Chodagam, S., B.S., TATA Institute of Fundmental Research, Mumbai, Maharashtra, India
Gregor, I., M.S., Oxford University, Oxford, United Kingdom
Hayward, N., B.S., Cambridge University, United Kingdom
Higashi, C., B.S., University of Tokyo, Japan
Park, J., B.S., Ph.D., Brandeis University, Waltham, Massachusetts
Riedl, C., B.S., York University, Ontario, Canada
Strigini, M., M.S., EMBL, Heidelberg, Germany
Tong, J., B.S., Cold Spring Harbor Laboratory
Voelkner, M., B.S., Max-Planck Institute, Frankfurt, Germany
Wildemann, B., B.S., Freie University Berlin, Germany

SEMINARS

Brand, A., University of Cambridge, United Kingdom. Neural cell fate determination.
Dickinson, M., University of California, Berkeley. Muscle function and flight.
Fortini, M., University of Pennsylvania, Philadelphia. Early neurogenesis.
Hall, J., Brandeis University, Waltham, Massachusetts. Behavioral mutants and genetics, an overview.
Kernan, M., State University of New York, Stony Brook. Mechanosensory systems.
Meinertzhagen, I., Dalhousie University, Halifax, Nova Scotia. Synaptic plasticity and the adult visual system.
O'Dowd, D., University of California, Irvine. Ion channels and neuronal diversity.
Patel, N., University of Chicago, Illinois. Embryogenesis and early pattern formation.
Ramaswami, M., University of Arizona, Tucson. Exocytosis and neuromuscular function.
Restifo, L., University of Arizona, Tucson. Metamorphosis and adult development.
Sink, H., New York University, New York. Axonal pathfinding.
Sokolowski, M., York University, Ontario, Canada. Larval behaviors.
Strauss, R., Max-Planck Institute, Tübingen, Germany. Central processing centers and walking.
Sun, X.J., Cross Cancer Institute, Edmonton, Canada. Synaptic plasticity and the adult visual system.
Taghert, P., Washington University School of Medicine, St. Louis, Missouri. Neuropeptide signals: Regulators of development and behavior.
Taylor, B., Oregon State University, Corvallis. Sex differences and reproductive behaviors.
Tully, T., Cold Spring Harbor Laboratory. Learning and memory.
Treisman, J., N.Y. Medical Center/Skirball Institute of Molecular Medicine, New York. Visual system development.
Zhong, Y., Cold Spring Harbor Laboratory. Neuronal plasticity.

Brain Development and Function

July 7–20

INSTRUCTORS **McKay, Ronald,** Ph.D., National Institutes of Health
Schuman, Erin, Ph.D., California Institute of Technology

This advanced lecture and discussion course was on the development and function of the nervous system. Eighteen participants with diverse geographical and intellectual backgrounds met for 2 weeks with leading neuroscience researchers. The lectures provided both a comprehensive introduction as well as a detailed presentation of current research. There were many opportunities for discussion among the students and lecturers. The following topics were discussed: axon guidance and recognition, cytoskeleton and protein trafficking, neurotransmitter release, organization of synaptic structures, visual system development, structural plasticity, hippocampal function, olfactory signaling and coding, and modulatory brain systems.

PARTICIPANTS

Diaz, E., B.A., Stanford University, Stanford
Furuyashiki, T., M.D., Kyoto University, Japan
Huber, A., B.S., M.S., University of Zurich, Switzerland
Karam, S., B.S., M.S., University of Washington, Seattle
Kools, P., B.S., Ph.D., University of Gent, Belgium
Malyala, A., B.A., M.A., George Washington University, Washington, D.C.
Metta, G., M.S., University of Genova, Italy
Muehlner, U., B.S., Research Institute of Molecular Pathology, Vienna, Austria

Nagano, T., M.D., Ph.D., Niigata University, Japan
Raab-Graham, K., B.A., Ph.D., University of California, Santa Barbara
Smidt, M., M.D., Ph.D., Rudolf Magnus Institute for Neuroscience Utrecht, The Netherlands
Widenfalk, J., M.D., Ph.D., Karolinska Institute, Stockholm, Sweden
Wilson, S., B.S., Ph.D., National Cancer Institute, Frederick, Maryland
Wulf, P., B.A., Swiss Federal Institute of Technology, Zurich, Switzerland
Yamaguchi, M., Ph.D., RIKEN Brain Research Institute, Wako Saitama, Japan
Yu, X., B.A., MRC Laboratory of Molecular Biology, Cambridge, United Kingdom
Zhang, K., M.D., Helsinki University, Finland
Zhong, Z.-W., B.S., Ph.D., Laval University, Quebec, Canada

SEMINARS

Adolphs, R., University of Iowa College of Medicine, Iowa City. Lesion studies of human cortex.
Craig, A.M., University of Illinois, Urbana. Cell biology of synaptic structures.
Davis, G., University of California, Berkeley. Axon guidance and control of neuromuscular function.
Davis, M., Yale University, New Haven, Connecticut. Anxiety and fear.
Eichenbaum, H., Boston University, Massachusetts. Relational functions in hippocampus.
Feller, M., National Institutes of Health, Bethesda, Maryland. Retinal development.
Ginty, D., Johns Hopkins University, Baltimore, Maryland. Signal pathways in neurons.
Harris, K., Harvard University, Boston, Massachusetts. Organization of synaptic structures.
Huganir, R., Johns Hopkins University, Baltimore, Maryland. Molecular biology and function of glutamate receptors.
Kolodkin, A., Johns Hopkins University, Baltimore, Maryland. Axon guidance and recognition I.
Lichtman, J., Washington University, St. Louis, Missouri. Synaptic competition at the neuromuscular junction.
Lippincott Schwartz, J., National Institutes of Health, Bethesda, Maryland. Membrane dynamics.
Lurent, G., California Institute of Technology, Pasadena. Spike coding.
McKay, R., National Institutes of Health, Bethesda, Maryland. Stem and progenitor cells.
Mitchison, T., Harvard University, Boston, Massachusetts. The neuronal cytoskeleton.
Muller, R., SUNY Health Science Center, Brooklyn, New York. Hippocampal place cells.
Ngai, J., University of California, Berkeley. Olfaction.
Posner, M., University of Oregon, Eugene. Imaging human brain function.
Reichardt, L., University of California, San Francisco. Neurotrophin in development.
Ryan, T., Cornell University, Ithaca, New York. Synaptic vesicle recycling.
Schuman, E., California Institute of Technology, Pasadena. Synaptic modulation.
Serafini, T., University of California, Berkeley. Axon guidance and recognition II.
Wong, R., Washington University, St. Louis, Missouri. Visual system, structure, and plasticity.
Yuste, R., Columbia University, New York. Imaging neuronal function.

Molecular Embryology of the Mouse

June 10–30

INSTRUCTORS	**Koopman, Peter,** Ph.D., University of Queensland, Australia **Magnuson, Terry,** Ph.D., Case Western Reserve University
CO-INSTRUCTORS	**Nagy, Andras,** Ph.D., Samuel Lunenfeld Research Institute/Mount Sinai Hospital, Ontario, Canada **Tam, Patrick,** Ph.D., Children's Medical Research Institute, Sydney, Australia
ASSISTANTS	**Bowles, Josephine,** University of Queensland, Australia **Bultman, Scott,** Case Western Reserve University

This intensive course was designed for biologists interested in broadening 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

current research. The procedures described included isolation and culture of pre- and postimplantation embryos, in situ hybridization to whole mounts of embryos, LacZ staining, skeletal preparation, making aggregation chimeras, establishment, culture, and genetic manipulation of embryonic stem cell lines, electrofusion, microinjection of DNA into pronuclei, microinjection of embryonic stem cells into blastocysts, and surgical transfer of manipulated embryos into oviducts and uteri of host mice.

PARTICIPANTS

Allen, A., B.S., Ohio State University, Columbus
Begue-Kirn, C., M.S., Ph.D, University of Texas/M.D. Anderson Cancer Center, Houston
Beverdam, A., M.S., Netherlands Institute for Developmental Biology, Utrecht, The Netherlands
Casey, B., B.A., M.D., Baylor College of Medicine, Houston, Texas
Cunningham, S., B.S., Ph.D., University of Warwick, Coventry, United Kingdom
Hietanen, K., M.S., University of Helsinki, Finland
Hornyak, T., B.A., M.D., Ph.D., New York University Medical Center, New York
Jungbluth, S., B.S., Ph.D., United Medical and Dental School/Guy's Hospital, London, United Kingdom
Mirotsou, M., B.S., University College London Medical School, London, United Kingdom
Salisbury, J., B.S., Ph.D., Mayo Clinic, Rochester, Minnesota
Schneider, R., B.A., M.S., Duke University, Durham, North Carolina
Smith, K., B.S., University of Virginia, Charlottesville
Vasioukhin, V., B.S., Ph.D., University of Chicago, Illinois
Yang, T., B.A., Ph.D., University of Florida College of Medicine, Gainesville

SEMINARS

Behringer, R., The University of Texas/M.D. Anderson Cancer Center, Houston. Genetic regulation of mammalian embryogenesis. Transgenic mice in biomedical research.
Bradley A., Baylor College of Medicine, Houston. Introduction to ES cells. Screening the genome for tumor suppressor genes.
Eppig, J., The Jackson Laboratory, Bar Harbor, Maine. The mouse and the Web: Exploring information resources.
Hogan, B., Vanderbilt University Medical School, Nashville, Tennessee. Role of BMPs and *Forkhead* genes in cell-cell interactions during mouse development.
Joyner, A., N.Y. Medical Center/Skirball Institute of Biomolecular Medicine, New York. Genetic analysis of mid/hindbrain patterning and the Shh pathway in mice.
Koopman, P., The University of Queensland, Australia. Sex determination. Sox9 and the development of the skeleton.
Jenkins, N., National Cancer Institute, Frederick, Maryland. Molecular genetic analysis of mouse coat color mutations provides new insight into intracellular vesicle transport.
Jessell, T., Columbia University, New York. Inductive signals, transcriptional responses, and the control of neuronal identity in the vertebrate CNS.
Krumlauf, R., National Institute for Medical Research, London, United Kingdom. Patterning mechanisms in the vertebrate hindbrain.
Lovell-Badge, R., National Institute for Medical Research, London, United Kingdom. Functional analysis of mouse sex-determining genes. *Sox* genes in early development of the nervous system.
Magnuson, T., Case Western Reserve University, Cleveland, Ohio. Genetic approaches to mouse development.
Mann, J., Beckman Research Institute/City of Hope Medical Center, Duarte, California. Imprinting and its role in development.
Martin, G., University of California, San Francisco. Vertebrate limb development. Multiple uses of Cre recombinase to study mouse development.
McLaren, A., Wellcome/CRC Institute, Cambridge, United Kingdom. Origin and development of germ cells. Mouse reproductive cycle and husbandry.
McMahon, A., Harvard University, Cambridge, Massachusetts. Signaling pathways in vertebrate limb morphogenesis. Manipulating gene expression in utero.
Mobraaten, L., The Jackson Laboratory, Bar Harbor, Maine. Embryo and gamete cryopreservation.
Nagy, A., Mount Sinai Hospital, Ontario, Canada. Applications of gene knockout technology in mice.
Olson, E., University of Texas/Hamon Center for Basic Cancer Research, Dallas. Molecular control of cardiac development and disease.
Papaioannou, G., Columbia University, New York. Chimeras in experimental embryology. T-box genes in mouse development.
Solter, D., Max-Planck Institute of Immunobiology, Freiberg, Germany. Preimplantation development overview from egg to blastocyst: Hard road to follow.
Soriano, P., Fred Hutchinson Cancer Research Center, Seattle, Washington. Insertional mutagenesis. Growth factor signaling during mouse development.
Tam, P., Children's Medical Research Institute, Wentworthville, Australia. Postimplantation development overview. Gastrulation, staging, landmarks of development. Functional analysis of the mouse organizer.
Wilkinson, D., National Institute for Medical Research, London, United Kingdom. In situ hybridization: Methods and research applications. Segmentation of the hindbrain and neural crest.

Molecular Mechanisms of Human Neurological Diseases

July 23–29

INSTRUCTORS **Gandy, Sam,** M.D., Ph.D., New York University, Orangeburg
Sisodia, Sangram, Ph.D., University of Chicago

How and why do neurons die in specific acute or chronic human neurological disorders? What are the molecular and biochemical manifestations of specific genetic lesions in specific neurodegenerative disorders? Do different pathological deaths share common mechanisms? What practical treatments can be 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, amyotrophic lateral sclerosis, prion diseases, Huntington's disease, epilepsy, and stroke. Taking advantage of small class size and extensive discussion, invited faculty lecturers examined critical issues in their areas of expertise. Overview was provided, and course participants did not need to have familiarity with neurological diseases. A basic introduction to central and peripheral neurons system structure and biology (including developments) was provided. The course focused principally on the specific hypotheses and approaches driving current research. Emphasis was placed on the highly dynamic interface between basic and clinical investigation, including the interdependence of clinical research and disease model development, and the value of disease research in understanding the function of the normal nervous system.

PARTICIPANTS

Auburger, G., B.S., M.D., National Human Genome Research Institute, Bethesda, Maryland
Beagles, K., B.S., University of Kentucky, Lexington
De Feo, P., B.A., M.S., Zeneca Pharmaceuticals, Wilmington, Delaware
Gaspar, C., B.S., Ph.D., Montreal General Hospital Research Institute, Canada
Goto, J., B.S., University of California, Los Angeles
Heyes, M., B.S, Ph.D., National Institutes of Mental Health, Bethesda, Maryland
Hotvedt, C., B.S., M.A., Ketchum Public Relations, New York
Kolmerer, B., B.S., Ph.D., Schering AG, Berlin, Germany
Kubota, K., B.A., Ph.D., Yamanouchi Pharmaceutical Co., Ibaraki, Japan
Lin-Chao, S., M.S., Ph.D., Academia Sinica, Taiwan, Republic of China
Malyala, A., B.A., M.A., Ph.D., George Washington University, Washington, D.C.
Orth, M., M.D., Ph.D., Otto Von Guericke University, Magdeburg, Germany
Pitossi, F., M.S., Ph.D., University of Buenos Aires, Argentina
Pramatarova, A., B.S., Ph.D., Montreal General Hospital Research Institute, Canada
Sacrestano, D., B.S., Rockefeller University, New York
Shanley, J., B.S., Zeneca Pharmaceuticals, Wilmington, Delaware
Terai, K., B.A., M.S., Ph.D., Yamanouchi Pharmaceutical Co., Ibaraki, Japan
Vincenz, C., B.S., Ph.D., University of Michigan, Ann Arbor
Zacco, A., B.S., Zeneca Pharmaceuticals, Wilmington, Delaware
Zhong, Z., B.S., Ph.D., R.W. Johnson Parmaceutical Research Institute, Raritan, New Jersey

SEMINARS

Aguzzi, A., University Hospital of Zurich, Switzerland. Prion diseases.
Catteno, E., University of Milan, Italy. Molecular biology of neurogenesis.
Ehrlich, M., New York University, New York. Molecular biology of neurogenesis.
Fischbeck, K., National Institutes of Health, NINDS, Bethesda, Maryland. Neurobiology and pathology of hereditary peripheral neuropathies.
Gandy, S., New York University, Orangeburg. Molecular pathology of familial Alzheimer's disease.
Griffin, J., Johns Hopkins University, Baltimore, Maryland. Structure and pathobiology of the PNS.
Lee, V., University of Pennsylvania School of Medicine, Philadelphia. Intracellular fibrous structures as a common feature of some neurodegenerative disease.
MacDonald, M., Massachusetts General Hospital, Charlestown. Huntington's disease and triple repeat disorders.
McNamara, J., Duke University Medical School, Durham, North Carolina. Neurobiology and pathology of the epilepsies.
Nixon, R., New York University, Orangeburg. Approaching sporadic Alzheimer's disease.
Orr, H., University of Minnesota, Minneapolis. Hereditary ataxias, DRPLA, and other triplet diseases.
Price, D., Johns Hopkins University School of Medicine, Baltimore, Maryland. Approaches to modelling late-onset human neurodegeneration.
Rothman, S., St. Louis Children's Hospital, St. Louis, Missouri. Pathobiology of hypoxic-ischemic neural injury.
Rothstein, J., Johns Hopkins University School of Medicine, Baltimore, Maryland. Molecular biology of motor neuron disease.
Salzer, J., New York University, New York. Molecular cell biology of myelin.
Sisodia, S., University of Chicago, Illinois. Molecular pathology of familial Alzheimer's disease.
Snider, W., Washington University School of Medicine, St. Louis, Missouri. Neurobiology of neurotrophins.
Walsh, C., Harvard Medical School, Boston, Massachusetts. Cerebral cortical development and dysgenesis.

Eukaryotic Gene Expression

July 28–August 17

INSTRUCTORS	**Carey, Michael,** Ph.D., University of California, Los Angeles **Gill, Grace,** Ph.D., Harvard Medical School **Gilmour, David,** Ph.D., Pennsylvania State University, University Park **Goodrich, James,** Ph.D., University of Colorado, Boulder
ASSISTANTS	**Auerbach, Scott,** Pennsylvania State University, University Park **Johnson, Barbra,** Harvard Medical School **Klube, Shelley,** University of Colorado, Boulder **Mitsouras, Katherine,** University of California, Los Angeles

This course was designed for students, postdocs, and professors who had recently ventured into the dynamic area of gene regulation. The course focused on state-of-the-art strategies and techniques employed in the field including in vitro transcription with cell-free extracts and transfection of mammalian tissue culture cells. Analytical techniques for measuring gene expression included primer extension and enzymatic analyses of reporter proteins. An emphasis was placed on biochemical studies of protein-DNA and protein-protein interactions. A site-specific transcription factor was expressed in *E. coli* and purified by affinity chromatography. A detailed characterization of the DNA-binding properties of the protein was carried out using electrophoretic mobility shift, DNase I footprinting, and methylation interference assays. Students also learned strategies and techniques for executing a structure-function study by generating site-directed mutants in the transcription factor and analyzing them both biochemically and by computer modeling. The factor was subjected to affinity chromatography to under-

stand how it interacts with the general transcription machinery. Over the past few years, the gene regulation field has begun to emphasize the importance of in vivo approaches to studying protein-DNA and protein-protein interactions. Students were exposed to in vivo footprinting, mapping of DNase-I-hypersensitive sites and the yeast two-hybrid methodologies. 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 molecular biology and technical approaches to their solution.

PARTICIPANTS

Andrew, S., B.S., Kolling Institute of Medical Research, Sydney, Australia
Del Mar Pena, L., B.S., Northwestern University of Medical School, Chicago, Illinois
Frank, R., B.A., M.D., Memorial Sloan Kettering Cancer Center, New York
Germain, S., B.S., M.S., Ph.D., INSERM, College de France, Paris, France
Le Saux, A., D.E.A., Ph.D., Max-Planck Institute, Koln, Germany
McDonald, M., B.S., Brandeis University, Waltham, Massachusetts
McLellan, A., B.S., University of Cambridge, United Kingdom
Monteiro, A., B.S., Ph.D., Rockefeller University, New York
Rodzik, S., B.S., North Carolina State University, Raleigh
Rosonina, E., B.S., University of Toronto, Ontario, Canada
Shieh, P., B.A., M.A., Johns Hopkins University School of Medicine, Baltimore, Maryland
Skopicki, H., B.A., M.D., Ph.D., Columbia University College for Physicians & Surgeons, New York
Theuns, J., B.S., University of Antwerp, Belgium
Tienhaara, A., M.D., Ph.D., University of Turku, Finland
Worley, T., B.A., Ph.D., University of Texas, Dallas
Yeung, F., B.S., University of Virginia, Charlottesville

SEMINARS

Bentley, D., Amgen Institute/University of Toronto, Ontario, Canada. Transcription elongation complexes.
Berger, S., Wistar Institute, Philadelphia, Pennsylvania. Histone acetylases.
Burley, S./Roeder, R., Rockefeller University, New York. X-ray crystallographic studies of eukaryotic transcription factors.
Carey, M., University of California, Los Angeles. Biochemical mechanisms of gene activation.
Ebright, R., Rutgers University, New Brunswick, New Jersey. Protein-DNA interactions in eukaryotic transcription initiation.
Gill, G., Harvard Medical School, Boston, Massachusetts. A transcriptional repressor that antagonizes E1A-mediated activation.
Gilmour, D., Pennsylvania State University, University Park. In vivo and in vitro analysis of the hsp70 promoter of *Drosophila*.
Goodrich, J., University of Colorado, Boulder. RNA polymerase II transcriptional regulation.
Haykinson, M., University of California, Los Angeles. Computer modeling and simulation of transcription factor–DNA interactions.
Lees, J., Massachusetts Institute of Technology, Cambridge. Role of the E2F transcription factors in cell cycle control.
Lis, J., Cornell University, Ithaca, New York. Multiple mechanisms of transcriptional regulation.
Maniatis, T., Harvard University, Cambridge, Massachusetts. The IFN-β enhanceosome.
Ptashne, M., Memorial Sloan Kettering Cancer Center, New York. The recruitment model for gene activation.
Roeder, R., Rockefeller University, New York. Role of general and gene-specific cofactors in transcriptional regulation.
Shi, Y., Harvard Medical School, Boston, Massachusetts. Function and mechanism of action of a CBP/p300 homolog in *C. elegans*.
Smale, S., University of California/HHMI, Los Angeles. Transcriptional regulation in the immune system.
Struhl, K., Harvard University, Boston, Massachusetts. Molecular mechanisms of yeast transcriptional regulation.
Workman, J., Pennsylvania State University, University Park. Multiprotein complexes that regulate transcription by modifying chromatin structure.
Zaret, K., Brown University, Providence, Rhode Island. Specifying tissues and gene expression patterns in development.

Imaging Structure and Function in the Nervous System

July 28–August 17

INSTRUCTORS **Denk, Winfried,** Ph.D., Lucent Technologies Bell Labs, Murray Hill, New Jersey
Halpain, Shelley, Ph.D., Scripps Research Institute
Kay, Steve, Ph.D., Scripps Research Institute

ASSISTANTS **Graber, Simone,** Scripps Research Institute
Helmchen, Fritjof, Lucent Technologies Bell Labs, Murray Hill, New Jersey
Ozer, Rachel, Scripps Research Institute

Advances in light microscopy, digital image processing, and the development of a variety of powerful fluorescent probes present expanding opportunities for visualizing and measuring the structure and function of neurons, synapses, and networks in the brain. This intensive laboratory and lecture course provided participants with the theoretical and practical knowledge to utilize emerging imaging technologies. The primary emphasis of the course was on vital light microscopy. Students learned the principles of light microscopy, as well as the use of different types of electronic cameras, laser-scanning systems, functional fluorophores, delivery techniques (such as microinjection), and digital image-processing software. The synergistic combination of different imaging modalities, such as fluorescence and infrared differential interference contrast combined with different functional probes, such as calcium-sensitive probes, voltage-sensitive dyes, photo-activated ("caged") compounds, and exocytosis tracers, with electrophysiological techniques was emphasized. Particular weight was given to multiphoton laser-scanning microscopy and to newly available biological fluorophores such as green fluorescent protein (GFP) and its variants and derivatives. A variety of neural systems were used, including

living animals, brain slices (e.g., hippocampus, cerebellum, and neocortex), and cultured cells. Applicants had a strong background in the neurosciences or in cell biology.

PARTICIPANTS

Aakalu, G., B.A., California Institute of Technology, Pasadena
Arnth-Jensen, N., B.S., University of Oxford, United Kingdom
Bausch, S., B.A., Ph.D., Duke University Medical Center, Durham, North Carolina
Brecht, M., B.S., Ph.D., Max-Planck Institute for Brain Research, Frankfurt, Germany
Cochilla, A., B.S., Ph.D., University of Colorado Medical School, Denver
Di Cristo, G., B.S., Scuola Normale Superiore di Pisa, Italy
Ehrlich, I., B.S., M.S., University of Frankfurt, Germany
Huang, J., B.S., Ph.D., Massachusetts Institute of Technology, Cambridge
Kaksonen, M., M.S., University of Helsinki, Finland
Kim, S., B.A., University of Texas Health Science Center, Houston
Mermelstein, P., B.S., Ph.D., Stanford University, California
Nascimento, A., B.S., M.S., Ph.D., University of Sao Paulo, Brazil
Spors, H., M.D., Max-Planck Institute, Heidelberg, Germany
Zhang, L., B.S., M.S., University of California, San Diego

SEMINARS

Betz, W., University of Colorado, Denver. Vesicle release with FM-143.
Block, S., Princeton University, New Jersey. Optical tweezers and applications.
Bonhoeffer, T., Max-Planck Institute for Neurobiology, Munich, Germany. Intrinsic imaging.
Brand, A., Cambridge University, United Kingdom. GFP in *Drosophila* development.
Cline, H., Cold Spring Harbor Laboratory. In vivo imaging of CNS neuronal development.
Cohen, L., Yale University, New Haven, Connecticut. Voltage-sensitive dyes, diode arrays.
Day, R., University of Virginia Health Science Center, Charlottesville. Application lecture: GFP, BFP as genetically encoded fluorophores. Application lecture: FRET.
Denk, W., Lucent Technologies Bell Labs, Murray Hill, New Jersey. Basic Microscopy I. Basic Microscopy II.
Dodt, H.-U., Max-Planck Institute, Munich, Germany. Contrast methods in tramsmitted-light microscopy I. Contrast methods II.
Fetcho, J., Cold Spring Harbor Laboratory. Functional imaging of neural circuits in living zebrafish.
Forscher, P., Yale University, New Haven, Connecticut. Imaging structural dynamics using video-enhanced DIC. Neuronal growth cone dynamics.
Halpain, S., The Scripps Research Institute, La Jolla, California. Tissue fixation and staining. Neuronal tissue preparations for light microscopy.
Harris, K., Children's Hospital/Harvard University, Boston, Massachusetts. Electron microscopy and synapse structure/function
Helmchen, F., Bell Laboratories, Murray Hill, New Jersey. Fundamentals of electrophysiological recording and calcium imaging in neurons.
Hess, G., Cornell University, Ithaca, New York. Caged neurotransmitters.
Hockerberger, P., Northwestern University, Chicago, Illinois. Basic photochemistry and phototoxicity.
Katz, L., Duke University, Durham, North Carolina. Mapping circuits with caged compounds. Biolistic gene transfer.
Kleinfeld, D., University of California, La Jolla. Imaging cerebral blood flow in vivo.
Konnerth, A., University of Saarlandes, Germany. Functional imaging, Ca^{++} channels.
Lanni, F., Carnegie Mellon University, Pittsburgh, Pennsylvania. Standing wave microscopy.
Lictman, J., Washington University, St. Louis, Missouri. Confocal microscopy I. Confocal microscopy II and long-term time lapse studies in vivo.
Matus, A., Friedrich Miescher Institute, Basel, Switzerland. Use of GFP-fusion proteins to study neuronal cytoskeleton in vivo.
Piston, D., Vanderbilt University, Nashville, Tennessee. Two-photon imaging of GFP to study insulin action.
Stelzer, E., European Molecular Biology Laboratory, Heidelberg, Germany. Image deconvolution. Theta/4PI imaging.
Svoboda, K., Cold Spring Harbor Laboratory. In vivo two-photon/GFP.
Tsien, R., University of California, San Diego. Development and use of fluorescent indicators to study second messengers. GFP and its cousins—the next generation of fluorescent indicators for in vivo imaging.
Watt, W., Cornell University, Ithaca, New York. Three-proton/multi-photon autofluorescence.
White, J., University of Wisconsin, Madison. Multi-photon imaging in particular of developing animals development.
Yuste, R., Columbia University, New York. Two-photon imaging of synapse dynamics.

Yeast Genetics

July 28–August 17

INSTRUCTORS
Dawson, Dean, Ph.D., Tufts University
Gottschling, Daniel, Ph.D., Fred Hutchinson Cancer Research Center
Stearns, Timothy, Ph.D., Stanford University

ASSISTANTS
Byrnes, Micheal, Stanford University
Kemp, Benedict, Tufts University
Stellwagon, Anne, Fred Hutchinson Cancer Research Center

The major laboratory techniques used in the genetic analysis of yeast were studied, including the isolation and characterization of mutants, tetrad analysis, complementation, and mitotic recombination. Micromanipulation used in tetrad analysis was carried out by all students. Molecular genetic techniques, including yeast transformation, gene replacement, analysis of gene fusions, and generation of mutations in cloned genes, were studied. Indirect immunofluorescence experiments were done to identify the nucleus, microtubules, and other cellular components. Lectures on fundamental aspects of yeast genetics were presented along with seminars given by outside speakers on topics of current interest.

PARTICIPANTS

Adler, E., B.A., Ph.D., University of California, San Diego
Basu, S., B.S., University of Medicine and Dentistry of New Jersey, Piscataway
Beletskii, A., M.S., Wayne State University, Detroit, Michigan
Carbrey, J., B.S., Johns Hopkins University School of Medicine, Baltimore, Maryland
Carmel-Harel, O., M.A., Ph.D., National Institutes of Health, Bethesda, Maryland
Collins, J., B.S., Brandeis University, Natick, Massachusetts
Couvreur, M., M.S., Catholic University, Leuven, Belgium
Fries, B., M.D., Albert Einstein College of Medicine, Bronx, New York
Frosco, M.B., B.S., M.D., Bristol Myers Squibb Pharmaceutical Research Institute, Wallingford, Connecticut
Li, Y.-C., B.S., Ph.D., Baylor College of Medicine, Houston, Texas
Mirels, L., B.A., M.D., Stanford University Medical Center, California
Morillon, A., B.S., M.S., CNRS, French Research Council, Paris, France
Nicholas, D., B.S., University of Idaho, Moscow
Shen, X., B.S., Ph.D., National Institutes of Health, Bethesda, Maryland
Sjostrom, I., M.S., University of Gothenburg, Sweden
Ungermann, C., M.S., Ph.D., Dartmouth Medical School, Hanover, New Hampshire

SEMINARS

Burke, D., University of Virginia, Charlottesville. DNA damage and the cell cycle.
Chant, J., Harvard University, Cambridge, Massachusetts. Cell polarity.
Cyert, M., Stanford University, California. Role of calcineurin in yeast.
Drubin, D., University of California, Berkeley. The actin cytoskeleton.
Futcher, B., Cold Spring Harbor Laboratory. The cell-cycle-regulated genes of yeast.
Haber, J., Brandeis University, Waltham, Massachusetts. Recombination and chromosome mechanics.
Hinnebusch, A., National Institutes of Health, Bethesda, Maryland. Translational control.
Hicke, L., Northwestern University, Evanston, Illinois. Ubiquitination and endocytosis.
Hoyt, A., Johns Hopkins University, Baltimore, Maryland. Meiotic checkpoints.
Kaiser, C., Massachusetts Institute of Technology, Cambridge. Protein sorting and transport.
Koshland, D., Carnegie Institute of Washington, Baltimore, Maryland. Sister chromatid cohesion and chromosome condensation.
Michaelis, S., Johns Hopkins University, Baltimore, Maryland. Pheromone response and production.
Mitchell, A., Columbia University, New York. Meiotic regulation.
Silver, P., Dana Farber Cancer Institute, Boston, Massachusetts. Nuclear targeting.
Rose, M., Princeton University, New Jersey. Nuclear fusion and fission.
Tyers, M., Samuel Lunenfeld Research Institute, Ontario, Canada. Ubiquitin and cell cycle regulation.

Advanced *Drosophila* Genetics

July 30–August 12

INSTRUCTORS **Ashburner, Michael,** Ph.D., University of Cambridge, United Kingdom
Hawley, R. Scott, Ph.D., 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 covered included chromosome mechanics, the design and execution of genetic screens, and the use of transposable elements as genetic tools.

PARTICIPANTS

Ballard, J.W., B.S., Ph.D., The Field Museum, Chicago, Illinois
Baum, B., B.A., Ph.D., Harvard Medical School, HHMI, Boston, Massachusetts
Camonis, J., M.D., Ph.D., CNRS, Institute Curie, Paris, France
Chavez, V., B.S., Ph.D., University of Minnesota, Minneapolis
Dobie, K., B.S, Ph.D., The Salk Institute, La Jolla, California
Gur, D., B.S., Hebrew University, Jerusalem, Israel
Hendengren, M., M.S., Stockholm University, Sweden
Huang, A., B.A., University of California, Berkeley

Johnson, M., B.S., M.S., Harvard Medical School, Boston, Massachusetts
Lincecum, J, B.A., Ph.D., Harvard Medical School, Boston, Massachusetts
Onfelt, T., M.S., Stockholm University, Sweden
Page, S., B.S., Ph.D., University of California, Davis
Parnell, T., B.S., University of Iowa, Iowa City
Renaud, O., B.S., M.S., IGBMC, Illkirch France
Robert, V., B.S., CNRS, GSM., Gif Sur Yvette, France
Shimmi, O., B.S., Ph.D., University of Minnesota, Minneapolis
Song, Z., B.S., Ph.D., Massachusetts Institute of Technology, Cambridge
Tickoo, S., B.S., M.S., Tata Institute of Fundamental Research, India
Vandaele, C., M.S., University of Claude Bernard Lyon I, Villeurbanne, France
Wayson, S., B.S., University of California, Davis
Wu, X., B.S., New York University, New York
Zarnescu, D., M.S., Pennsylvania State University, University Park
Zhang, Y., B.A., Ph.D., University of Utah, Salt Lake City

SEMINARS

Ashburner, M., University of Cambridge, United Kingdom. Introduction to *Drosophila* biology, phylogeny. Fly base: Where to find information. Chromosomes, cytogenetics, chromosome abberations, mapping (genetic and cytogenetic).
Cherbas, P., Indiana University, Bloomington. Somatic cell genetics.
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.
Ganetzky, B., University of Wisconsin, Madison. Neurogenetics.
Golic, K., University of Utah, Salt Lake City. Mosaic systems: FRT/FLP.
Hall, J., Brandies University, Waltham, Massachusetts. Genetic analyses of behavior.
Hawley, S., University of California, Davis. Genetics of meiosis; methods of study an exploitation; distributive pairing.
Lehman, R., New York University Medical Center/Skirball Institute, New York. Genetics screens for mutations affecting embryogenesis (including maternal effect mutations); methods for their analysis.
Karpen, G., The Salk Institute, La Jolla, California. *cis* and *trans* regulators of chromosome inheritance in *Drosophila*.
Roth, P., EMBL, Heidelberg, Germany. P-element technologies.
Rubin, G., University of California, Berkeley. The fly genome.
Spradling, A., Carnegie Institute of Washington, Baltimore, Maryland. P-element technologies.
Sullivan, W., University of California, San Francisco. Crosses and columns: Complementary approaches for analyzing the embryonic cytoskeleton.

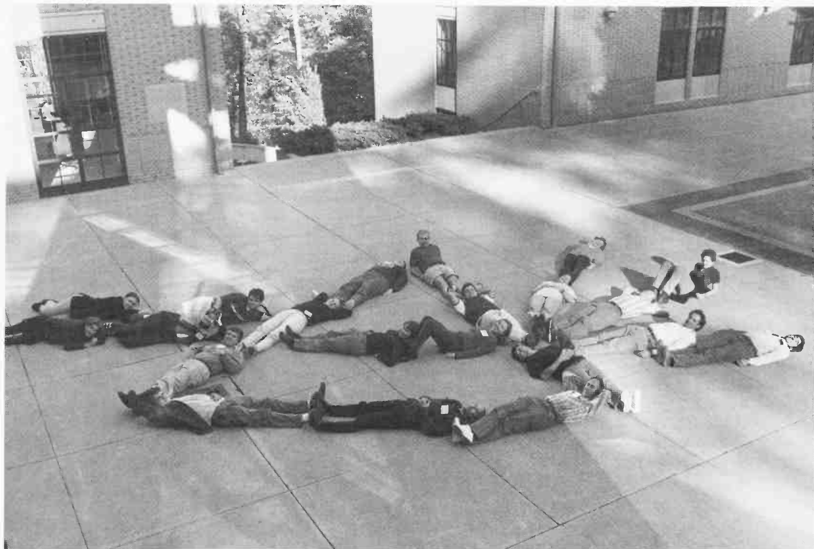
Advanced In Situ Hybridization and Immunocytochemistry

October 14–27

INSTRUCTORS
Ochs, Robert, Ph.D., Scripps Research Institute
Murray, John, M.D., Ph.D., University of Pennsylvania
Spector, David, Ph.D., Cold Spring Harbor Laboratory
Ried, Thomas, Ph.D., National Institutes of Health
Schrock, Evelin, Ph.D., National Institutes of Health

ASSISTANTS
Howard, Tamara, Cold Spring Harbor Laboratory
Quinlin, Margot, University of Pennsylvania

This course focused on specialized techniques in microscopy related to localizing DNA and RNA sequences and proteins in cells for microscopic examination. It emphasized the use of the latest equipment and techniques in epifluorescence microscopy, confocal laser scanning microscopy, electron microscopy, and digital image processing. 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 genome organization and cellular and molecular biology. The course was designed for the molecular biologist who is in need of microscopic approaches and for the cell biologist who is not famil-



iar with the practical application of the advanced techniques presented in the course. Among the methods presented were the preparation of tagged nucleic acid probes, fixation methods, detection of multiple DNA sequences in single nuclei or chromosome spreads, comparative genomic hybridization, spectral karyotyping, cellular localization of RNA, use of a variety of reporter molecules and nonantibody fluorescent tags, indirect antibody labeling, detection of multiple proteins in a single cell, and immunoelectron microscopy. 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 gave up-to-the-minute reports on current methods and research using the techniques that were presented in the course.

PARTICIPANTS

Andresen, V., B.S., University of Bergen, Norway
Anneser, J., M.D., University of Munich, Germany
Binnie, A., B.S., Sir William Dunn School of Pathology, Oxford, United Kingdom
Bjork, P., B.S., Stockholm University, Sweden
Boorer, K., B.S., Ph.D., VivoRX Inc., Santa Monica, California
Chamberlin, M., B.A., Ph.D., Pioneer Hi-Bred International, Johnston, Iowa
Duca, K., B.S, Ph.D., University of Wisconsin, Madison
Dunn, D., B.S., M.S., University of Western Australia, Perth
Ermakova, O., B.S., M.S., Albert Einstein College of Medicine, New York
Fuchs, J., B.S., Ph.D., University of Vienna, Austria
Kosak, S., B.A., Ph.D., University of Chicago, Illinois
Montagna, C., B.S., Ph.D., Consiglio Nazionale Delle Richerche, Milan, Italy
Oklu, R., B.S., University of Cambridge, United Kingdom
Seandel, M., B.A., State University of New York, Stony Brook
Thomas, J., B.A., M.D., Vanderbilt University, Nashville, Tennessee
Van Tine, B., B.S., University of Alabama, Birmingham

SEMINARS

Deerinck, T., University of California, San Diego. Electron microscopy as a tool in cell and molecular biology.
Hell, S., Max-Planck, Gottingen, Germany. 4 Pi microscopy.
Huang, S., Northwestern University Medical School, Chicago, Illinois. RNA in situ hybridization.
Lippincott-Schwartz, J., National Institutes of Health, Bethesda, Maryland. GFP and FRAP analysis.
Murray, J., University of Pennsylvania, Philadelphia. Basic introduction to light and fluorescence microscopy. Principles of confocal microscopy and deconvolution techniques.
Pinkel, D., University of California, San Francisco. Filters and fluorochromes for fluorescence microscopy.
Ried, T., National Institutes of Health, Bethesda, Maryland. CGH and SKY.
Rieder, C., New York State Department of Health, Albany. Mitosis.
Singer, R., Albert Einstein College of Medicine, New York. Cytoplasmic organization of mRNA.
Spector, D., Cold Spring Harbor Laboratory. Immunocytochemistry.
Svoboda, K., Cold Spring Harbor Laboratory. 2-Photon microscopy.

Macromolecular Crystallography

October 14–27

INSTRUCTORS	**Furey, William,** Ph.D., V.A. Medical Center, Pittsburgh, Pennsylvania **Gilliland, Gary,** Ph.D., National Institute of Standards and Techniques, Rockville, Maryland **McPherson, Alexander,** Ph.D., University of California, Riverside **Pflugrath, James,** Ph.D., Molecular Structure Corporation, The Woodlands, Texas
ASSISTANT	**Chu, Seung,** National Institute of Standards and Techniques, Rockville, Maryland

Crystallography and X-ray diffraction yield a wealth of structural information unobtainable through other methods. This "intensive" laboratory/computational course focused on the major techniques used to determine the three-dimensional structures of macromolecules. It was designed for scientists with a working knowledge of protein structure and function, but who were new to macromolecular crystallography. Topics covered included crystallization (proteins, nucleic acids, and complexes), crystal characterization, X-ray sources and optics, synchrotrons, crystal freezing, data collection, data reduction, multiple isomorphous replacement, multiwavelength anomalous diffraction phase determination, sol-

vent flattening, molecular replacement and averaging, electron density interpretation, structure refinement, molecular graphics, noncrystallographic summary, simulated annealing, and coordinate desposition. Participants learned through extensive hands-on experiments. They crystallized and determined a protein structure, with lectures on the theory and informal discussions behind the techniques.

PARTICIPANTS

Brunskill, A., B.S., Rutgers University, Newark, New Jersey
Buckler, D., B.A., Ph.D., University of Dentistry and Medicine of New Jersey, Piscataway
Conn, G., B.S., Ph.D., Johns Hopkins University, Baltimore, Maryland
Di Marco, S., B.S., Ph.D., Institute of Molecular Biology Research, Pomezia, Italy
Fu, D,X., B.A., Ph.D., University of California, San Francisco
Grollman, A., B.A., M.D., State University of New York, Stony Brook
Handel, T., B.S., Ph.D., University of California, Berkeley
Kostyukova, A., B.S., Ph.D., Matsushita Electric Industrial Co., Ltd., Kyoto, Japan
Lin, S.-X., B.S., Ph.D., Laval University Medical Center, Quebec, Canada
O'Neill, J., B.A., Fred Hutchinson Cancer Research Center, Seattle, Washington
Podell, E., B.A., M.S., University of Colorado, Boulder
Schwartz, J.C., B.S., Albert Einstein College of Medicine, New York
Walke, S., B.S., Medical Research Council Laboratory of Molecular Biology, Cambridge, United Kingdom
Wilson, N., B.A., Ph.D., National Jewish Medical and Research Center, Denver, Colorado
Wucherpfennig, K., B.S., M.D., Ph.D., Dana Farber Cancer Institute, Boston, Massachusetts
Yaffe, M., B.S., Ph.D., M.D., Harvard Medial School, Boston, Massachusetts

SEMINARS

Berman, H., Rutgers University, Piscataway, New Jersey. DNA: Relationship of structure to function.
Bounre, P., University of California, San Diego. 3-D comparison methods: Theory and practice.
Brunger, A., Yale University, New Haven, Connecticut. Structural insights into synaptic vesicle fusion.
De La Fortelle, E., MRC Laboratory of Molecular Biology, Cambridge, United Kingdom. SHARP.
Fitzgerald, P., Merck Research Laboratories, Rahway, New Jersey. Metalo-β-lactamase as a target for structure-based drug design.
Ginell, S., Argonne National Laboratory, Illinois. Cryo-crystallography and the "immortal crystal."
Gilliland, G., National Institutes of Standards and Technology, Rockville, Maryland. Crystal structure of *E. coli* uracil DNA glycosylase and its complexes with uracil and glycerol: Structure and glycosylase mechanism revisited.
Hendrickson, W., Columbia University, New York. MAD phasing. HIV gp120.
Howell, P.L., Hospital for Sick Children, Ontario, Canada. Desperately seeking selenium: The structure determination of *S*-adenosyl-homocysteine.
Kjeldgaard, M., Aarhus University, Denmark. The EF-Tu:tRNA complex.
Lee Mor, J.-T., Cold Spring Harbor Laboratory. Bleomycin hydrolase: Intriguing structure, enigmatic activities, and links to diseases.
Perrakis, A., Grenoble, Germany. WARP.
Tronrud, D., University of Oregon, Eugene. Macromolecular refinement: Past, present, and future.
Xu, R., Cold Spring Harbor Laboratory. A novel structure of human mitochondrial protein p32.

Positional Cloning: Contig to Candidate Gene

October 14–27

INSTRUCTORS	**Jacob, Howard,** Ph.D., Medical College of Wisconsin, Milwaukee **Silverman, Gary,** M.D., Ph.D., Harvard University **Spencer, Forrest,** Ph.D., Johns Hopkins University School of Medicine
CO-INSTRUCTORS	**Church, Deanna,** Samuel Lunenfeld Research Institute, Toronto, Canada **Parimoo, Satish,** Johnson & Johnson, Skillman, New Jersey
ASSISTANTS	**Brownlie, Alison,** Harvard University **Kwitek-Black, Anne,** Medical College of Wisconsin, Milwaukee

This laboratory-based course was designed for investigators using genetic and physical mapping tools or functional assays to isolate genes of interest, with emphasis placed on those procedures used to isolate and evaluate a candidate gene after it has been localized genetically. Principal procedures included physical mapping by contig construction using YACs and BACs/PACs, STS-content mapping, DNA fingerprinting, pulsed-field gel electrophoresis, and end sequence rescue. Additional procedures illustrating the use of yeast as a host organism for YAC analysis and manipulation were covered, including YAC transfer between yeast strains to facilitate isolation of pure clone DNA, the generation of high-density physical maps using a YAC fragmentation strategy, and the introduction of mammalian selectable markers or specific mutations into YACs. Techniques for preparing large-insert DNA for

transfer into ES or other cell lines via lipofection, spheroplast fusion, or pronuclear injection were included. Gene identification from large cloned DNA segments was accomplished by laboratory exercises in cDNA selection and/or exon trapping. Candidate gene evaluation through computer-based sessions designed to access data and applications available on the World Wide Web were emphasized, including map, similarity, and expression profile information. Studies in mutational analysis for identifying the gene of interest were discussed in depth. The laboratory-based component of the course was supplemented by lectures by invited speakers who are prominent investigators and utilized many of the procedures taught in the course. All participants presented their own research topics, which were used as the basis for structured discussions of how to apply current technologies to specific research projects.

PARTICIPANTS

Attie, A., B.S., Ph.D., University of Wisconsin, Madison
Ayele, M., B.S., M.S., Texas Technical University, Lubbock
Baranzini, S., B.S., M.S., Ph.D., University of California, San Francisco
Berghmans, S., D.V.M., University of Liege, Belgium
Budiman, M., B.S., Clemson University, South Carolina
Feuerman, M., B.A., Ph.D., State University of New York, Brooklyn
Gardner, K., B.F.A., M.D., University of Pittsburgh, Pennsylvania
Hornum, L., M.S., Ph.D., Hagedorn Research Institute, Gentofte, Denmark
Lan, H., B.S, Ph.D., University of Wisconsin, Madison
Maumenee, I., B.S., M.D., Johns Hopkins University, Baltimore, Maryland
Pegoraro, E., B.S., M.D., University of Padova, Italy
Roth, S., M.S., University of Helsinki, Finland
Saitta, F., B.S., Ph.D., Johns Hopkins University, Baltimore, Maryland
Villafuerte, S., M.S., Ph.D., University of Antwerp, Belgium
Winkler, S., B.S., EMBL, Heidelberg, Germany
Wise, R., B.S., Ph.D., United States Department of Agriculture, Ames, Iowa

SEMINARS

Baxevanis, A., National Institutes of Health, Bethesda, Maryland. Computational analysis I: Alignment tools and assigning function to candidate proteins. Computational analysis II: Gene finding in genomic sequence.
Birren, B., Whitehead Institute/Massachusetts Institute of Technology, Cambridge. BAC and methods of library screening. Map building.
Church, D., Mount Sinai Hospital, Toronto, Canada. Functional genomics: Generating a biological map of the mouse genome.
Dietz, H., Johns Hopkins University, Baltimore, Maryland. Mutational screening.
Green, E., National Institutes of Health, Bethesda, Maryland. Mapping and sequencing a human chromosome: How and why.
Jacob, H., Medical College of Wisconsin, Madison. Comparative mapping to facilitate positional cloning.
Parimoo, S., Johnson & Johnson, Skillman, New Jersey. Postional cloning of hair follicle genes.
Roe, B., University of Oklahoma, Norman. The Human Genome Project: So many bases, so little time—A view from the trenches.
Spencer, F., Johns Hopkins University, Baltimore, Maryland. Genome fidelity: View from a model system.
Stein, L., Cold Spring Harbor Laboratory. Map building.
Trask, B., University of Washington, Seattle. Genomic insights through cytogenetics.
Reeves, R., Johns Hopkins University, Baltimore, Maryland. Comparative genomic strategies in the investigation of complex genetic traits.
Silverman, G., Harvard Medical School, Boston, Massachusetts. Now that I found it, what does it do? Functional analysis of single genes.

Caenorhabditis elegans

November 11–24

INSTRUCTORS	**Hengartner, Michael,** Ph.D., Cold Spring Harbor Laboratory **Jorgensen, Erik,** Ph.D., University of Utah, Salt Lake City **Plasterk, Ronald,** Ph.D., Netherlands Cancer Institute, Amsterdam
ASSISTANTS	**Harris, Todd,** University of Utah, Salt Lake City **Korswagen, Hendrik,** Netherlands Cancer Institute, Amsterdam

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*, as well as students with some prior worm experience who wished to expand their repertoire of expertise. The following topics were covered both in the laboratory and by lectures from experts in the field: worm pushing, *C. elegans* databases and worm bioinformatics, anatomy and development, forward genetics, chemical and transposon mutagenesis, generation of transgenic animals, expression pattern analysis, reverse genetics, con-

struction 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

Berezikov, E., M.S., Novosibirsk State University, Russia
Calvo, D., B.S., Ph.D., Harvard Medical School, Boston, Massachusetts
Graves, B., B.A., Ph.D., University of Utah, Salt Lake City
Hagen, F., B.S, Ph.D., University of Rochester, New York
Herlitze, S., B.S., Ph.D., University of Tübingen, Germany
Herr, W., B.A., Ph.D., Cold Spring Harbor Laboratory
Lamphear, B., B.S., Ph.D., Louisiana State University, Shreveport
Nass, R., B.A., B.S., Ph.D., Vanderbilt University, Nashville, Tennessee
Peraus, G., B.S., Ph.D., Hoechst Marion Roussel AG, Germany
Silverman, G., B.A., M.D., Ph.D., Harvard University, Boston, Massachusetts
Trupp, M., B.S., Ph.D., Karolinska Institute, Stockholm, Sweden
Van Gilst, M., B.S., Ph.D., University of California, San Francisco
Vietor, I., Ph.D., I.M.P., Research Institute of Molecular Pathology, Vienna, Austria
Walker, A., B.S., Ph.D., Harvard University, Boston, Massachusetts
Westerlaken, J., M.S., University of Nijmegen, The Netherlands
Zhang, Z., B.M., Ph.D., Harvard University, Boston, Massachusetts

SEMINARS

Blumenthal, T., University of Colorado Medical Center, Denver. Splicing—Worm biochemistry.
Chalfie, M., Columbia University, New York. Mechano-sensation—Use of GFP for reporters.
Durbin, R., Wellcome Trust, Sanger Center, Cambridge, United Kingdom. The *C. elegans* genome—AceDB.
Hengartner, M., Cold Spring Harbor Laboratory. Programmed cell death—Suppression genetics.
Jorgensen, E., University of Utah, Salt Lake City. Synaptic transmission—Nomenclature, forward genetics.
Kemphues, K., Cornell University, Ithaca, New York. Early embryonic development—RNA inhibition.
Meyer, B., University of California, Berkeley. Sex determination/ Dosage compensation —Transgenics.
Okkema, P., University of Illiniois, Chicago. Muscle development/gene expression—Reporter gene constructs, ectopic expression.
Plasterk, R., Netherlands Cancer Center, Amsterdam, The Netherlands. G proteins in *C. elegans*—Reverse genetics.
Ruvkun, G., Harvard Medical School, Boston, Massachusetts. Dauer formation, genome analysis.
Seydoux, G., Johns Hopkins University, Baltimore, Maryland. Regulation of gene expression—In situ hybridization.

Computational Genomics

November 5–10

INSTRUCTORS **Pearson, William,** Ph.D., University of Virginia, Charlottesville
Smith, Randall, Ph.D., SmithKline Pharmaceuticals

ASSISTANT **Retief, Jacques,** University of Virginia, Charlottesville

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 of 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 was taught using Windows NT and Macintosh workstations connected to the World Wide Web and Unix server. Participants were expected to be comfortable using the Unix operating system, programs, and a Unix text editor (programming knowl-

edge was not required). The course was ideal for biologists seeking advanced training in biological sequence analysis, computer core directors, and staff for molecular biology or genetics resources and for scientists in other disciplines, such as computer scientists, who wished to survey current research problems in biological sequence analysis.

PARTICIPANTS

Bongcam, R., B.S., Ph.D., Uppsala University, Sweden
Cabot, E., B.A., Ph.D., Genetics Computer Group, Inc., Madison, Wisconsin
Chen, R., B.S., M.S., Allergan, Inc., Irvine, California
Christoffels, A., B.S., M.S., University of Western Cape, Cape Town, South Africa
Cusick, M., B.S., Ph.D., Wadsworth Center, Albany, New York
Dwyer, R., B.A., M.S., Ph.D., North Carolina State University, Raleigh
Fink, L., B.S., University of California, San Diego
Gardiner, K., B.S., Ph.D., Eleanor Roosevelt Institute, Denver, Colorado
GuhuThakurta, D., M.S., Johns Hopkins University, Baltimore, Maryland
Hazard, E.S. III, B.S., M.S., Ph.D., Medical University of South Carolina, Charleston
Knowlton, R., B.A., Ph.D., SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania
Long, M., B.S., Ph.D., University of Chicago, Illinois
Lopez, L., B.S., Ph.D., University of Sao Paolo, Brazil
Metz, T., B.S., M.S, Ph.D., GSF National Research Center, Neuherberg, Germany
Morley, M., B.A., M.S., Children's Hospital of Philadelphia, Pennsylvania
Nachman, I., B.S., Ph.D., Compugen, Petach Tikva, Israel
Person, C., M.S., Lexicon Genetics, Inc., The Woodlands, Texas
Radelof, U., B.S., Max-Planck Institute, Berlin, Germany
Raumann, B., B.A., Ph.D., University of California, San Francisco
Sasinowski, M., B.S., Ph.D., Clemson University Genomics Institute, South Carolina
Schaid, D., B.S., Ph.D., Mayo Clinic, Rochester, Minnesota
Snyder, E., B.A., Ph.D., Genomica Corporation, Boulder, Colorado
Waters, E., B.A., Ph.D., Marquette University, Milwaukee, Wisconsin
Wintero, A.K., M.S., Ph.D., The Royal Veterinary & Agricultural University, Frederiksberg, Denmark

SEMINARS

Altschul, S., National Institutes of Health, Bethesda, Maryland. Statistics of sequence similarity scores. Identifying distant relationships with BLAST2 and PSI-BLAST. Statistic of patterns and profiles.
Eddy, S., Washington University, St. Louis, Missouri. Blocks, motifs, domains, and other databases. Multiple sequence comparison with hidden markov models.
Henikoff, S., Fred Hutchinson Cancer Research Center, Seattle, Washington. Using BLOCKS to identify very distant relationships.
Neuwald, A., Cold Spring Harbor Laboratory. Exploiting domains.
Pearson, W., University of Virginia, Charlottesville. Introduction and overview. Protein evolution– biology. Algorithms for pairwise sequence comparison.
Smith, R., SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania. Introduction to multiple sequence comparison.
Stormo, G., University of Colorado, Boulder. Identifying sites in unaligned sequences. New approaches to discovering features in biological sequences. Eukaryotic gene identification.
Zhang, M., Cold Spring Harbor Laboratory. Computing on yeast promoters.

Phage Display of Combinatorial Antibody Libraries

November 11–24

INSTRUCTORS
Barbas, Carlos, Ph.D., Scripps Research Institute
Siegel, Donald, M.D., Ph.D., University of Pennsylvania Medical Center, Philadelphia
Silverman, Gregg, M.D., University of California, San Diego

ASSISTANTS
Carey, Stephen, University of California, San Diego
Fuller, Roberta, Scripps Research Institute

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 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 covered. The lecture was series-presented by a number of invited speakers, who emphasized PCR amplification 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, the

whole biology 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

Balyasnikova, I., M.S., Ph.D., University of Illinois, Chicago
Bubis, M., B.S., M.S., Ph.D., Weizmann Institute of Science, Rehovot, Israel
DiDonato, J., B.A., Ph.D., Lerner Research Institute, Cleveland, Ohio
Dincq, S., M.S., University of Leuven, Belgium
Engel, A., B.S., Ph.D., Roche Diagnostics, Penzberg, Germany
Fernsten, P., B.S., Ph.D., Wyeth-Lederle Vaccines and Pediatrics, West Henrietta, New York
Goodyear, C., B.S., Glasgow Caledonian University, United Kingdom
Martin, G., B.S., M.S., Ph.D., Hoechst Marion Roussel, Inc., Bridgewater, New Jersey
Moeck, G., B.S., Ph.D., Universite Paris-Sud, France, Orsay, France
Pupo-Antunez, M., B.S., M.S., Pedro Kouri Tropical Medicine Institute, Havana, Cuba
Raats, J., M.S., Ph.D., University of Nijmegen, The Netherlands
Reiersen, H., B.S., M.S., University of Bath, United Kingdom
Rookey, K., B.S., M.S., Scriptgen Pharmaceuticals, Waltham, Massachusetts
Thaler, D., B.A., Ph.D., Rockefeller University, New York
Wachtel, M., B.A., Ph.D., United States Department of Agriculture, Albany, California
Zhang, X.K., B.S., Ph.D., Medical University of South Carolina, Charleston

SEMINARS

Barbas, C., Scripps Research Institute, La Jolla, California. Phage display of antibodies and zinc finger proteins.
Kelsoe, G., Duke University, Durham, North Carolina. B-cell development: Phenotype, anatomy location, and diversification of the antibody gene repertoire.
Lowman, H., Genetech, Inc., San Francisco, California. Extending antibody contacts to VEGF by phage display.
Nestler, P., Cold Spring Harbor Laboratory. Encoded combinatorial libraries: Chemistry and beyond.
Pasqualini, R., La Jolla Cancer Research, California. In vivo panning.
Scott, J., Simon Fraser University, British Columbia, Canada. Phage display of peptides.
Siegel, D., University of Pennsylvania Medical Center, Philadelphia. Cell surface selection of combinatorial Fab libraries.
Silverman, G., University of California, San Diego. Repertoire cloning of SLE autoantibodies.
Webster, R., Duke University, Durham, North Carolina. The biology of filamentous phage.
Wilson, I., Scripps Research Institute, La Jolla, California. Structural biology of the immune system.

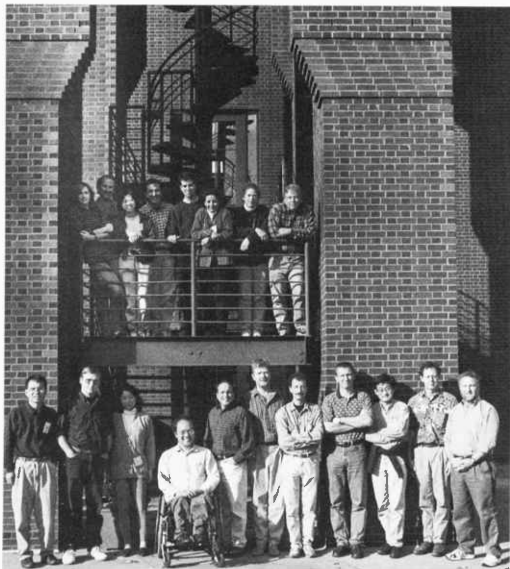
Mouse Behavorial Anaylsis

December 2–15

INSTRUCTORS
Gallagher, Michela, Ph.D., Johns Hopkins University, Baltimore
Silva, Alcino, Ph.D., University of California, Los Angeles

ASSISTANTS
Bourtchuladze, Roussudan, Columbia University
Han, Jung-Soo, Johns Hopkins University, Baltimore
Kogan, Jeff, University of California, Los Angeles
Wolfer, David, University of Zurich, Switzerland

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 especially 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, the rotor-rod, and other activity tests. In addition, there were demonstrations of several aspects of in vitro and in vivo electrophysiology (fields and whole-cell recordings of synaptic plasticity and hippocampal place cell recordings).



PARTICIPANTS

Anokhin, K., M.D., Ph.D., Institute of Normal Physiology, Moscow, Russia
Bartsch, D., B.S., Ph.D., Columbia University, New York
Berke, J., B.A., Ph.D., National Institutes of Health, Bethesda, Maryland
Bito, H., B.A., M.D., Ph.D., Kyoto University, Japan
Boulter, J., B.A., Ph.D., University of California, Los Angeles
De Lacalle, S., M.D., Ph.D., Beth Israel Deaconess Medical Center, Boston, Massachusetts
Hsiao, K., B.A., M.D., Ph.D., University of Minnesota, Minneapolis
Huang, T.-T., B.S., Ph.D., University of California, San Francisco
Huynh, D., B.S., Ph.D., Cedars-Sinai Medical Center, Los Angeles, California
Martinez, A., B.S., Ph.D., University of Texas, San Antonio
Simpson, E., B.S., Ph.D., The Jackson Laboratory, Bar Harbor, Maine
Wetsel, W., B.A., Ph.D., Duke University, Durham, North Carolina

SEMINARS

Alcino, S., University of California, San Francisco. Introduction—Orientation.
Basbaum, A., University of California, San Francisco. The neurobiology of pain and its control.
Burwell, R., Brown University, Providence, Rhode Island. Cortical/hippocampal anatomy.
Davis, M., Emory University, Atlanta, Georgia. Neural system involved in fear and anxiety.
Dudai, Y., Weizmann Institute of Science, Rehovot, Israel. Chemical senses and ecological behaviors in rodents: Paradigms for the analysis of normal and abnormal learning. Interim reevaluation of the contribution of neurogenetics to the analysis of learning and memory.
Eichenbaum, H., Boston University, Massachusetts. Hippocampal place cells: Space and more. The nature of hippocampal memory representation.
Holland, P., Duke University, Durham, North Carolina. Behavioral analysis of learning and memory.
Gallagher, M., Johns Hopkins University, Baltimore, Maryland. The aging brain.
Laroche, S., CNRS-Universite Paris-SUD, Orsay, France. A molecular biological approach to synaptic plasticity and learning.
McClelland, J., Carnegie Mellon Institute, Pittsburgh, Pennsylvania. Why there are complementary learning systems in hippocampus and neocortex: Insights from the success and failures of connectionist models of learning and memory.
Muller, R., State University of New York, Brooklyn. Site visit.
Squire, L., University of California, San Diego. The measurement of mammalian memory functions: Lessons from humans.
Price, D., Johns Hopkins University, Baltimore, Maryland. Transgenic models of neurodegenerative disease.
Thompson, R., University of Southern California, Los Angeles. Genetic approaches to mechanisms of cerebellar-dependent memory.
Wilson, M., Massachusetts Institute of Technology, Cambridge. Rodent hippocampal function.
Wolfer, D., University of Zurich, Switzerland. Meta-analysis of behavior in large numbers of mutant and wild-type mice: Conclusions concerning genetic background.

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
Access Graphics
ALA Scientific Instruments, Inc.
American Laser Corporation
Amersham Life Science Incorporated
Amersham Pharmacia Biotech, Inc.
Amresco
Applied Precision
Applied Scientific Instrumentation, Inc.
Applied Spectral Imaging
Atto Instruments
Axon Instruments
Bachem Biosciences
Beckman-BREA
Becton Dickinson Labware
Bio-101
Bio-Rad Laboratories
Bioptechs
Boehringer Mannheim Corp.
Burleigh Instruments, Inc.
Carl Zeiss
CBS Scientific
Cell & Molecular Technologies, Inc.
Chroma Technology Corporation
Clontech Laboratories, Inc.
Codonics Inc.
Coherent Laser
Cooke Corporation
Cora Styles
Corning Costar Corporation
Corning Inc.
Diagnostic Instruments, Inc.
Diruscio & Associates
Drummond Scientific Company
DuPont NEN
Dynal
Dynex
E-Y Laboratories
E-Y Laboratories, Inc.
Eastman Kodak Company
Epicentre Technologies
Eppendorf Scientific
Ericomp
Fine Science Tools
Fisher Scientific
FMC Corporation
Fostec Inc.
Fotodyne
General Valve
Gibco/Life Technologies
Hamamatsu Photonic Systems
Hampton Research
Havard Apparatus Inc.
Hewlett Packard
Hoeffer Pharmacia
HVS Image Ltd.
Inovision
Instrutech
Invitrogen Corporation
Jackson Immunoresearch Laboratories
Jouan, Inc.
Kopf Instruments
Lecroy
Leica, Inc.
Lexicon Genetics Incorporated
LiCor, Inc.
Life Technologies, Inc.
Ludl Electronic Products
Luigs & Neumann
MatTek Corporation
Med Associates
Media Cybernetics
Medical Systems Corporation
Meta Systems
Micro Video Instruments
Millipore
MJ Research, Inc./
Modulation Optics, Inc.
Molecular Dynamics
Molecular Probes
Nalge Labware
Nalge Nunc International
Narishige International USA, Inc.
NEN Life Science Products
New England Biolabs
Nikon
Novagen
Novex
Ocean Optics
Olympus America, Inc.
Omega Optical
Operon Technologies Inc.
Optronics Engineering
Orinvchm Packard Instruments
Owl Scientific, Inc.
Packard Instruments
PE Biosystems
Perceptive Biosystems
Perkin-Elmer Applied Biosystems
PerSeptive Biosystems
Pfanstiehl Laboratories, Inc.
Photometrics, Inc.
Pierce
Polyfiltronics
Princeton Separations
Promega Corporation
Qiagen Inc.
Robbins Scientific Corporation
Savant Instruments
Scanalytics Inc.
Schleicher & Schuell
Seikagaku America, Inc.
Sherwood-Davis & Geck
Shimadzu
Sigma-Aldrich
Sony Medical Systems
Sooner Scientific, Inc.
Sorenson
Spectra-Physics Lasers, Inc.
Stoelting Co.
Stratagene
Sutter Instruments
Techne, Inc.
Technical Manufacturing Corp.
Tektronix
Tropix
Upstate Biotechnology Inc.
Vector Laboratories
Vorhies Technologies, Inc.
VWR Scientific Products
Vysis
Wallac, Inc.
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. Gary Karpen, Salk Institute. Are centromere identity and function determined by an epigenetic mechanism? (Host: Tatsuya Hirano)

Dr. Daniel Leahy, Department of Biophysics and Biophysical Chemistry, Johns Hopkins University. Structure and function of the developmental signaling molecule hedgehog. (Host: Leemor Joshua-Tor)

Dr. Gary Stormo, University of Colorado, Boulder. Specificity in protein/DNA interactions: Experimental and computational approaches. (Host: Michael Zhang)

Dr. Barbara Meyer, HHMI/University of California, Berkeley. Sex determination and X-chromosome dosage compensation in *C. elegans*. (Host: Tatsuya Hirano)

February

Dr. Tak Mak, Ontario Cancer Institute, Canada. Three dead mice, see how they run. (Host: Scott Lowe)

Dr. Barry Gumbiner, Memorial Sloan Kettering Cancer Center. Signaling and morphogenesis mediated by cadherins and catenins. (Host: David Helfman)

Dr. Denise Montell, Johns Hopkins University School of Medicine. Molecular genetics of cell migration in *Drosophila*. (Host: Tim Tully)

Dr. Phil Green, University of Washington, Seattle. Assembly of genome sequences and ESTs. (Host: Michael Zhang)

March

Dr. Stephen Johnston, University of Texas, Southwestern Medical Center. Genetic to genomic immunization: A technological approach to making vaccines. (Host: Leemor Joshua-Tor)

Dr. Peter Kim, HHMI/MIT/Whitehead Institute. Invasion of cells by viruses. (Host: James Moore)

Dr. Michael Greenberg, Harvard Medical School, Division of Neuroscience. Signal transduction pathways that regulate differentiation and survival in the developing nervous system. (Host: Hollis Cline)

Dr. William Bialek, NEC Research Institute, Princeton, New Jersey. What do neurons compute? Low-dimensional features in complex signals. (Host: Karel Svoboda)

April

Dr. Gerald Joyce, Scripps Research Institute. Order from randomness in the in vitro evolution of a DNA enzyme. (Host: Adrian Krainer)

Dr. Lewis C. Cantley, Harvard Medical School. Structural basis for specificity in signal transduction. (Host: Michael Wigler)

Dr. Mark Peifer, University of North Carolina, Chapel Hill. Cell adhesion, signal transduction, and cancer: The Armadillo connection. (Hosts: Nick Tonks/David Helfman)

Dr. Xiaodong Wang, University of Texas, Southwestern Medical Institute. Biochemical studies of apoptosis: Putting a colorful puzzle together. (Host: Yuri Lazebnik)

October

Dr. Robert Pruitt, Harvard University. Regulating cellular interactions by modifying extracellular properties. (Host: Ueli Grossniklaus)

Dr. Richard Roberts, New England Biolabs. The real world of restriction enzymes or why I love bugs. (Host: Adrian Krainer)

November

Dr. Robert Darnell, Rockefeller University. Functional studies of neuron-specific paraneoplastic disease antigens. (Host: Karel Svoboda)

Dr. Roland Somogyi, Incyte Pharmaceuticals, Inc. Inference of genetic programs in CNS development and injury. (Host: Michael Zhang)

December

Dr. Alan Weiner, Yale University. Role of p53 and CSB proteins in virally induced chromosome fragility. (Host: Nouria Hernandez)

Dr. Gabriel Nunez, University of Michigan. Regulation of programmed cell death. (Host: Michael Hengartner)

Dr. Steven Smith, State University of New York, Stony Brook. How light activates rhodopsin: Structural insights into the mechanism of a G-protein-coupled receptor. (Host: Winship Herr)

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

Mark Settles (Martienssen Lab): Analogies in protein translocation: What maize can tell us about *E. coli*.
Tom Misteli (Spector Lab): Pre-mRNA splicing in the nuclear landscape.
Qiong Liu (Hengartner Lab): Potential signal transduction protein CED-6 is required for engulfment of apoptotic cells in *C. elegans*.
Meena Selvakumar (Helfman Lab): Regulation of alternative RNA splicing via exon sequences.

February

Yi Liu (Herr Lab): The HSV VP16 associated protein HCF has a conserved function in cell proliferation control.
Jean-Philippe Vielle-Calzada (Grossniklaus Lab): Confessions of a botanical voyeur: Altering the sexual life of *Arabidopsis*.
Dick McCombie: Our first megabase: How we got there and what we found.
Lee Zou (Stillman Lab): One step closer to initiation: The story of CDC45.

March

Michael Gutch (Tonks Lab): A genetic analysis of protein tyrosine phosphatase function and signal transduction in *C. elegans*.
Karel Svoboda: Imaging neuronal function.
Hollis Cline: Imaging mechanisms of neuronal plasticity in vivo.

April

Rui-Ming Xu: The crystal structure of a human mitochondrial protein.
Gregory Hannon: *myc* activates telomerase.
Masaaki Hamaguchi: RICH: Looking for the gene.
Ethan Ford (Hernandez Lab): How do transcriptional activators function? Oct-1 as a model.

October

Marcia Belvin (Yin Lab): Role of the dCREB2 transcription factor in circadian rhythms in *Drosophila*.
Andy Neuwald: Sequence-based prediction of protein structure and function: Chaperone-like ATPases related to the δ′ subunit of DNA polymerase III.
Ana Losada (Hirano Lab): Cohesins: Protein complexes holding sister chromatids together.

November

Bill Tansey: Myc: Dead at 30 (minutes).
Michael Myers (Tonks Lab): PTEN: Sometimes taking it off is better than putting it on.

December

Yalin Wang (Zhong Lab): Calcium imaging of olfactory responses in the mushroom body of adult *Drosophila*.
Michael Greenberg (Skowronski Lab): Nef uses different strategies to down-regulate surface expression of CD4 and MHC class I molecules.
Tracy Kuhlman (Hernandez Lab): The general transcription factors in snRNA transcription: Making sense of the alphabet soup.

UNDERGRADUATE RESEARCH

Program Director: Michael Hengartner

Program Assistant: Jane Reader

An important aspect of the summer program at the Laboratory is the participation of college undergraduate students in active research projects under the supervision of full-time laboratory staff members. The program was initiated in 1959. Since that year, 508 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 459 applicants, took part in the program:

Thomas Bridges, Gonville and Caius College, Cambridge University
Advisor: **Timothy Tully**
Sponsor: Burroughs Wellcome
The cloning of the human homolog of the *Drosophila* learning gene *linotte*.

Brian Chan, Harvard University
Advisor: **Michael Zhang**
Sponsor: National Science Foundation
Computational analysis of intronic elements involved in alternative splicing.

Curtis Robert Chong, Harvard University
Advisor: **Leemor Joshua-Tor**
Advisor: National Science Foundation
Crystal structure of D-cysteine bound to carboxypeptidase A at 1.75 Å resolution.

Serafin Colmenares III, University of Hawaii at Manoa
Advisors: **Nouria Hernandez and Shannon Pendergrast**
Sponsor: Frederica von Stade
Structure-function analysis of the FBI-1 zinc finger domain.

Ruth Cosgrove, New Hall, Cambridge University
Advisor: **David Spector**
Sponsor: Burroughs Wellcome
Visualization of RNA in the living cell.

Justin Cross, Queens' College, University of Cambridge
Advisor: **Linda Van Aelst**
Sponsor: Burroughs Welcome
The functional characterization of AF-6, a Ras-binding protein.

Wilson W. Cui, University of California, San Diego
Advisor: **Peter Nestler**
Sponsor: Olney Fund
Detection by fluorescence of protease substrate specificity using an encoded combinatorial library.

Rachel Dodes, College of Agriculture and Life Sciences, Cornell University
Advisors: **Michael Hengartner and Mona Spector**
Sponsor: National Science Foundation
Toward determining function of CED-9 interacting proteins in *Caenorhabditis elegans*.

Maitreya Dunham, Massachusetts Institute of Technology
Advisor: **Robert Martienssen**
Sponsor: National Science Foundation
Molecular genetics of *asymmetric leaves 1* in *Arabidopsis thaliana.*

Kristina Gremski, Yale University
Advisor: **Ueli Grossniklaus**
Sponsor: Garfield Fund
tlazolteotl: A mutation affecting ovule development and female fertility in *Arabidopsis thaliana*.

Kristin Hendren, Duke University
Advisor: **Rui-Ming Xu**
Sponsor: Jephson Educational Trust
Purification and crystallization studies of the human cell cycle protein hCDC34.

Zainab Khalfan, Cedar Crest College
Advisor: **David Jackson**
Sponsor: Dr. H. Bentley Glass
Determination of cell-to-cell trafficking of the maize KNOTTED-1 protein via grafting.

Shujin Luo, College of Life Sciences, Peking University
Advisor: **Hong Ma**
Sponsor: Emanuel Ax
Isolation of genes expressed in flower development using enhancer trap and gene trap.

Todd Morgan, Harvard University
Advisor: **Bruce Stillman**
Sponsor: Burroughs Wellcome
Human CDC45: The homolog of a yeast replication origin protein.

Jason Moss, Duke University
Advisor: **Ryuji Kobayashi**
Sponsor: Burroughs Wellcome
Improved techniques for MALDI-MS analysis of large proteins.

Masafumi Muratani, University of Tsukuba
Advisor: **William Tansey**
Sponsor: Read Fellowship
Transcriptional activation domains that signal protein destruction.

Sabine Nicoleau, Wesleyan University
Advisor: **Winship Herr**
Sponsor: National Science Foundation
The protein interactions that occur with a specific region of a nuclear host-cell factor called HCF.

Audra Norris, Reed College
Advisor: **W. Richard McCombie**
Sponsors: Libby Internship, Bliss Memorial Fund
Sequence analysis of maize ESTs.

Rithwick Rajagopal, Cornell University
Advisor: **Grigori Enikolopov**
Sponsor: National Science Foundation
Mapping *Drosophila* nitric oxide synthase using the yeast two-hybrid system.

Matthew S. Robbins, Yale University
Advisor: **Gregory Hannon**
Sponsor: Burroughs Wellcome
Identification of secreted proteins overexpressed in human breast cancer using a secretion trap screen.

Patrice Saunders, Howard University
Advisor: **David Helfman**
Sponsor: National Science Foundation
Adhesion-dependent signaling transduction: Normal versus transformed cells.

Markus Seeliger, Universitaet Hannover
Advisor: **Yuri Lazebnik**
Sponsor: Shakespeare Fund
Studies on protein-protein interactions of caspase 9.

Eva M. Smietana, Indiana University–Purdue University at Indianapolis
Advisor: **Scott Lowe**
Sponsor: National Science Foundation
Genetic and biochemical analysis of c-Myc-induced apoptosis in primary mouse embryonic fibroblasts.

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 1998, a total of 355 students participated in 28 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 students' exploration of the local environment. Field classes are held on Laboratory grounds, St. John's Preserve, Fire Island National Seashore, Caumsett State Park, the Cold Spring Harbor Fish Hatchery and Aquarium, as well as other local preserves and sanctuaries.

In addition to the three 2-week sessions, the Adventure Education course meets on two Fridays for trips. The students go on a three-masted schooner to navigate and explore the waters of Long Island Sound and on a 12-mile canoe trip on the Nissequogue River.

PROGRAM DIRECTOR

William M. Payoski, M.A., Adjunct Professor, Nassau Community College

REGISTRAR

Amy Stiso, Cold Spring Harbor Laboratory

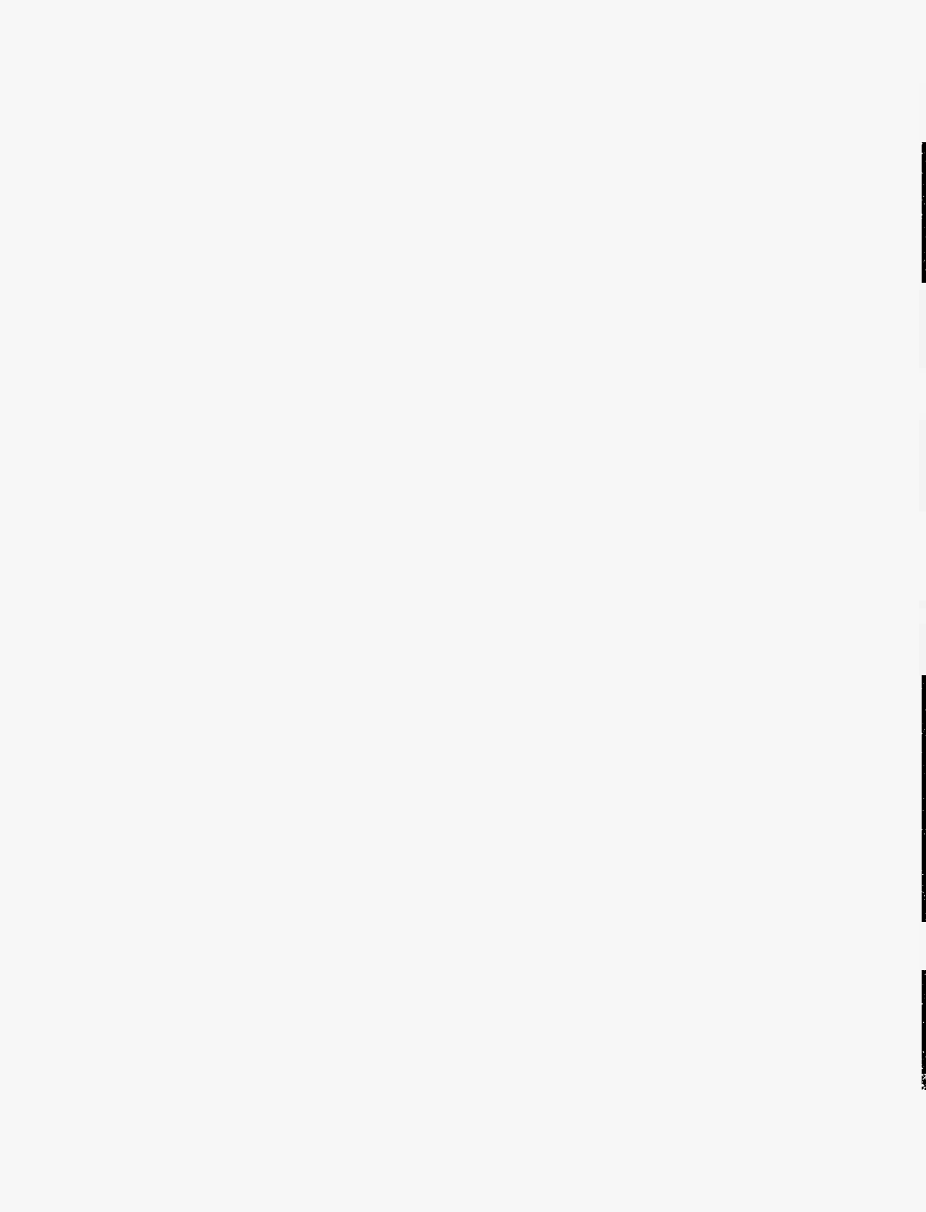
INSTRUCTORS

Alison Forte, B.S. in Marine Science, University of Rhode Island
Donna Pandaliano, M.S., Science Teacher, Valley Stream School District
Linda Payoski, M.S., Science Teacher, Uniondale School District
Marjorie Pizza, M.S., Science Teacher, Locust Valley School District
Brian Withers, Fine Arts Teacher, New York City School System

COURSES

Nature Bugs
Nature Detectives
Nature Discovery
Ecology Explorers
Frogs, Flippers, and Fins
Pebble Pups
Freshwater Life
Seashore Life
Flora, Fauna, and Fun with Watercolors
Marine Biology
Nature Photography
Adventure Education

BANBURY CENTER



BANBURY CENTER DIRECTOR'S REPORT

This was the 22nd year in which scientists from all over the world made their way to the beautiful estate that Charles Robertson so generously gave to Cold Spring Harbor Laboratory. And this year they made their way here in record numbers to participate in a record number of meetings. The Center was also used for meetings by Laboratory scientists and local community groups as well as for courses, so that no fewer than 40 events took place here.

Summary of Meetings and Participants

This was, indeed, a record year of 19 science meetings, 8 meetings for Laboratory-related purposes, 8 occasions when the Center was used for special meetings by local community groups, and the annual 5 neurobiology courses. There were 601 participants in the science meetings alone, of whom 474 were from the United States. Although, as usual, California, Maryland, Massachusetts, and New York accounted for more than 50% of these scientists, participants were drawn from no fewer than 36 states; 75 scientists came from Europe and our most distant visitors were from Japan and Australia. The Corporate Sponsors sent 75 scientists to the Center, and 41 scientists from other companies were invited participants.

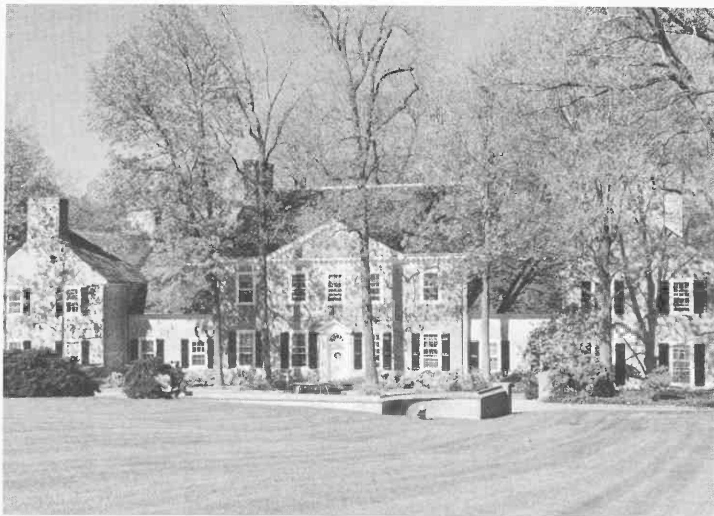
Human Genetic Disorders

We continue to hold meetings dealing with human genetic disorders and disorders in which there is likely to be a strong genetic component. Three of these meetings were designed to review critically specific research and provide guidance on what should be done next. *Superoxide Dismutase and Motor Neuron Disease* examined what is known of the role of superoxide dismutase in cells, its mechanism of action, and how it relates to cell death, and, most importantly, how useful the various animal models of ALS are for understanding the pathology of ALS and for testing therapeutic agents.

Therapeutic agents are not yet available for the treatment of Duchenne muscular dystrophy but major research is under way to exploit the fact that the muscle protein utrophin may be able to substitute for the dystrophin that is missing or nonfunctional in Duchenne muscular dystrophy. The key is to find a small molecule that will turn on the utrophin gene and that can be administered easily to patients. In *Muscle Gene Regulation and Its Therapeutic Potential*—the second in a series of meetings on this subject—we brought together experts from around the world to discuss what is known about the control of muscle gene expression. We were looking for common or special mechanisms that might provide clues as to how to turn on the utrophin gene and what sorts of molecules might work.

The third of these meetings—*Critical Issues in Marfan Research*—dealt with a very important strategic issue in all cases where advances have been made in the understanding of the genetic basis of a human disorder, namely, how to use that genetic knowledge to benefit patients. Thus, this meeting discussed not only continuing laboratory research, especially in mouse models, but also what clinically based research is needed and how new information can be used for diagnosis. Finally, there was discussion of research priorities and how collaborations might be set up to promote research.

Two other meetings in this group covered disorders where there is considerable molecular knowledge of both the genes involved and the systems affected. Because of this, researchers in these fields can begin to make rational, data-based choices of possible therapeutics. *The Molecular Basis of Asthma: Fundamental Processes with Potential Genetic and Therapeutic Targets* was particularly interesting in beginning to integrate all that is known about immunological mechanisms in asthma with the knowledge coming from the genetic analysis of the components of these systems.



Robertson House provides housing accommodations at Banbury Center.

The Molecular Physiology of Weight Regulation and Obesity meeting was equally interesting and exciting. Energy regulation has long been a field of research in physiology, but a revolution occurred some years ago when the first gene involved in obesity—leptin—was cloned. This has led to a remarkable synthesis of findings in genetics, endocrinology, and neuroendocrinology. The workshop explored what is known of leptin and its neuroendocrine control as well as the role of other molecules such as those derived from POMC (pro-opiomelanocortin).

Horse Genetics

At the same time that sequencing the human genome is forging ahead, programs have been established to do the same with animals of agricultural importance. The targeted livestock include cattle, sheep, chickens, and pigs, but one livestock animal that has not received the largesse of substantial funding is the horse. This is unfortunate not least because horses are interesting genetic animals in their own right and, through the horse-racing industry, an important economic force. The Banbury Center meeting on horse genetics was inspired by the claim that thoroughbred performance has not improved in recent years and that this failure may have a genetic basis. However, *Horse Genomics and the Genetics of Factors Affecting Horse Performance* went far beyond that one topic: It provided an occasion for evaluating the current state of horse genetics, for establishing collaborations, and for determined discussions on the future of horse genomics.

Genomics

Complete genome sequences are known for many bacteria, for parasites and yeast, and even for *Caenorhabditis elegans*, a multicellular organism. But all these millions of nucleotides are of little use unless we can identify the genes they contain and find out what the genes do. We held three techni-

cal meetings dealing with these questions, and the first was *Full-length cDNA Cloning: A Workshop on Problems and Solutions*. cDNA clones are made from mRNA that carries information from genes to sites of protein synthesis. They are very useful, representing as they do just genes without all of the sequences in chromosomal DNA that do not code for protein. Unfortunately, it is not easy to make complete cDNA clones or to make copies of all the mRNAs in a cell. Participants critically reviewed the current status of cDNA cloning and tried to identify new strategies for improving cloning. There was also a very interesting discussion of intellectual property rights that affect this work.

Finding human disease genes using mapping techniques has been very successful for disorders where one gene is involved. But it becomes increasingly difficult to do such mapping when several or even many genes may contribute to a disorder. *Large-scale Discovery and Genetic Applications of SNPs* was an in-depth look at single nucleotide polymorphisms, a relatively new tool for doing such mapping. Enthusiasts believe that SNPs will make it possible to find genes involved in complicated disorders, and although there have been some successes (e.g., in diabetes), much still needs to understood about how many SNPs will be needed, how they are to be found, and the theoretical basis for their use.

Finally, finding all of the genes in an organism will be of little use unless we can determine what they do. This has become a critical issue for those organisms for which the complete sequence is known (e.g., yeast). Beyond identifying the functions of genes, we also need to know how sets of genes interact in the life of the cell. Techniques are being developed to do this and *The Genome and Experimental Biology* reviewed a wide variety of approaches, especially those based on chip technology. One consequence of genomic-based research is that data are going to be produced at a very high rate. There was discussion of how to cope with this flood of information, which became very lively when it turned to the physicists' strategy of E-mail publishing.

Infectious Diseases

Banbury Center has long had an interest in Lyme disease and in the use of vaccines to combat infectious diseases. Our series of Lyme disease meetings shows how research in this area has changed—we now have the complete genomic sequence of *Borrelia burgdorferi*. The meeting this year dealt with a more prosaic topic but one of tremendous importance—*Laboratory Methods for the Diagnosis of Lyme Disease*. The meeting reviewed the various tests currently being used, including those based on immunological methods and those based on detecting spirochete DNA or RNA. In addition, there was considerable discussion of factors affecting the reliability of these tests. The meeting concluded with an overview of the key issues and how they might be tackled.

The goal of the Albert B. Sabin Vaccine Institute is to promote the use of vaccines. One obstacle in doing this—a problem common to many fields—is the difficulty of moving from research findings to applications in the "real world." Here, economic, social, and political factors come into play in ways not so evident in the laboratory. *From Bench to Bedside: A Colloquium on Translational Vaccine Control* was a case-based review of these problems and drew on a wide variety of vaccine developments, including vaccines directed against measles, influenza, viral hepatitis, Lyme disease, and even cancer.

Coping with Stress

It is not only human beings that feel stress. In fact, our cells lead very stressful lives and a complex metabolic system has evolved to help them cope. *Integrating Cellular Stress Responses* reviewed how cells detect and respond to a variety of stresses: heat, starvation, hypoxia, and poisoning by heavy metals, for example. The participants discussed the extent to which these challenges initiate the same or similar responses and how these responses evolved. Here is a case where the chip technology referred to above can be used to monitor all the changes in gene expression that go on in a yeast cell when it is stressed.

Our experience with boiling eggs demonstrates the effect of heat on proteins: It produces irreversible changes. And yet there are bacteria and other organisms that flourish in the boiling hot springs of Yellowstone Park or around the "black smoker" vents in the deepest oceans. How they are able to

survive there was the topic of *Biochemistry at 100ºC: How Are Enzymes and Their Substrates Stabilized?* The participants came from a wide variety of backgrounds including chemistry, biophysics, biochemistry, and evolutionary biology. The former dealt with the mechanisms by which life goes on at these high temperatures and the latter discussed the controversial questions of whether these organisms are "primitive" and what they can tell us about the origins of life. We were honored to have Stanley Miller, who carried out seminal experiments on the origin of life more than 40 years ago, participate in the meeting.

The Art of Science—State and Federal Judges

The Federal Judicial Center serves as the agency for research and continuing education for judges in the federal court system. In addition, its Interjudicial Affairs Office is charged with serving as an information clearinghouse for the Center's research and education programs with state judicial systems. These two functions come together in the *Basic Issues in Science* seminars that we hold here at Banbury Center. These are not intended to give specific information on scientific issues as they come before the courts, but rather to give state and federal judges a flavor of how scientists think and carry out their research. Thus, our program includes history and philosophy of science, as well as an education in genetics. We try also to cover contemporary issues—this year, for example, Lee Silver came to Banbury to discuss human cloning.

Genetics Learning on the Web

Banbury Center and the DNA Learning Center are collaborating on a very exciting project to provide a genetics text for the lay public and high school students on the World Wide Web. Although many examples of genetics sites exist on the Web, Dave Micklos and I believe that none of them provides the information at the appropriate level or in a manner that does not intimidate. The Josiah Macy, Jr. Foundation agreed and has provided funding to support the project. Part of this funding is for workshops at Banbury Center to explore how such a site might be designed and made and to assess our progress. Thus, the meeting *Genes, Teens, and the World Wide Web* brought together science educators, Web designers, science writers, and Web scientists. We were especially pleased that Bruce Alberts, President of the National Academy of Sciences, was able to participate. He has made improving science education a major goal of the Academy.

Eugenics Source Materials on the Web

Banbury Center is also collaborating with the DNA Learning Center on another project exploiting the power of the Web to deliver information and materials that would otherwise be inaccessible. Our project, funded by the National Human Genome Research Institute, will make available to teachers, researchers, and the general public up to 1000 images on eugenics. This material is currently stored in archives in academic institutions and only a very small amount has been published in books and journals. An advisory group is helping us prepare this site and providing us with guidance about how to annotate and provide context for the materials. It is a very mixed group, including historians, geneticists, and ethicists. The group met here in September to review what we had collected and to discuss the guidelines for display. For example, should all names and other identifiers be removed? Can this be done without compromising the data? We will now go on to produce some sample pages for further review.

The J.P. Morgan/Cold Spring Harbor Laboratory Executives' Seminar

When this series of meetings began in 1986, there can have been no expectation of their remarkable success. Indeed, this year, the acceptance rate was so high that we became worried that our participants would be flowing out of the door. The meeting was remarkable also for the range of physical

dimensions covered. Entitled *Imaging from Molecules to Brains*, we began with atomic force microscopy of molecules, moved through X-ray crystallography and tracking molecules in cells, to imaging the living human brain at work on various tasks. The topic was chosen in part because of the Laboratory's increasing interest in imaging, and Leemor Joshua-Tor, David Spector, and Karel Svoboda described their work. We are very grateful to David Deming and J.P. Morgan for their continuing support of this extraordinary event.

Other Meetings

On several occasions, scientists from the Laboratory used the Center for small meetings. In addition, we are happy to make the Center available on a limited basis for use by local community groups, as the pressure of our schedule permits. In 1998, such groups included the Lloyd Harbor Conservation Board, Holiday House, and Huntington Hospital Board of Trustees.

Funding

The Corporate Sponsor Program, now in its 15th year, continues to provide funding for six Banbury Center meetings. The participating companies make good use of their spaces at Banbury Center, sending 46 scientists to our meetings. Foundation support of meetings was especially strong in 1998 with no fewer than eight foundations coming to Banbury: the ALS Foundation, the William Theodore Denslow Foundation, the Dorothy Russell Havermeyer Foundation, the National Marfan Foundation, the Merck Genome Research Institute, the Oxnard Foundation, the Albert B. Sabin Vaccine Institute, Shriners Hospitals for Children, and the Swartz Initiative for Computational Neuroscience. Federal funding came from the Federal Judicial Center; the National Heart, Lung, and Blood Institute; the National Human Genome Research Institute; and the National Institute of Allergy and Infectious Diseases. Company support for Banbury Center meetings comes for specific meetings as well as through the

Banbury Conference Center

Corporate Sponsor Program. In 1998, Glaxo Wellcome, Inc.; Immunetics; Research Genetics, Inc.; SmithKline Beecham Pharmaceuticals; and Tularik Inc. provided funding for meetings.

Other News

The most significant change to Banbury Center is the addition of the Meier House to the estate. Immediately across from the Conference Room, this house belonged to Dr. Walter and Mrs. Anne Meier, who was one of the daughters of Charles and Marie Robertson. The house has been refurbished and will provide extra accommodation for our participants. This means that the capacity of the Conference Room is now matched by the accommodation in Sammis Hall and Robertson and Meier Houses and we will not need to ferry people to and from the main Laboratory campus.

A very welcome improvement in 1998 was the upgrade of our Internet connection to a T1 line. The Web has become our primary source of information, and although 10 years ago, the fax machine revolutionized communications with organizers and participants, the impact of E-mail is far, far greater. The increased speed of the T1 line is much appreciated by participants as well, especially for genomics-related meetings where Internet access is a sine qua non of research and presentations.

Acknowledgments

It is not easy coping with over 600 scientists, and Banbury Center could not possibly sustain such a high level of activity without the energy and commitment of many people. First and foremost, Bea Toliver and Ellie Sidorenko manage the Center, dealing with all that is needed to keep things running smoothly, while Katya Davey looks after Robertson House. In addition, Bea Toliver administers the Corporate Sponsor Program that provides support for all Laboratory meetings. The surroundings of the Center make a major contribution to the ambience of the meetings, and Chris McEvoy and Andy Sauer work hard to maintain the beauty of our environment. Participants much appreciate the food at Banbury Center meetings and Jim Hope and his staff continue to meet high standards. The demand for audiovisual equipment becomes increasingly complex as computers join the traditional slide and overhead projectors. The AV team handles it all. Finally, Art Brings and his Staff in Facilities and Housekeeping look after us all year round.

Jan Witkowski

Neurocomputational Strategies: From Synapses to Behavior

February 1–4

FUNDED BY **The Swartz Initiative for Computational Neuroscience**

ARRANGED BY **R. Malinow,** Cold Spring Harbor Laboratory
T. Sejnowski, The Salk Institute for Biological Studies

SESSION 1: Cellular and Molecular Mechanisms Underlying Synaptic Signaling, Synaptic Plasticity, and Axonal Pathfinding
Chairperson: T. Sejnowski, The Salk Institute for Biological Studies, San Diego, California

M. Poo, University of California, San Diego, La Jolla: Long-range propagation of LTP and LTD in neural networks.
G.J. Goodhill, Georgetown University Medical Center, Washington, D.C.: Theoretical modeling of axon guidance.
D. Willshaw, University of Edinburgh, Scotland: Competitive influences in the development of nerve connections.
R. Malinow, Cold Spring Harbor Laboratory: Silent synapses in plasticity.
Z.F. Mainen, Cold Spring Harbor Laboratory: Increase in functional AMPA receptors during LTP.
T. Bonhoeffer, Max-Planck Institute for Psychiatry, Munchen, Germany: Specificity of synaptic enhancement in the hippocampus.

SESSION 2: Spike Coding in Axons and Spike Decoding in Dendrites
Chairperson: R. Malinow, Cold Spring Harbor Laboratory

M. Wilson, University of California, Davis: The smallest unit of synaptic transmission.
K. Svoboda, Cold Spring Harbor Laboratory: Dendritic function in neocortex in vivo.
F. Gabbiani, California Institute of Technology, Pasadena: Multiplying with neurons.
D. Johnston, Baylor College of Medicine, Houston, Texas: Dendritic computations.
G.M. Shepherd, Yale University School of Medicine, New Haven, Connecticut: Action potential propagation in dendrites—Its significance for information processing in the olfactory system.
I. Segev, Hebrew University, Jerusalem, Israel: The noisy neuron.

P. Konig, T. Sejnowski, N. Kopell, A. Destexhe, K. Svoboda

SESSION 3: Thalamocortical Interactions and Short-term Synaptic Plasticity
Chairperson: R.E. Shrock, State University of New York, Stony Brook

S.M. Sherman, State University of New York, Stony Brook: Functional organization of thalamus and thalamocortical interactions.
A. Destexhe, Laval University, Quebec, Canada: How corticothalatic feedback and intrathalatic inhibition cooperate to synchronize oscillations over large cortical territories.
P.R. Adams, State University of New York, Stony Brook: The thalamocortical algorithm.
D.A. McCormick, Yale University School of Medicine, New Haven, Connecticut: Network and computational implications of short- and long-term changes in thalamic and cortical activity.
M.V. Tsodyks, Weizmann Institute of Science, Rehovot, Israel: Dynamics of neocortical circuits—Models and experiments.
L.F. Abbott, Brandeis University, Waltham, Massachusetts: The role of short-term synaptic plasticity in cortical processing.

SESSION 4: Mechanisms for Response Properties and Synchronization of Cortical Neurons
Chairperson: P.R. Adams, State University of New York, Stony Brook

D.L. Ferster, Northwestern University, Evanston, Illinois: Assembly of receptive fields in cat visual cortex.
K.D. Miller, University of California, San Francisco: Ocularly matched, contrast-invariant orientation tuning in cat V1: Development and mature circuitry.
G.G. Turrigiano, Brandeis University, Waltham, Massachusetts: Activity-dependent scaling of synaptic strengths in neocortical networks.
P. Konig, Institute for Neuroinformatics, Zurich, Switzerland: Is synchronization of neuronal activity relevant for behavior?
R.D. Traub, Birmingham University School of Medicine, United Kingdom: Synaptic plasticity induced by 40 Hz oscillations.
N. Kopell, Boston University, Massachusetts: Mechanisms for synchronization (and desynchronization) in networks of neurons.
R.E. Shrock, State University of New York, Stony Brook: Synaptic plasticity in neural networks modeling brain function.

SESSION 5: Neural Assemblies and Neural Population Codes
Chairperson: L.F. Abbott, Brandeis University, Waltham, Massachusetts

D. Horn, Tel Aviv University, Israel: Memory maintenance via neuronal regulation.
D.W. Tank, Bell Laboratories, Lucent Technologies, Murray Hill, New Jersey: Cellular and circuit mechanisms of persistent neural activity.
W.B. Kristan, University of California, San Diego: Population codes for directed behaviors in simple nervous systems.
T. Sejnowski, The Salk Institute for Biological Studies, San Diego, California: Limits on the accuracy of population codes.

SESSION 6: Whither Computational Neuroscience?
Discussion Leader: T. Sejnowski, The Salk Institute for Biological Studies, San Diego, California

Superoxide Dismutase and Motor Neuron Disease

February 22–25

FUNDED BY **Amyotrophic Lateral Sclerosis Association**

ARRANGED BY **R.H. Brown,** Massachusetts General Hospital
R. Horvitz, Massachusetts Institute of Technology

SESSION 1: Superoxide Dismutase—Structure and Related Issues
Chairperson: R.H. Brown, Massachusetts General Hospital, Charlestown

R.H. Brown, Massachusetts General Hospital, Charlestown: Introduction to ALS and SOD1—WT and mutant.
I. Fridovich, Duke University Medical Center, Durham, North Carolina: A superoxide-dependent proxidase activity of the H48Q variant.
L.J. Hayward, Massachusetts General Hospital-East, Boston: Introduction to ALS and SOD1—WT and mutant.
P.J. Hart, University of California, Los Angeles: Atomic structure of human SOD and effect of FALS mutations on this structure.
V.S. Culotta, Johns Hopkins University, School of Hygiene and Public Health, Baltimore, Maryland: The role of copper chaperone for superoxide dismutase.

SESSION 2: Superoxide Dismutase—Catalytic and Noncatalytic Mechanisms
Chairperson: J.S. Valentine, University of California, Los Angeles

J.S. Valentine, University of California, Los Angeles: The abnormal copper chemistry of ALS-mutant CuZnSODs.
E.R. Stadtman, National Heart, Lung, and Blood Institute, NIH, Bethesda, Maryland: Increased peroxidation.
M.B. Yim, National Heart, Lung, and Blood Institute, NIH, Bethesda, Maryland: Increased peroxidation.
J.P. Crow, University of Alabama at Birmingham: Aberrant metal binding by SOD mutants and possible functional consequences.
M.F. Beal, Massachusetts General Hospital, Boston: Oxidative pathology in ALS.
C. Kunst, Eleanor Roosevelt Institute, Denver, Colorado: Aberrant protein binding.
A.L. Goldberg, Harvard Medical School, Boston, Massachusetts: Selective degradation of abnormal proteins—Proteasome inhibitors and protection against cell damage.
K.J. Davies, University of Southern California, Los Angeles: Protein aggregation/recycling.

R. Brown, R. Horvitz

SESSION 3: Superoxide Dismutase—Cell Death Activation
Chairperson: H.R. Horvitz, HHMI, Massachusetts Institute of Technology, Cambridge

H.R. Horvitz, HHMI, Massachusetts Institute of Technology, Cambridge: Overview of programmed cell death.
C.M. Troy, College of Physicians & Surgeons of Columbia University, New York: Caspase specificity in different models of neuronal cell death.
R.P. Roos, University of Chicago, Illinois: Mechanisms of FALS-associated neuronal death and rescue.
D.A. Figlewicz, University of Rochester Medical Center, New York: Pathways of cell death in mutant–SOD-expressing spinal motor neurons.
R.M. Friedlander, Massachusetts General Hospital, Boston: Mechanisms and modulation of the ICE cell death cascade.
S. Przedborski, Columbia University, New York: (1) Programmed cell death. (2) Oxidative stress.
D.E. Bredesen, The Burnham Research Institute, La Jolla, California: Protein abnormalities in motor neuron diseases.

SESSION 4: Animal Models for ALS and Other Neurodegenerative Diseases
Chairperson: D. Cleveland, University of California, San Diego, La Jolla

M. Gurney, Pharmacia and Upjohn, Kalamazoo, Michigan: Excess in vivo free radical production in FALS transgenic mice.
D. Cleveland, University of California, San Diego, La Jolla: Mechanism of FALS-linked SOD1 mutant-mediated disease in mice.
D.L. Price, The Johns Hopkins University School of Medicine, Baltimore, Maryland: Models of ALS and other neurodegenerative disorders.
J.P. Phillips, University of Guelph, Ontario, Canada: Targeted expression of normal and FALS human SOD in motor neurons of wild-type and SOD-Null mutants of *Drosophila*.
S.W. Davies, University College London, United Kingdom: Neuronal intranuclear inclusions and trinucleotide repeat disease.

SESSION 5: Non-SOD1 ALS and Other Neurodegenerative Diseases
Chairperson: K.H. Fischbeck, University of Pennsylvania Medical School, Philadelphia

R.H. Brown, Massachusetts General Hospital, Charlestown: Other ALS genes.
K.H. Fischbeck, University of Pennsylvania Medical School, Philadelphia: Spinal and bulbar muscular atrophy (Kennedy's disease).
A.H.M. Burghes, Ohio State University, Columbus: Spinal muscular atrophy/SMN.

SESSION 6: General discussion: Future Research

Participants during meeting break.

Horse Genomics and the Genetics of Factors Affecting Horse Performance

March 8–11

FUNDED BY **The Dorothy Russell Havemeyer Foundation, Inc.**

ARRANGED BY **D. Antczak,** Cornell University
E. Bailey, University of Kentucky

SESSION 1: Background
Chairperson: W.R. Allen, University of Cambridge, United Kingdom

D.F. Antczak, Cornell University, Ithaca, New York and E.F. Bailey, University of Kentucky, Lexington: Introduction.
E.G. Cothran, University of Kentucky, Lexington: Genetic variability in the horse.

SESSION 2: Determinants of Performance
Chairperson: E.F. Bailey, University of Kentucky, Lexington

J.R. Rooney, Queenstown, Maryland: Structural and functional conformation and lameness.
S.G. Kamerling, Louisiana State University, Baton Rouge: Equine performance: Tests and targets.
T. Ivers, Equine Racing Systems, Inc. Washougal, Washington: Nongenetic factors in racehorse performance.

SESSION 3: Performance: What It Is: How To Measure It
Chairperson: D.F. Antczak, Cornell University, Ithaca, New York

E.P. Cunningham, Trinity College, Dublin, Ireland: Estimates of heritability and genetic gain in thoroughbreds.

COMMENTARIES

J. Leimbach, West Chester, Pennsylvania: Signs of inbreeding depression in the thoroughbred. Pedigree analysis. Mapping on full-sibling horse reference families.
A. Porter, Alan Porter Pedigree Research, Lynbrook, New York: The way the racing/breeding community recognizes the leading performers (the social divide—stakes winners and the rest).

J. Witkowski, E. Bailey, D. Antczak

SESSION 4: Horse Genomics

A. Chakravarti, Case Western Reserve University, Cleveland, Ohio: Problems and strategies of complex trait analysis.
A.T. Bowling, University of California, Davis: Synteny map.
M. Binns, Animal Health Trust, Suffolk, United Kingdom: Mapping on full-sibling horse reference families.
E.F. Bailey, University of Kentucky, Lexington: Status of the International Gene Mapping Workshop for the Horse: Completion of Phase I.
L.C. Skow, Texas A&M University College of Veterinary Medicine, College Station: Development of anchored type II markers in the horse map.

SESSION 5: Horse Genetics
Chairperson: S.J. O'Brien, National Cancer Institute, Frederick, Maryland

D.F. Antczak, Cornell University, Ithaca, New York: The maternal grandsire effect: Does it exist, and could it be caused by genomic imprinting.
E.P. Cunningham, Trinity College, Dublin, Ireland: Inbreeding and fertility in thoroughbreds.
S. Ohno, City of Hope, Duarte, California: The rule of mitochondrial inheritance dictates Eclipse and Matchem (two foundation sires of the racing thoroughbred) shared the identical mitochondrial sequence.
S. Valberg, University of Minnesota, St. Paul: Clinical application of equine genetics to neuromuscular disorders.
J. Mickelson, University of Minnesota, St. Paul: Genetic basis of the overo lethal white foal syndrome.
L.D. Van Vleck, University of Nebraska, Lincoln: Issues involved with improving quantitative traits such as racing ability.

SESSION 6: Genetics In Other Species
Chairperson: S. Ohno, City of Hope, Duarte, California

S.J. O'Brien, National Cancer Institute, Frederick, Maryland: Comparative genomics in mammals.
S.C. Heath, Rockefeller University, New York: Linkage analysis for large complex pedigrees.
M. Neff, University of California, Berkeley: From cottage industry to community: Canine molecular genetics.

SESSION 7: Future Developments—Scientific and Policy
Discussion Leaders: D.F. Antczak, Cornell University, Ithaca, New York, and **E.F. Bailey,** University of Kentucky, Lexington

Presentation during a meeting.

Full-length cDNA Cloning: A Workshop on Problems and Solutions

March 22-25

FUNDED BY **The Merck Genome Research Institute, National Cancer Institute, and Research Genetics, Inc.**

ARRANGED BY **M.B. Soares,** University of Iowa
P. Carninci, RIKEN, Ibaraki, Japan

SESSION 1: Meeting Overview and Discussion of Issues

M.B. Soares, University of Iowa, Iowa City: Meeting overview.

SESSION 2: Quality Assessment of Full-length cDNA Libraries
Chairperson: P. Carninci, RIKEN, Ibaraki, Japan

C. Auffray, Genetique Moleculaire et Biologie du Development, Villejuif, France: Generating and analyzing full-length cDNA clones for muscle and brain-specific transcripts.

R.A. Gibbs, Baylor College of Medicine, Houston, Texas: Sequencing and sequence analysis of full-length cDNAs.

M.A. Marra, Washington University School of Medicine, St. Louis, Missouri: Sequencing and sequence analysis of full-length cDNAs.

SESSION 3: Synthesis and Selection of Full-length cDNAs I
Chairperson: W. Szybalski, University of Wisconsin Medical School, Madison

M.B. Soares, University of Iowa, Iowa City: Introductory overview.

J. Jessee, Life Technologies, Inc., Rockville, Maryland: New reverse transcriptases for cDNA synthesis.

P. Carninci, RIKEN, Ibaraki, Japan: First-strand synthesis of cDNAs by thermoactivated reverse transcriptase.

C. Schneider, National Laboratory CIB, Trieste, Italy: Strategies to select full-length cDNA copies for cloning.

J. Pelletier, McGill University, Montreal, Canada: Affinity selection of full-length cDNA: RNA product.

P. Carninci, RIKEN, Ibaraki, Japan: Full-length cDNA selection by biotinylated cap trapper.

M. Lovett, University of Texas Southwestern Medical Center, Dallas: CAP trapping as a full-length cDNA methodology.

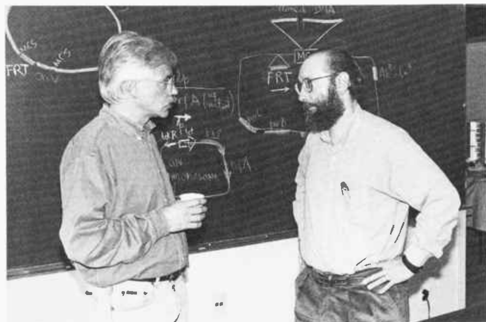
S. Sugano, The University of Tokyo, Japan: Oligo-capping method and its use in construction of full-length cDNA libraries.

S. Kato, Sagami Chemical Research Center, Sagamihara-shi, Japan: Synthesis of full-length cDNA using a DNA-RNA oligo capping method.

N. Nomura, Kazusa DNA Research Institute, Chiba, Japan: Complete sequencing of 610 human nearly full-length cDNA clones which correspond to long transcripts.

D.B. Krizman, National Cancer Institute, Bethesda, Maryland: Analysis of transcript size from various sources of RNA.

G.G. Lennon, Gene Logic, Inc. Columbia, Maryland: Imagene: Clustering of ESTs and full-length clones.



C. Schneider, M. Soares

SESSION 4: Synthesis and Selection of Full-length cDNAs II
Chairperson: S. Weissman, Yale University School of Medicine, New Haven, Connecticut

L. Stubbs, Lawrence Livermore National Laboratory, California: Constructing libraries with large inserts using size-selected mouse cDNA.

M.B. Soares, University of Iowa, Iowa City: cDNA synthesis and cloning from size-fractionated mRNA.

SESSION 5: Critical Issues Pertaining to Cloning and Amplification of Full-length cDNAs: From Single Cells to Bulk Tissues I
Chairperson: S. Weissman, Yale University School of Medicine, New Haven, Connecticut

W. Szybalski, University of Wisconsin Medical School, Madison: Introductory overview.

K.W. Beisel, Boys Town National Research Hospital, Omaha, Nebraska: Construction of cDNA libraries from single cells.

D.B. Krizman, National Cancer Institute, Bethesda, Maryland: Construction of cDNA libraries from a few thousand cells derived from microdissected tissues.

M. McClelland, Sidney Kimmel Cancer Center, San Diego, California: Critical issues pertaining to PCR amplification of full-length cDNAs.

F.S. Hagen, Icogen Corporation, Redmond, Washington: Full-length representative cDNA libraries.

M. Metzker, Merck Genome Research Institute, West Point, Pennsylvania: Full-length cloning and sequencing at Merck.

SESSION 6: Critical Issues Pertaining to Cloning and Amplification of Full-length cDNAs: From Single Cells to Bulk Tissues II
Chairperson: R.A. Gibbs, Baylor College of Medicine, Houston, Texas

W. Szybalski, University of Wisconsin Medical School, Madison: Development of conditionally amplifiable pBAC vectors for cloning of full-length cDNAs.

M.B. Soares, University of Iowa, Iowa City: Full-length normalized libraries. Are they needed? Can they be constructed with existing methods?

V. Prasad, Albert Einstein College of Medicine, Bronx, New York: Factors affecting processivity and fidelity of RT.

S. Weissman, Yale University School of Medicine, New Haven, Connecticut: cDNA display from small numbers of hematopoietic cells.

S. Wiemann, German Cancer Research Center, Heidelberg: Identification and characterization of full-length cDNA.

SESSION 7: Toward the Development of a Unigene Set of Full-length cDNAs
Chairperson: R.A. Gibbs, Baylor College of Medicine, Houston, Texas

J.R. Hudson, Research Genetics, Inc., Huntsville, Alabama: Clone distribution, availability, and intellectual property.

SESSION 8: Revisiting the Critical Issues: Critical Overview, Coordination, and Future Planning
Chairperson: M. Boguski, National Center for Biotechnology Information, NLM, Bethesda, Maryland

S. Weissman, Yale University School of Medicine, New Haven, Connecticut: A critical overview.

M.J. Finley Austin, Merck Genome Research Institute, West Point, Pennsylvania: Coordination among different public and private institutions.

R.L. Strausberg, National Cancer Institute, Bethesda, Maryland: The Cancer Genome Anatomy Project: Coordination challenges. What have we learned from CGAP that could facilitate this project?

E.A. Feingold, National Human Genome Research Institute, Bethesda, Maryland: Role of NHGRI.

N. Nomura, Kazusa DNA Research Institute, Japan: Proposal for cDNA symposiums in Kazusa.

Round Table Discussion on Funding Interests/Opportunities

The Molecular Basis of Asthma: Fundamental Processes with Potential Genetic and Therapeutic Targets

March 29–April 1

FUNDED BY **The William Theodore Denslow Foundation, with additional support from the National Heart, Lung, and Blood Institute, NIH, Tularik, Inc., and private contributions**

ARRANGED BY **J.M. Drazen,** Brigham and Women's Hospital
S.B. Liggett, University of Cincinnati College of Medicine

Goals of Conference:
S.B. Liggett, University of Cincinnati College of Medicine

SESSION 1: Interlukin 5
Chairperson: C.J. Sanderson, TVW Telethon Institute for Child Health Research, Perth, Australia

J. Tavernier, University of Gent Faculty of Medicine, Belgium: Expression and activation of the IL-5 receptor: The close relationship between agonism and antagonism.
A.E.I. Proudfoot, Glaxo Wellcome Research & Development SA, Geneva, Switzerland: Structure-function studies of the IL-5/IL-5R interaction.
P.S. Foster, John Curtin School of Medical Research, Canberra, Australia: IL-5 and allergic airways disease as assessed in IL-5 knockout mice.
C.J. Sanderson, TVW Telethon Institute For Child Health Research, Perth, Australia: IL-5 production.
R.W. Egan, Schering-Plough Research Institute, Kenilworth, New Jersey: Biology of IL-5 blockade.

P. Foster, M. Rothenberg, L. Glimcher

SESSION 2: IL-4 and the TH2 Phenotype-basic Immunobiology
Chairperson: L.H. Glimcher, Harvard School of Public Health, Boston, Massachusetts

L.H. Glimcher, Harvard School of Public Health, Boston, Massachusetts: Transcriptional regulation of IL-4 gene expression.

W.E. Paul, National Institutes of Health, Bethesda, Maryland: IL-4 signaling mechanisms with regard to TH commitment.

R.M. Locksley, University of California, San Francisco: In vivo origins of TH2 cells in murine parasitic infestation.

R.L. Coffman, DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, California: T cell development in murine models of allergic disease.

G.K. Khurana Hershey, Children's Hospital Medical Center, Cincinnati, Ohio: Biology of the R576 IL-4 receptor allele: A gain-of-function variant.

SESSION 3: Polymorphisms of Target Genes in Asthma
Chairperson: S.B. Liggett, University of Cincinnati College of Medicine, Ohio

N.J. Schork, Case Western Reserve University, Cleveland, Ohio: Analysis of target gene variations in complex diseases.

M.T. Boyce-Jacino, Molecular Tool, Inc., Baltimore, Maryland: Methods of rapid detection of polymorphisms.

J.M. Drazen, Brigham and Women's Hospital, Boston, Massachusetts: Genetic variants of 5-lipoxygenase.

S.M. Prescott, University of Utah, Salt Lake City: Platelet activating factor acetylhydrolase gene variation.

S.B. Liggett, University of Cincinnati College of Medicine, Ohio: Polymorphisms of the β-adrenergic receptor.

SESSION 4: Chemokines
Chairperson: J. Oppenheim, NCI-Frederick Cancer Research and Development Center, Maryland

T.J. Williams, National Heart and Lung Institute, London, United Kingdom: Regulation of eotaxin.

M.E. Rothenberg, Children's Hospital Medical Center, Cincinnati, Ohio: Control of eosinophil trafficking by chemokines.

D.M. Center, Boston University School of Medicine, Massachusetts: Regulation of $CD4^+$ T-cell accumulation and activation in the lung by IL-16.

J.A. Elias, Yale University School of Medicine, New Haven, Connecticut: Transgenic modeling: Lessons from IL-11 and IL-16 mice.

N.W. Lukacs, University of Michigan Medical School, Ann Arbor: Role of chemokines on leukocyte recruitment, airway reactivity and lymphokine profiles.

J. Oppenheim, NCI-Frederick Cancer Research and Development Center, Maryland: Chemokine-induced neutrophil release of immunoadjuvants: Defensins and cathepsin G.

SESSION 5: IgE Function and Regulation
Chairperson: S.J. Galli, Beth Israel Deaconess Medical Center, Boston, Massachusetts

S.J. Galli, Beth Israel Deaconess Medical Center, Boston, Massachusetts: Role of mast cells and IgE- and FαRI-dependent amplification and positive feedback mechanisms.

J.V. Ravetch, The Rockefeller University, New York: FCαRII-dependent regulation of the allergic response.

K.J. Moore, Millennium Pharmaceuticals Inc., Cambridge, Massachusetts: Analysis of the defective IgE response in a mouse inbred strain.

J.-P. Kinet, Beth Israel Deaconess Medical Center, Boston, Massachusetts: Positive and negative regulation of signaling via the FαRI.

Critical Issues in Marfan Research

April 17–19

FUNDED BY **National Marfan Foundation, with additional support from Shriners Hospitals for Children**

ARRANGED BY
P. Byers, University of Washington School of Medicine
R.B. Devereux, New York Hospital/Cornell Medical Center
H.C. Dietz, The Johns Hopkins Hospital
Uta Francke, HHMI, Stanford University Medical Center
R.E. Pyeritz, Allegheny University of the Health Sciences
F. Ramirez, Mount Sinai School of Medicine
L.Y. Sakai, Shriners Hospital for Children

SESSION 1: Clinical Research in Marfan Syndrome and Related Disorders
Chairperson: R.E. Pyeritz, Allegheny University of the Health Sciences, Pittsburgh, Pennsylvania

R.E. Pyeritz, Allegheny University of the Health Sciences, Pittsburgh, Pennsylvania: Introduction and overview.

Brief Reports

D.M. Alcorn, Stanford University Medical Center, California
A.C. Braverman, Washington University School of Medicine, St. Louis, Missouri
P. Byers, University of Washington School of Medicine, Seattle
H.C. Dietz, The Johns Hopkins Hospital, Baltimore, Maryland
D.M. Milewicz, University of Texas Medical School at Houston
S.D. Shapiro, Washington University School of Medicine, St. Louis, Missouri

SESSION 2: Lessons from Animal Models
Chairperson: F. Ramirez, Mount Sinai School of Medicine, New York

F. Ramirez, Mount Sinai School of Medicine, New York: Introduction.
H.C. Dietz, The Johns Hopkins Hospital, Baltimore, Maryland: Vascular pathogenesis of MFS and mice.
S.D. Shapiro, Washington University School of Medicine, St. Louis, Missouri: Metalloproteinases which degrade elastic fibers.
P. Carmeliet, KU Leuven, Belgium: Proteinases and aneurysm formation.
H.C. Dietz, The Johns Hopkins Hospital, Baltimore, Maryland: Phenotypic variability in MFS and mice.
R. Akhurst, Onyx Pharmaceuticals, Richmond, California: Identification of modifiers in mice.

P. Byers, R. Akhurst, D. Rifkin, L. Sakai

SESSION 3: Molecular Diagnostics and Databases

A. Diagnostic Issues—Clinical

All participants: Revisit the clinical diagnostic criteria for MFS—experience using the Ghent criteria.
R. Pyeritz, D.M. Milewicz, P. Byers, H.C. Dietz: Define MFS/CCA overlap syndromes.
All participants: Clinical subtypes or continuum?

B. Diagnostic Issues—Laboratory

M. Godfrey, University of Nebraska Medical Center, Omaha, H.C. Dietz: Immunofluorescence with FBN1 antibodies.
D.M. Milewicz: ^{35}Cys pulse-chase studies.

All participants: MFAPS, LTBPS screening methods.
C. Boileau, H.C. Dietz, R. Pyeritz: FBN1.
D.M. Milewicz: FBN2.
M. Godfrey: LTBP2.

C. Database Issues

P. Byers, R. Pyeritz: NMF-supported clinical database.
C. Boileau, INSERM-Clinique Maurice Lamy, Paris, France: Marfan database.
H.C. Dietz: Consortium database.

D. Discussion of Research Priorities and Possible Collaborations

SESSION 4: Summaries and General Discussion
Chairperson: P. Byers, University of Washington School of Medicine, Seattle

R.E. Pyeritz, Allegheny University of the Health Sciences, Pittsburgh, Pennsylvania
F. Ramirez, Mount Sinai School of Medicine, New York

General Discussion
Moderator: P. Byers, University of Washington School of Medicine, Seattle

Coffee break

Muscle Gene Regulation and Its Therapeutic Potential

April 26–29

FUNDED BY **Oxnard Foundation**

ARRANGED BY **K.E. Davies,** University of Oxford
S.D. Hauschka, University of Washington
D. Helfman, Cold Spring Harbor Laboratory
P.W.J. Rigby, MRC National Institute for Medical Research

SESSION 1: Muscle Transcription
Chairperson: P.W.J. Rigby, MRC National Institute for Medical Research, London, United Kingdom

K.E. Davies, University of Oxford, United Kingdom: Introduction to Utrophin Project.
J.D. Haley, OSI Pharmaceuticals, Inc. Uniondale, New York: Searching for small molecules to regulate gene expression.
W. Herr, Cold Spring Harbor Laboratory: Introduction to general transcription factors.
O. Pourquie, Developmental Biology Institute of Marseilles, France: Evidence for a molecular clock linked to somitogenesis.
C.P. Emerson, University of Pennsylvania School of Medicine, Philadelphia: Developmental regulation of MyoD.

SESSION 2: Muscle Gene Regulation I
Chairperson: K.E. Davies, University of Oxford, United Kingdom

M. Buckingham, Institut Pasteur, Paris, France: Myf-5.
P.W.J. Rigby, MRC National Institute for Medical Research, London, United Kingdom: Regulation of Myf-5.
H.H. Arnold, University of Braunschweig, Germany: Control of the Myf-5 gene.
S.F. Konieczny, Purdue University, West Lafayette, Indiana: Positive and negative regulators of myogenesis.
L. Kedes, University of Southern California, Los Angeles: Conformation and acetylation control of MyoD activity.
M. Buckingham, Institut Pasteur, Paris, France: Attaining in vivo levels of muscle gene transcription.

J. Rafael, K. Davies

SESSION 3: Muscle Gene Regulation II
Chairperson: S.D. Hauschka, University of Washington, Seattle

S.J. Tapscott, Fred Hutchinson Cancer Research Center, Seattle, Washington: Expression of genes in native chromatin in skeletal muscle cells.
C.P. Ordahl, University of California, San Francisco: Transcriptional repression and activation via MCAT elements.
P. Maire, INSERM, Paris, France: Characterization of MEF3 proteins and their role in controlling muscle gene expression.
N. Rosenthal, Massachusetts General Hospital-East, Charlestown: The molecular basis of muscle cell diversity.
M.A. Rudnicki, McMaster University, Ontario, Canada: Regulations of myogenic determination and differentiation.
J. Robbins, Children's Hospital Research Foundation, Cincinnati, Ohio: Control of muscle protein levels during transgenic overexpression.

SESSION 4: Muscle Gene Regulation III
Chairperson: D. Helfman, Cold Spring Harbor Laboratory

S.D. Hauschka, University of Washington, Seattle: Are there new muscle gene control elements and transcription factors still to be discovered?
R.J. Schwartz, Baylor College of Medicine, Houston, Texas: Regulation of muscle gene activity.
M. Antoniou, UMDS, Guy's Hospital, London, United Kingdom: The value of locus control regions in integrating and nonintegrating gene therapy vectors.
L. Schaeffer, Pasteur Institute, Paris, France: Implication of a multisubunit Ets-related transcription factor in synaptic expression of the nicotinic acetylcholine receptor.
S. Burden, Skirball Institute, New York University Medical Center: Neuregulin-activated gene expression.
A. Buonanno, National Institute of Child Health and Human Development, Bethesda, Maryland: Activity-dependent and fiber-type-specific regulation of muscle genes.

SESSION 5: Implications for Therapies
Chairperson: T.A. Partridge, Imperial College School of Medicine, London, United Kingdom

B. Jasmin, University of Ottawa, Ontario, Canada: Regulation of utrophin gene expression in skeletal muscle cells.
J.M. Tinsley, University of Oxford, United Kingdom: Multifunctional analysis of utrophin transgenes in mouse models.
J.M. Leiden, The University of Chicago, Illinois: Gene transfer into skeletal and cardiac muscle in vivo.
G. Cossu, University of Rome La Sapienza, Italy: Nonsomitic myogenic progenitors.
J.S. Chamberlain, University of Michigan Medical School, Ann Arbor: Development of muscle-specific adenoviral vectors.

Roundup/General Discussion
Moderator: T.A. Partridge, Imperial College School of Medicine, London, United Kingdom

Cocktails at Robinson House

Laboratory Methods for the Diagnosis of Lyme Disease

August 30–September 2

FUNDED BY **National Institute of Allergy and Infectious Diseases, NIH, Centers for Disease Control and Prevention, and ORD, with additional support from Immunetics and SmithKline Beecham Pharmaceuticals**

ARRANGED BY **P. Baker,** National Institute of Allergy and Infectious Diseases, NIH

SESSION 1: ELISA and Western Blot Assays
Chairperson: B.J. Luft, State University of New York, Stony Brook

A.C. Steere, New England Medical Center, Boston, Massachusetts: Prospective evaluation of two-tiered testing in early Lyme disease.
M. Aguero-Rosenfeld, Westchester County Medical Center, Valhalla, New York: Critique of recommended two-tiered testing.
M.E. Schriefer, Centers for Disease Control and Prevention, Ft. Collins, Colorado: Can two-tiered testing do any better?
J. Glass, Brook Biotechnologies, Inc., Stony Brook, New York: Use of recombinant antigens in diagnosis.
A. de Silva, Yale University School of Medicine, New Haven, Connecticut: Differential *Borrelia burgdorferi* gene expression: Applications in serodiagnosis.
B.J.B. Johnson, Centers for Disease Control and Prevention, Fort Collins, Colorado: Evaluation of recombinant antigens.
A.E. Levin, Immunetics, Inc., Cambridge, Massachusetts: Neural network interpretation of Lyme Western blots: Human versus Deep Green.
F.C. Cabello, Viro Dynamics, New York: Potential use of BmpC, a *Borrelia burgdorferi* protein of the 39 kD family, as a reagent in diagnosis of Lyme disease.

SESSION 2: Nucleic-acid-based Approaches and Applications I
Chairperson: J.L. Goodman, University of Minnesota, Minneapolis

J.L. Goodman, University of Minnesota, Minneapolis: PCR in *Borrelia burgdorferi* infection: Principles, practice, and critical review.
G.P. Wormser, Westchester County Medical Center, Valhalla, New York: PCR and the culturing of *Borrelia burgdorferi*.

SESSION 3: Nucleic-acid-based Approaches and Applications II
Chairperson: J.L. Goodman, University of Minnesota, Minneapolis

I. Schwartz, New York Medical College, Valhalla, New York: Mining the *Borrelia burgdorferi* genome sequence for improved diagnostic targets.
A.C. Steere, New England Medical Center, Boston, Massachusetts: New strategies for improved diagnosis.

SESSION 4: Other Approaches for Diagnosis
Co-Chairpersons: A.G. Barbour, University of California, Irvine and **A.C. Steere,** New England Medical Center, Boston, Massachusetts

E.A. Davidson, Georgetown University School of Medicine, Washington, D.C.: Rapid detection and early diagnosis.
M.S. Klempner, Tufts-New England Medical Center, Boston, Massachusetts: Matrix metalloproteinases in the cerebrospinal fluid of patients with Lyme disease.
P.K. Coyle, State University of New York, Stony Brook: Antigen-capture and immune.
S.E. Schutzer, UMDNJ-New Jersey Medical School, Newark: Immune complex analysis for the diagnosis of early and active Lyme disease.
A.G. Barbour, University of California, Irvine: Bacteriocidal assays.
S. Lesley, Promega Corporation, Madison, Wisconsin: Use of phage display to isolate biotinylated detection reagents.

SESSION 5: Evaluating the Influence of Coinfection and Other Factors
Co-Chairpersons: G.P. Wormser, Westchester County Medical Center, Valhalla, New York and **A. Weinstein,** George Washington University Medical Center, Washington, D.C.

G.P. Wormser, Westchester County Medical Center, Valhalla, New York: Addressing coinfection.
J.W. IJdo, Yale University School of Medicine, New Haven, Connecticut: Serologic diagnosis of human granulocytic ehrlichiosis.
D.T. Dennis, Centers for Disease Control and Prevention, Ft. Collins, Colorado: Evaluating testing in primary care practice.
R.R. Porwancher, Infectious Disease Consultants, P.C., Trenton, New Jersey: A probabilistic approach to the diagnosis of Lyme disease.

SESSION 6: Overview and Panel Discussion
Chairperson: D.J. Gubler, Centers for Disease Control and Prevention, Ft. Collins, Colorado

Panel Members: J.L. Goodman, G.P. Wormser, A.G. Barbour, B.J. Luft, A.C. Steere

Image Archive on the American Eugenics Movement Editorial Advisory Panel Workshop

September 20–22

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: Introduction and Overview

D. Micklos, DNA Learning Center, Cold Spring Harbor Laboratory, and J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory: Introduction—Description of the project and summary of what has been done, review of the functions of the EAP as defined in the grant application, and review of important issues as identified by the NIH Review Panels.

SESSION 2: Round Table Discussion

An opportunity for each participant to raise other issues based on their professional and personal perspectives and making a list of key points to be kept in mind during the meeting.

SESSION 3: "Focus" Talks

M.L. Levitt, The American Philosophical Society, Philadelphia, Pennsylvania: The American Philosophical Society and the Project.

E.J.Thompson, National Human Genome Research Institute, Bethesda, Maryland: Privacy and confidentiality—A view from NHGRI.

P. Reilly, Shriver Center for Mental Retardation, Waltham, Massachusetts: Privacy and confidentiality—A geneticist's view.

H. Friedlander, Brooklyn College/City University of New York, Brooklyn: A historian's view.

SESSION 4: Review Selected Images

Examine selected images in the light of the morning's discussions, with particular reference to: themes, keywords, privacy, context.

Tour of Laboratory and DNA Learning Center; Review Macy Foundation *DNA from the Beginning* Project

SESSION 5: Review Images

Examine as many examples as possible, with particular reference to interest, coverage of themes, technical quality.

SESSION 6: Summary

An opportunity for critical review of the meeting, suggestions for the next stage of the project, date and goals of next meeting.

Gathering outside the Banbury Conference Center.

Y Chromosome in Disease and Evolution

October 13–16

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **H. Cooke,** University of Edinburgh

SESSION 1

J.A. Marshall Graves, La Trobe University, Melbourne, Australia: Evolution of the mammalian Y chromosome, spermatogenesis and sex determining genes.

D.S. Guttman, University of Chicago, Illinois: An X-linked gene with a degenerate Y-linked homolog in the dioecious plant *Silene latifolic*.

M. Mitchell, INSERM, Marseilles, France: The organization of genes on the mouse Y chromosome.

J. Schmidtke, Institute of Human Genetics, Hannover, Germany: Evolution of TSPY and related genes.

S.C. Maxson, University of Connecticut, Storrs: The mouse Y chromosome and behavior.

SESSION 2

P.S. Burgoyne, National Institute for Medical Research, London, United Kingdom: The multiple-copy Y-gene families Rbm and Ssty in the mouse.

Y.-F.C. Lau, University of California, San Francisco: Expression of Y chromosome genes in human prostate cancers.

W.R. Rice, University of California, Santa Cruz: Evolutionary decay of the Y sex chromosome.

B. Charlesworth, University of Edinburgh, United Kingdom: The population genetics of Y chromosome evolution.

D.C. Page, HHMI, Whitehead Institute for Medical Research, Cambridge, Massachusetts: The fall and rise of the human Y chromosome.

SESSION 3

G.A. Rappold, Institute of Human Genetics, Heidelberg, Germany: SHOX—A pseudoautosomal gene involved in growth and development.

W. Schempp, Universitat Freiburg, Germany: Fiber-FISH mapping of DAZ, RBM, and CDY gene family members within deletion interval 6 of the human Y chromosome.

W.-H. Li, University of Texas, Houston: Male-driven evolution of DNA sequences in mammals.

H. Cooke, University of Edinburgh, United Kingdom: Lack of detectable differences in function between DAZ and Dazl.

M.F. Hammer, University of Arizona, Tucson: Evolutionary history of the human Y chromosome.

P. Burgoyne, S. Maxson, E. Simpson

SESSION 4

M.A. Jobling, University of Leicester, United Kingdom: Identification and dating of Y chromosomal lineages, and the influence of selection on Y haplotypes.

E.E. Eichler, Case Western Reserve University, Cleveland, Ohio: Pericentromeric gene duplications and the complex architecture of the human genome.

B.D. McKee, University of Tennessee, Knoxville: Role of gene conversion in maintenance of sequence homogeneity in Su (Ste) and rDNA clusters of *D. melanogaster* X and Y chromosomes.

S. Henikoff, HHMI, Fred Hutchinson Cancer Research Center, Seattle, Washington: Evolution and utility of satellite repeats.

P.A. Underhill, Stanford University, California: Y chromosome SNP halotype diversity.

C.E. Bishop, Baylor College of Medicine, Houston, Texas: Sequencing the mouse Y chromosome.

SESSION 5

E.M. Simpson, The Jackson Laboratory, Bar Harbor, Maine: Toward a physical map of the mouse Y chromosome: Enrichment, sizing, and cloning by bivariate flow cytometry.

D. Vollrath, Stanford University, California: DNA sequence diversity of the human Y chromosome.

S.R. Haynes, National Institute of Child Health and Human Development, NIH, Bethesda, Maryland: Rb97D, a *Drosophila* RNA-binding protein that associates with a specific region of the Y chromosome in spermatocytes.

M. Steinemann, Henrich Heine Universitat Dusseldorf, Germany: *Drosophila miranda:* A model system for Y chromosome evolution and differential gene activity.

P.H. Yen, University of California, Los Angeles, Torrance: Deletion interval 6 of the human Y chromosome: Spermatogenesis genes and large repeats.

J.A. Marshall Graves, La Trobe University, Melbourne, Australia: Meeting overview.

Participants outdoors discussing the meeting during a break.

The Molecular Physiology of Weight Regulation and Obesity

October 18–21

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **R. Cone,** Oregon Health Sciences University
J.S. Flier, Beth Israel Deaconess Medical Center

Goals of the Conference:
J.S. Flier, Beth Israel Deaconess Medical Center, Boston, Massachusetts

SESSION 1: Leptin
Chairperson: R. Cone, Oregon Health Sciences University, Portland

J.M. Friedman, HHMI, The Rockefeller University, New York: Leptin and the neural circuits regulating weight.
J.S. Flier, Beth Israel Deaconess Medical Center, Boston, Massachusetts: Leptin and the regulation of neuroendocrine function.
R.A. Steiner, University of Washington, Seattle: Leptin as a neuroendocrine signal to the reproductive system.
J.L. Cameron, Oregon Regional Primate Research Center, Beaverton: Neural control of food intake in nonhuman primates: Comparison with rodent species.
M.W. Schwartz, Puget Sound VA Health Care System, Seattle, Washington: Leptin, hypothalamic neuropeptides, and energy homeostasis.

SESSION 2: Central Regulation of Energy Balance I
Chairperson: M.W. Schwartz, Puget Sound VA Health Care System, Seattle, Washington

J.K. Elmquist, Beth Israel Deaconess Medical Center, Boston, Massachusetts: Linking mediobasal and lateral hypothalamic feeding centers in the rodent and human brain.
R. Palmiter, HHMI, University of Washington, Seattle: Role of NPY and dopamine in regulation of feeding behavior.
G. Barsh, Stanford University School of Medicine, California: Genetics and biochemistry of endogenous

M. Yanagiswa

melanocortin receptor antagonists.

K.L. Stark, Amgen, Inc., Thousand Oaks, California: AGRP and the control of feeding.

B.E. Levin, VA Medical Center, East Orange, New Jersey: Neuropeptides and the defense of body weight set-point in diet-induced obesity.

SESSION 3: Central Regulation of Energy Balance II
Chairperson: L. Van der Ploeg, Merck Research Laboratories, Rahway, New Jersey

R. Cone, Oregon Health Sciences University, Portland: Melanocortins and energy balance.

K. Gudmundsson, Children's Hospital, Boston, Massachusetts: CRH-leptin interactions.

P.J. Larsen, Novo-Nordisk, Maalov, Denmark: Hypothalamic CART: A new anorectic peptide regulated by leptin.

E. Maratos-Flier, Joslin Diabetes Center, Harvard Medical School, Boston, Massachusetts: Melanin concentrating hormone.

M. Yanagisawa, HHMI, University of Texas Southwestern Medical Center, Dallas: Orexins: Novel lateral hypothalamic neuropeptides that regulate feeding behavior.

SESSION 4: GLP-1/Energy Expenditure
Chairperson: J.M. Friedman, HHMI, The Rockefeller University, New York

S. Bloom, Hammersmith Hospital, London, United Kingdom: GLP-1 and body weight regulation.

D. Drucker, The Toronto Hospital, Canada: Essential neuroendocrine role of GLP-1 in vivo.

M.L. Reitman, National Institutes of Health, Bethesda, Maryland: Role of adipose tissue in metabolic regulation: Lessons from a no-fat mouse.

B. Spiegelman, Dana-Farber Cancer Institute, Boston, Massachusetts: PGC1: A transcriptional regulator of energy dissipation.

G.S. McKnight, University of Washington, Seattle: Role of PKA IIB isoform in weight regulation.

SESSION 5: Genetics of Obesity
Chairperson: J.S. Flier, Beth Israel Deaconess Medical Center, Boston, Massachusetts

J. Naggert, The Jackson Laboratory, Bar Harbor, Maine: Fat and tub.

R.L. Leibel, Columbia University, New York: Molecular physiology of human obesity.

C. Bouchard, Laval University, Ste-Foy, Quebec, Canada: Genetics of human obesity.

A.G. Comuzzie, Southwest Foundation for Biomedical Research, San Antonio, Texas: Genome scanning for obesity genes in humans.

S. O'Rahilly, Addenbrooke's Hospital, Cambridge, United Kingdom: Genetics of early-onset obesity in humans.

A. Gruters-Kieslich, Humboldt University, Berlin, Germany: POMC defects in human obesity.

J.P. Morgan & Co. Incorporated/Cold Spring Harbor Laboratory Executive Conference on Imaging: From Molecules to Brains

October 23–25

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.
C. Bustamante, University of California, Berkeley: Seeing molecules—The scanning force microscope in biology.

SESSION 2

P. Sigler, HHMI, Yale University, New Haven, Connecticut: Proofreading and editing protein folding—Imaging the final step in gene expression.
R. Tsien, HHMI, University of California, San Diego: Fluorescent sensors of intracellular signal transduction—Applications to pharmaceutical screening.
M. Ellisman, National Center for Microscopy and Imaging Research, University of California, San Diego: Cellular and subcellular components of nervous systems—3-D microscopy.

SESSION 3

B. Stillman, Cold Spring Harbor Laboratory: Introduction—Imaging at Cold Spring Harbor Laboratory
K. Svoboda, Cold Spring Harbor Laboratory: Two-photon Imaging.
D. Spector, Cold Spring Harbor Laboratory: Imaging RNA processing in cells.

SESSION 4

B. Rosen, MGHG-NMR Center, Harvard Medical School, Cambridge, Massachusetts: Functional imaging of the working brain.
J. Gabrieli, Stanford University, California: Imaging memory in the brain.
J.D. Watson, Cold Spring Harbor Laboratory: Closing remarks.

H. Solomon, C. Bustamante

The Art of Judging: Perspectives of Science

October 27–30

FUNDED BY **The Federal Judicial Center, Judiciary Leadership Development Council, and Cold Spring Harbor Laboratory**

ARRANGED BY **J.G. Apple,** Federal Judicial Center, Washington, D.C.
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1

D. Micklos, DNA Learning Center, Cold Spring Harbor Laboratory: Cold Spring Harbor Laboratory and its place in science.

SESSION 2

J. Maienschein, Arizona State University, Tempe: From Darwin to Dolly: Developments in the biological sciences in the 20th century.
L. Silver, Princeton University, New Jersey: Cloning: The biological and social implications of new science.

SESSION 3

D. Wilkinson, Princeton University, New Jersey: Life in an inhospitable universe.
S. Feinberg, Carnegie Mellon University, Pittsburgh, Pennsylvania: Statistics and probabilities in science.

SESSION 4

D. Wilkinson, Princeton University, New Jersey: New concepts of the universe.

SESSION 5

J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory: A look at the past.
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory: DNA and the Human Genome Project.
Visit to DNA Learning Center and Cold Spring Harbor Laboratory
Tour Leader: J. Kruper, DNA Learning Center, Cold Spring Harbor Laboratory

SESSION 6

P. Reilly, Shriver Center for Mental Retardation, Waltham, Massachusetts: Social implications of genetic research.

SESSION 7

R. Shapiro, New York University, New York: Origins of life.
M. Gallo, Robert Wood Johnson Medical School, Piscataway, New Jersey: Toxicology, the environment, and risk assessment.

SESSION 8

J. Horgan, *Scientific American*, Washington, D.C.: Science in the 21st century.

Overhead slide projection presentation.

Integrating Cellular Stress Responses

November 1–4

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **M.-J.H. Gething,** University of Melbourne
R.I. Morimoto, Northwestern University

SESSION 1
Chairperson: N.C. Jones, Imperial Cancer Research Fund, London, United Kingdom

R.I. Morimoto, Northwestern University, Evanston, Illinois: Introduction
J.A. King, Massachusetts Institute of Technology, Cambridge: How protein folding fails at higher temperatures.
T. Baker, Massachusetts Institute of Technology, Cambridge: Specificity determinants in the Clp/Hsp100 protein family.
R.I. Morimoto, Northwestern University, Evanston, Illinois: Protein homeostasis and the heat shock response.
J. Bardwell, University of Michigan, Ann Arbor: Heat shock proteins, beyond the chaperone paradigm.
P. Spellman, Stanford University School of Medicine, California: Examination of stress responses in yeast using DNA microarrays.

SESSION 2
Chairperson: J.A. King, Massachusetts Institute of Technology, Cambridge

S. Hultgren, Washington University, St. Louis, Missouri: Link between pilus biogenesis, pathogenesis, and host responses.
M.-J.H. Gething, University of Melbourne, Victoria, Australia: Unfolded protein and inositol starvation responses: Modulation by the cell integrity MAPK pathway.
N.C. Jones, Imperial Cancer Research Fund, London, United Kingdom: Gene regulation by stress-activated map kinases.
D. Ron, The Skirball Institute of Biomolecular Medicine, New York: CHOP and the ER stress response.

SESSION 3
Chairperson: D.R. Green, La Jolla Institute for Allergy and Immunology, California

U. Jakob, University of Michigan, Ann Arbor: Hsp33-A Redox-regulated molecular chaperone.
G.L. Semenza, Johns Hopkins Hospital, Baltimore, Maryland: Regulation of oxygen homeostasis by hypoxia-inducible factor 1.
C. Prives, Columbia University, New York: Signaling to the p53 tumor suppressor protein.
T.V. O'Halloran, Northwestern University, Evanston, Illinois: Managing heavy metal stress: Copper and zinc trafficking pathways.
W. Schaffner, Universitat Zurich, Switzerland: MTF-1, a mammalian zinc finger transcription factor, is required for correct response to heavy metal and oxidative stress.

SESSION 4
Chairperson: C. Prives, Columbia University, New York

D. Ingber, Children's Hospital Medical Center, Boston, Massachusetts: Cellular signal integrations.
D.L. Levens, National Cancer Institute, Bethesda, Maryland: How does DNA sense stress?
D.R. Green, La Jolla Institute for Allergy and Immunology, California: The apoptotic response to stress.
G.I. Evan, Imperial Cancer Research Fund, London, United Kingdom: Cytokines and oncogenes: Decisions for growth control.

SESSION 5
Chairperson: T. Baker, Massachusetts Institute of Technology, Cambridge

M.E. Feder, University of Chicago, Illinois: Organismal and evolutionary limits to inducible responses.
S.L. Rutherford, HHMI, University of Chicago, Illinois: Hsp90 as a capacitor of evolution.
M.-J.H. Gething, University of Melbourne, Victoria, Australia: Closing comments and summary.

Large-scale Discovery and Genetic Applications of SNPs

November 10–13

FUNDED BY **Glaxo Wellcome Inc. and the National Human Genome Research Institute, NIH**

ARRANGED BY **A. Chakravarti,** Case Western Reserve University
E. Lander, Whitehead Institute for Biomedical Research

SESSION 1: Genomic Variation
Chairperson: A. Chakravarti, Case Western Reserve University, Cleveland, Ohio

A. Chakravarti, Case Western Reserve University, Cleveland, Ohio: Introduction.
C.H. Langley, University of California, Davis: Lessons from SNP association studies in *Drosophila*.
M.E. Kreitman, University of Chicago, Illinois: Toward a DNA polymorphism database.
C.F. Aquadro, Cornell University, Ithaca, New York: DNA diversity and rates of recombination across the human genome.
R. Harding, John Radcliffe Hospital, Oxford, United Kingdom: Patterns of nucleotide diversity, recombination, and linkage disequilibrium in the beta-globin gene.
A.G. Clark, Pennsylvania State University, University Park: Fine-scale structure of linkage disequilibrium in LPL, ACE, and ApoE.

SESSION 2: Genome-wide Mapping and Association Studies
Chairperson: E.S. Lander, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts

D. Cohen, Genset SA, Paris, France and Nicholas Schork, Case Western Reserve University, Cleveland, Ohio.
L. Kruglyak, Fred Hutchinson Cancer Research Center, Seattle, Washington: Prospects for the use of SNPs in whole-genome linkage disequilibrium mapping.
A. Chakravarti, Case Western Reserve University, Cleveland, Ohio: Quantitating genome sharing using SNPs.
A.J. Brookes, University of Uppsala, Sweden: Association study design based on SNPs: Optimizing chances of success.

D. Cox, A. Roses, J. Witkowski

SESSION 3: Current Status of SNP Discovery
Chairperson: D. Nickerson, University of Washington, Seattle

D.G. Wang, Bristol-Myers Squibb, Princeton, New Jersey: Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms.
P.-Y. Kwok, Washington University School of Medicine, St. Louis, Missouri: Strategies for SNP discovery and testing.
E. Lai, Glaxo Wellcome Inc., Research Triangle Park, North Carolina: Large-scale SNP identification.
J. Hacia, National Human Genome Research Institute, NIH, Bethesda, Maryland: Evolutionary sequence analysis of human SNP sites.
P.J. Oefner, Stanford University, California: Geographic distribution and frequency of SNPs.

SESSION 4: Future Technologies for SNP Discovery
Chairperson: D.R. Cox, Stanford University School of Medicine, California

L. Fors, Third Wave Technologies, Inc., Madison, Wisconsin: Scalable, high-throughput technology for SNP analysis directly from genomic DNA.
U. Landegren, University of Uppsala, Sweden: Strategies for high-throughput SNP analysis.
L.M. Smith, University of Wisconsin, Madison: Mass spectrometric analysis of genetic variations.
M.P. Weiner, Glaxo Wellcome, Inc., Research Triangle Park, North Carolina: SNP analysis using mobile solid support.
R. Lipshutz, Affymetrix, Santa Clara, California: Chips and SNPs.
C. Tynan, PE-Applied Biosystems, Foster City, California: High-density TaqMan arrays and "Zip Chips."

SESSION 5: Intellectual Property Issues
Chairperson: A.R. Williamson, London, United Kingdom

A.R. Williamson, London, United Kingdom: Introduction.
M. Freire, National Institutes of Health, Rockville, Maryland: NIH perspective on ESTs and SNPs intellectual property.
D.R. Cox, Stanford University School of Medicine, California: A high-resolution SNP map of the Human Genome.
R. Eisenberg, University of Michigan Law School, Ann Arbor: Competition between public and private research funding for SNP discovery.

SESSION 6: General Discussion and Summing Up
Chairpersons: A. Chakravarti, Case Western Reserve University, Cleveland, Ohio and **E.S. Lander,** Whitehead Institute for Biomedical Research, Cambridge, Massachusetts

Coffee break

Genes, Teens, and the World Wide Web

November 22–24

FUNDED BY **The Josiah Macy, Jr. Foundation**

ARRANGED BY **J. Kruper,** DNA Learning Center, Cold Spring Harbor Laboratory
D. Micklos, DNA Learning Center, Cold Spring Harbor Laboratory
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Opening Remarks and Keynote Address I

J.D. Watson, Cold Spring Harbor Laboratory: Thirty years of biology publishing.
A. Kay, Walt Disney Imagineering, Glendale, California: Helping children learn.

SESSION 2: The Web in Biology Education and Publishing

N. Campbell, University of California, Riverside: The biology place: Using the Web to build investigative learning communities.
L.J. Chaput, Cogito Learning Media, Inc., San Francisco, California: Making cutting edge science accessible to students.
V.L. Ward, Genentech Access Excellence, South San Francisco, California: Preparing teachers for genomic biology.
R.J. Semper, The Exploratorium, San Francisco, California: Live@the Exploratorium: Communicating scientific discovery through webcasting.

SESSION 3: Keynote Address II

B.M. Alberts, National Academy of Sciences, Washington, D.C.: Why it's important to communicate biology.

SESSION 4: How Do We and How Should We Use the Web to Learn?

C. Marshall, Xerox Palo Alto Research Center, California: A reader's-eye view of the Web: Hypertext, annotation, collaboration, and the real world.
T.M. Kahn, Design Worlds for Learning, Inc., San Jose, California: Building virtual learning communities.
P. Greenspun, Massachusetts Institute of Technology, Cambridge: Building scalable online communities.
B. Berenfeld, The Concord Consortium, Massachusetts: Authentic science for kids: The global laboratory curriculum.

SESSION 5: Emerging Technologies and Opportunities

J. McEntyre, National Center for Biotechnology Information, NIH, Bethesda, Maryland: The Human Genome Project and opportunities to integrate research into education.
J. Kruper, DNA Learning Center, Cold Spring Harbor Laboratory: DNA from the beginning.
K. Jones, Magnet Interactive Studios, Washington, D.C.: PBS online: Bringing educational programming to the digital age.
T. Hass, Applications Development for Internet2, Ann Arbor, Michigan: High bandwidth and opportunities for learning.

The Genome and Experimental Biology

December 1–4

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **P.O. Brown,** Stanford University
G.M. Rubin, University of California

SESSION 1: How Has the Genome Sequence Changed the Way We Look At Yeast?

G.N. Giaever, Stanford University School of Medicine, California: Drug-induced haploinsufficiency, drug target identification.
P. Spellman, Stanford University Medical Center, California: Identification of cell-cycle-regulated genes in yeast.
R.A. Young, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Dissecting the regulatory circuitry of the yeast genome.
M. Martin, Rosetta Inpharmatics, Kirkland, Washington: Genomics and drug discovery.

SESSION 2: Comprehensive Genetic Surveys and Screens—Methods and Models

G.M. Rubin, University of California, Berkeley: Using genetics to analyze gene function in *Drosophila*.
E.H. Ruley, Vanderbilt Medical Center, Nashville, Tennessee: Genetic analysis of cultured cells and mice by tagged sequence mutagenesis.
B.J. Wold, California Institute of Technology, Pasadena: Challenges for functional genomics in the mouse: Single cell expression states and dispersed *cis*-regulatory elements.
L.I. Zon, HHMI, Children's Hospital, Boston, Massachusetts: Zebrafish genetics and human disease.

SESSION 3: Dealing with a Flood of Data: Understanding, Integrating, Publishing

M.B. Eisen, Stanford University School of Medicine, California: Exploring gene expression space.
D.J. Lipman, National Library of Medicine, Bethesda, Maryland: Computing discoveries in biology.
P.H. Ginsparg, Los Alamos National Laboratory, New Mexico: Knowledge network for physics?
M. Ashburner, European Bioinformatics Institute, Cambridge, United Kingdom: Integrating information across organisms—On the representation of information concerning gene functions in databases.

P. Brown, L. Zon, J.D. Watson

SESSION 4: Experimental Approaches with a "Genomic" Style

O. Kallioniemi, National Human Genome Research Institute, NIH, Bethesda, Maryland: Tissue microarrays for high-throughput molecular profiling.

M. Wigler, Cold Spring Harbor Laboratory: Representational approaches to genomic analysis.

J.S. Minden, Carnegie Mellon University, Pittsburgh, Pennsylvania: Difference gel electrophoresis: A rapid method for identifying proteosome changes.

T.S. Heuer, Massachusetts General Hospital, Boston: Functional screening of cDNA libraries with mRNA-protein fusions.

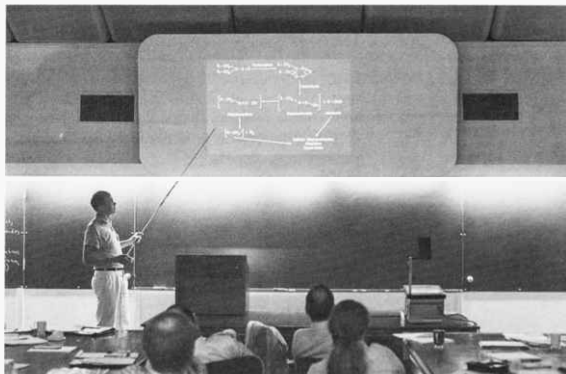
SESSION 5: Experimental Genomic Approaches to Understanding, Diagnosing, and Treating Human Disease

D. Gerhold, Merck & Company, West Point, Pennsylvania: A drug metabolism and safety gene-chip database; on silica to in silica.

L. Staudt, National Cancer Institute, NIH, Bethesda, Maryland: Genomic-scale views of gene expression in normal and malignant lymphocytes using the Lumphochip cDNA microarray.

L.T. Williams, Chiron Corporation, Emeryville, California: A high throughput gene expression study in cancer.

P.O. Brown, HHMI, Stanford University Medical School, California: The DNA microarray as a vehicle for genome exploration.



Making a presentation.

Biochemistry at 100°C: How Are Enzymes and Their Substrates Stabilized?

December 6–9

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **M.W. Adams,** University of Georgia
R.M. Kelly, North Carolina State University

Welcoming Remarks:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Goals of the Conference:
M.W. Adams, University of Georgia, Athens
Robert M. Kelly, North Carolina State University, Raleigh

SESSION 1: Origins, Environments and Organisms
Chairpersons: D.S. Clark, University of California, Berkeley and **F.T. Robb,** University of Maryland, Baltimore

S.L. Miller, University of California, La Jolla: A cold origin for life: Pros and cons.
J. Wiegel, University of Georgia, Athens: Thermal ranges of microbial growth.
M.W. Adams, University of Georgia, Athens: Hyperthermophilic metabolisms and pathways: Unusual or standard?
R.W. Kelly, North Carolina State University, Raleigh: Carbohydrate utilization by hyperthermophiles: What do the genome sequences tell us?

SESSION 2: Biochemistry
Chairperson: R. Jaenicke, University of Regensburg, Germany

H. Santos, Universidade Nova de Lisboa, Oeiras, Portugal: Organic solutes from hyperthermophiles: Stabilizing effects on enzymes.
J. Trent, NASA Ames Research Center, Moffett Field, California: Role of heat-shock proteins in vivo—Do hyperthermophilic enzymes need chaperones?
F.B. Perler, New England BioLabs, Inc., Beverly, Massachusetts: Protein splicing in hyperthemophiles.
G. Antranikian, Technical University, Hamburg, Germany: Hydrolytic enzymes from hyperthermophiles.
M.J. Danson, University of Bath, United Kingdom: Staying together—The importance of subunit interactions in enzyme hyperstability.

R. Jaenicke, F. Perler

SESSION 3: Protein Structure
Chairpersons: M.J. Danson, University of Bath, United Kingdom and **A. Karplus,** Cornell University, Ithaca, New York

R. Jaenicke, University of Regensburg, Germany: Stability and folding of hyperthermophilic proteins.
F.T. Robb, University of Maryland, Baltimore: Rational placement of mutations that enhance protein thermostability.
T. Oshima, Tokyo University of Pharmacy and Life Science, Japan: Stabilization of mesophilic enzymes by a combination of theoretical design and evolutionary molecular engineering.
J.G. Zeikus, Michigan State University, East Lansing: Molecular determinants for activity and stability of xylose isomerase, amylase, and alkaline phosphatase.
R. Sterner, University of Gottingen, Germany: How do (ba)8-barrel enzymes protect themselves and their thermolabile substrates at temperatures close to 100°C?

SESSION 4: Biophysical Approaches
Chairpersons: J.N. Reeve, Ohio State University, Columbus and **H. Santos,** Universidade Nova de Lisboa, Oeiras, Portugal

M. Rossi, University of Naples, Italy: Studies on molecular bases of the thermostability of the β-glycosidase from *Sulfolobus solfataricus*.
W. Englander, University of Pennsylvania, Philadelphia: Hydrogen exchange as a tool to assess protein stability.
G.N. La Mar, University of California, Davis: Solution NMR study of the novel molecular structure of the ferredoxin from *Pyrococcus furiosus*.
D.S. Clark, University of California, Berkeley: Pressure stabilization of proteins near 100°C: Implications for structure-function relationships.
J.W. Shriver, Southern Illinois University School of Medicine, Carbondale: Global analysis of the linkage of pH and salt concentration to protein folding: Application to core packing mutants of Sac7d and Sso7d.

SESSION 5: Genetics/Protein–DNA Interactions/Genomics
Chairperson: F.B. Perler, New England Biolabs, Inc., Beverly, Massachusetts

J.N. Reeve, Ohio State University, Columbus: Histone-fold stabilization.
P. Lopez-Garcia, Universite Paris-Sud, Orsay, France: Control of DNA superhelical changes during heat shock in hyperthermophilic Archaea.
K.M. Noll, University of Connecticut, Storrs: Recent developments in genetic transfer methods in *Thermotoga*.
D.W. Grogan, University of Cincinnati, Ohio: Genetic processes in thermophilic Archaea.
H.-P. Klenk, University of Gottingen, Germany: Extreme thermophilia: Ancient conserved feature or phylogenetic pitfall?

Participants discussing presentation.

From Bench to Bedside: Colloquium on Translational Vaccine Research

December 15–17

FUNDED BY **The Albert B. Sabin Vaccine Institute, Inc. at Georgetown University**

ARRANGED BY **H.B. Herscowitz,** Georgetown University
P.K. Russell, Potomac, Maryland

Introductory Remarks:
H.R. Shepherd, The Albert B. Sabin Vaccine Institute, Inc. at Georgetown University, Washington D.C.
P. Hotez, Yale University School of Medicine, New Haven, Connecticut
P.K. Russell, Potomac, Maryland

SESSION 1: Historical Perspective
Chairperson: P. Hotez, Yale University School of Medicine, New Haven, Connecticut

P. Hotez, Yale University School of Medicine, New Haven, Connecticut: Welcoming remarks.
R. Rabinovich, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland: Setting the stage.

SESSION 2: Viral Vaccines
Chairperson: P. Hotez, Yale University School of Medicine, New Haven, Connecticut

D.E. Griffin, Johns Hopkins School of Public Health, Baltimore, Maryland: Issues in developing new measles vaccines.
J.L. Gerin, Georgetown University Medical Center, Rockville, Maryland: Vaccines for viral hepatitis.
A.R. Shaw, Merck Research Laboratories, West Point, Pennsylvania: Development of a live oral vaccine for rotavirus.
R.B. Belshe, St. Louis University School of Medicine, Missouri: Efficacy of live attenuated influenza vaccine given intranasally.

P. Hotez, J. Gerin, D. Sack

SESSION 3: Arthropod-borne Infection Vaccines
Chairperson: P. Hotez, Yale University School of Medicine, New Haven, Connecticut

W.R. Ballou, Walter Reed Army Institute of Research, Washington, D.C.: Malaria vaccines: Translational obstacles.

A.C. Steere, New England Medical Center, Boston, Massachusetts: Vaccination for Lyme disease with recombinant *Borrelia burgdorferi* OspA with adjuvant.

S. Hoffman, Naval Institute of Medical Research, Bethesda, Maryland: Translating genomic sequence data into vaccines: Malaria as a model system.

N. Kanesa-Thasan, Walter Reed Army Institute of Research, Washington, D.C.: Progress and problems in Dengue virus vaccine development.

P. Hotez, Yale University School of Medicine, New Haven, Connecticut: Approaches to vaccinating against Helminth infections.

SESSION 4: Bacterial Vaccines

M.M. Levine, University of Maryland School of Medicine, Baltimore: Attenuated *Salmonella typhi* as live vector vaccines expressing foreign antigens.

D.A. Sack, Johns Hopkins University, Baltimore, Maryland: Formulation issues with cholera vaccine: Making efficacious vaccines effective.

SESSION 5: Cancer Vaccines and Immunomodulators

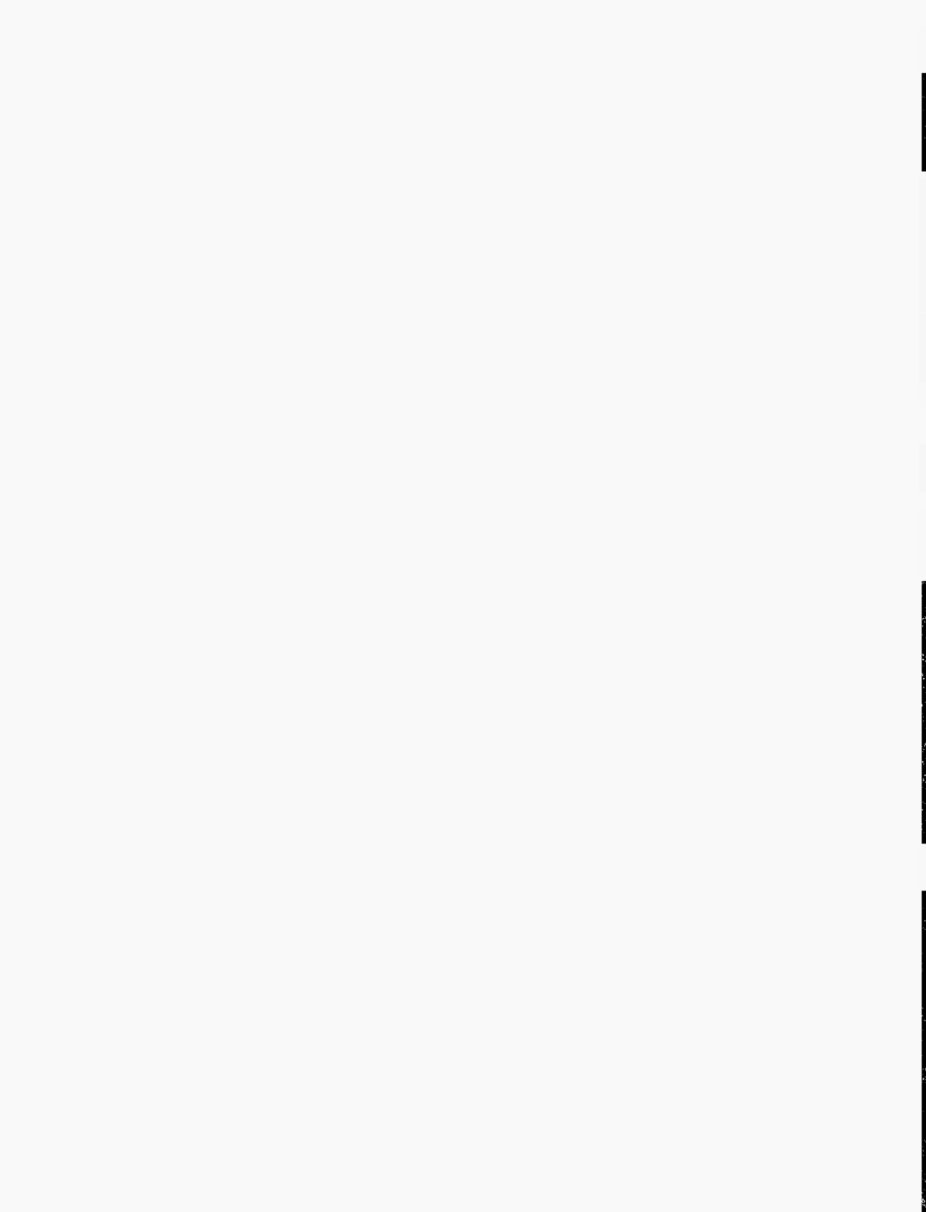
J. Schlom, National Cancer Institute, NIH, Bethesda, Maryland: T-cell costimulation in vaccine design and development.

R. Bucala, The Picower Institute for Medical Research, Manhasset, New York: A novel circulating cell type with potent antigen presenting properties: basic and clinical studies.

M.M. Levine, University of Maryland School of Medicine, Baltimore, and P.K. Russell, Potomac, Maryland: Wrap up and discussion.

Robertson House Library

DNA LEARNING CENTER



DNA LEARNING CENTER

David A. Micklos	**John A. Kruper**	**Scott Bronson**
Judy Cumella-Korabik	**Susan Lauter**	**Patricia Harrison**
Janeen Russo	**Shirley Chan**	**Amanda Broege**
	Matt Christensen	**Martha Mullally**
	Gisella Walter	**Andrew Morotti**
	Mark Feingold	

Laboratory President James Watson is fond of saying, "You get bigger or you get smaller." Behind this deceptively simple adage lies the business plan for any good institution. Organizations rarely stay the same size for very long. Those that respond to change and take advantage of new opportunities, evolve into large ones. Those that fail to respond and adapt, shrink.

1998 was a year of remarkable change that put the DNALC solidly on the up side of that simple equation. Income increased by 59%, from $878,400 in 1997 to $1,393,100 in 1998. In the past several years, the DNALC has shown a deficit roughly equal to depreciation costs. However, the 1998 budget was balanced after full payment of depreciation. The greatest single source of growth in 1998 was funding from the Josiah Macy, Jr. Foundation. Other sources of strength were three major federal grants, annual giving by the Corporate Advisory Board, and increased receipts for lab instruction.

The organization of our staff masthead is also emblematic of the year. The DNALC staff grew from 9 to 14 positions in 1998. These were in addition to a similar number of personnel changes in the second half of 1997. The large number of new staff members and new grant commitments dictated a functional reorganization. In the past, staff functions were divided by grade level into elementary–middle school instruction and high school–college instruction. Under the reorganization, the staff was divided into three functional groups: Core Administration (three positions), Laboratory Education (five positions), and Multimedia Communications (six positions). One major effect of the reorganization was to move away from our reliance on part-time instructors to a staff of full-time instructors. These changes have simplified administration and increased collaboration among staff members.

Scott Bronson assists a participant at the new *Genomic Biology & PCR* Workshop

Despite an almost complete changeover in instructional staff since mid-1997, our service to local school systems increased in 1998. Most notable was a 34% increase in students performing labs at the DNALC. This increase was mainly due to bringing the middle-school genetics lab into full use, with up to four classes per day. We had our busiest summer yet, administering 25 student workshops, including eight minority workshops held at Stuyvesant High School and Mt. Sinai School of Medicine (Manhattan), John F. Kennedy High School (Bronx), Intermediate School 109 (Queens), and Central Islip High School (Suffolk County). We also collaborated with Mt. Sinai School of Medicine to conduct two Gateway Summer Teacher Institutes, drawing 100 faculty from 15 New York City high schools. With support from Howard Hughes Medical Institute and the William Randolph Hearst Foundation, we have been making a concerted effort to increase learning opportunities for students in New York City. Thanks primarily to multi-year collaborations with Community School District 29 and Mt. Sinai's Gateway to Higher Education Program, minorities comprised 35% of students participating in 1998 lab programs.

	1994	1995	1996	1997	1998	1997–1998 Change
Student Labs (on-site)	3,961	4,682	6,088	7,105	9,540	34%
Student Labs (off-site)	1,434	2,328	5,045	7,665	8,195	7%
Teacher Labs	302	379	302	392	482	23%
Student Workshops	361	503	437	402	429	7%
Teacher Workshops	177	101	151	245	190	–22%
Lab Subtotal	6,235	7,993	12,023	15,809	18,836	19%
Student Lectures	575	520	575	407	568	40%
Exhibit/LI Discovery	9,943	10,366	10,122	11,150	12,062	8%
Total	**16,753**	**18,879**	**22,720**	**27,366**	**31,466**	**15%**

DNA from the Beginning Goes Online

By year's end, we had made a limited online release of the first installment of *DNA from the Beginning*, an Internet primer on genetics funded by the Josiah Macy, Jr. Foundation. The initial release covers 14 key concepts in classical genetics, including Mendelian inheritance, the physical basis of heredity, genetic linkage, and the beginning of human genetics. Additional releases in 1999 and 2000 will cover concepts in molecular genetics, gene regulation, genetic manipulation, and genomic biology.

Initially, a confidential Internet address was provided for review by a select group of biology researchers, scholars, and teachers. After incorporating reviewers' feedback, the World Wide Web (WWW) site was officially launched with registration on major search engines and a 4700-piece postcard mailing that included 3000 high school teachers from our national database of leaders in genetics education. We also added links to major genetics education programs, medical and genome research centers, and genetics support groups.

The title of *DNA from the Beginning* (*DNAFTB*) can be taken as a metaphor on several levels. On a conceptual level, *DNAFTB* stresses DNA as the beginning of human life and health, and as the mediator of the evolution of all life. On a pedagogical level, *DNAFTB* builds knowledge following the historical development of genetics—from the beginning, one experiment at a time. *DNAFTB* is targeted at the level of a bright teenager and is intended to provide basic information that anyone would find useful in facing a "personal genetic dilemma."

DNAFTB is designed to support the learning styles of two types of Internet users: the casual browser, who wants a quick synopsis of key materials, and the active learner, who wants to build a deeper understanding. We hope that the consistent organization and novel features of the site will actually encourage browsers to become active learners.

In contrast to books, which are organized around chapters, *DNAFTB* is organized around key concepts. Approximately 75 concepts will form the narrative backbone of the work—a *Readers' Digest* of genetics from Mendel to molecular cloning. Thus, the casual reader can take *DNAFTB* as a continuous story or work to master "a concept a day." Each concept is carefully chosen to emphasize the progressive development of genetics. Concepts are presented as pure ideas and in relatively nonscientific language. In keeping with research showing that Internet users tend to skim Web pages and get confused by too many "clicks," each concept is limited to a single screen containing a title, collage illustration, and maximum 150 words of text.

Layered behind each concept screen are multimedia elements that allow the active learner to discover the experiments and people behind the concept:

- *Animations* created with Macromedia Flash software illustrate exemplar experiments upon which the concept is based, with scientists describing their own experiments in the first person. Animation is especially effective in illustrating multistep experiments and molecular events that are difficult to capture in static text illustrations. We believe that these animations are unique in biology education; similar animations are the most requested and downloaded pages at our WWW site.

- *Gallery* contains still images collected from primary scientific archives, often rare photographs not found in science textbooks. The images are displayed in a linear, scrolling loop, which gives the sense of browsing pictures on a gallery wall. Each image can be enlarged for closer inspection.
- *Audio/Video* contains primarily short video clips from interviews with scientists and historians that highlight the human side of discovery. Clips are served to users as "streaming video." This system avoids long download delays by quickly delivering an initial segment, and then loading the remaining footage while the scene is playing.
- *Bio* provides details about the scientists closely associated with the concept. Biographies highlight scientific "heroes" and attempt to reflect the person behind the science.
- *Links* provides several jumping off points to related WWW sites outside of *DNAFTB*. Our editors have judged each link especially relevant and appealing. A review is provided for each external resource.
- *Problem* provides an animated tutorial that tests comprehension, usually by means of an experiment that builds upon experiments presented in the *Animation* section. Each problem presents a series of connected investigations. At appropriate checkpoints within the problem, multiple-choice questions test for concept mastery before allowing the user to continue.
- *Gene the Gene* is an animated cartoon character who presents entertaining facts and stimulating thought questions that challenge users to make associations or to consult other resources.

DNAFTB incorporates a number of unique programming features. Each "page" downloaded by a user is dynamically constructed, at the time of a user request, from individual narrative and media elements stored on the *DNAFTB* database server. A customized authoring shell allows the project editors to easily edit, add, or delete elements without reconstructing or reprogramming an entire page layout. The site's dynamic structure also makes possible a unique *Relational Navigator*, which allows users to explore *DNAFTB* according to their own interests, in a nonlinear manner. Depending on the page from which it is activated, the *Relational Navigator* draws a unique "map" of internal links to related concepts and media elements.

Although anyone may access *DNAFTB* as a "guest," users are encouraged to register to take advantage of additional features, including setting browser preferences, bookmarking pages, tracking sections read, and searching by key words. Registered users are also provided a personalized score sheet on *Problems* they have answered, which can be printed out and turned in to the classroom teacher as a homework assignment. The registered user database also provides accurate measures of *DNAFTB* site use, according to both content viewed and user demographics.

Genes, Teens, and the World Wide Web Affirms Our Methods

In preparation for the initial release of *DNAFTB*, in November we collaborated with our favorite Brit Jan Witkowski to sponsor a Banbury meeting on the future of online genetics education. *Genes, Teens, and the World Wide Web* drew together experts from the worlds of biological research, science publishing, and Internet computation. The meeting was cohosted by CSHL president James Watson and Bruce Alberts, President of the National Academy of Sciences. Keynote speaker Alan Kay, Vice President of Walt Disney Imagineering, was a member of the Xerox Palo Alto Research Center (PARC), which invented modern personal and networked computing. Other participants included Lubert Stryer and Neil Campbell, authors of mega-selling biochemistry and biology texts; Phillip Greenspun, who constructed database-driven WWW sites for Fortune 500 companies; Robert Semper, Vice President of the San Francisco Exploratorium; and Ted Hanss, Vice President of Internet II.

In many ways, discussion at the meeting affirmed our methods for online publishing. First, our home page, *Gene Almanac*, was reconceived in July as "the source for timely information about genes in edu-

(Left) Dr. Alan Kay, Walt Disney Imagineering, addresses participants of the *Genes, Teens, and the World Wide Web* meeting in November. *(Right)* Philip Greenspun (seated), Matt Christensen, and John Kruger of the DNALC, Joe Perpich of Howard Hughes Medical Institute, and Cathy Marshall of Xerox PARC browse *DNA from the Beginning*.

cation." Far from the standard organizational mouthpiece, it is a dynamic information source that changes daily. *Gene News* posts four to six daily news articles generated by a customized Internet search engine, which scans numerous national news sources, including *The New York Times, Los Angeles Times, Boston Globe, MSNBC, Fox News,* and *BBC Online* feature articles, that highlight science methods and people in the news, using animations and video elements to make the topics engaging to nonscientific audiences.

Several *Gene Almanac* elements aim to involve students in research and build a community of online learners. *Student Allele Database* and the new *DNA Sequence Server* allow students to use their own DNA "fingerprints" as a starting point for online investigations. *BioForms* provide case studies on DNA sequence analysis and custom interfaces that allow students to easily use statistical tools available at genome servers. *Gene Talk* includes bulletin boards and chat rooms, including special areas for teacher groups trained at summer workshops.

The freshness of the *Gene Almanac* content is made possible because each page is "made from scratch," drawing items and design features from a number of sources to create the page seen by a user. Some pages draw information from databases maintained at the DNALC, and others send requests to other WWW sites and package the returned information in our own forms. This sort of dynamic page construction is common to the most advanced sites on the WWW. Users seem to appreciate the changes. Traffic at the site increased threefold during the year, to more than 18,500 unique user sessions per month, including visitors from 50 countries.

We Look Forward to a Major Addition to the DNALC

Since opening in 1988, the DNALC has been the largest provider of genetics and biotechnology labs. Now, our new multimedia projects have made the DNALC one of the largest Internet providers of multimedia learning materials for biology education. However, realistically, our current facilities will not allow us to maintain this advantage very far into the future. In 1998, our two teaching labs were saturated, and our super-talented Internet developers joined the rest of the staff in our dreary basement office.

So, we were buoyed by news in the spring that the Dolan Family Foundation had agreed to provide $1 million toward construction of a 7600-square-foot *BioMedia* addition that will double the size of the

DNALC. *BioMedia* expresses our goal to explore the creative use of computer and telecommunication technology in modern biology education. Computer and video facilities in the addition will allow students to move effortlessly between multimedia experiences and hands-on science in adjacent biochemistry and genetics laboratories.

Centerbrook Architects and Planners, of Essex, Connecticut, are designing the addition. This firm has done the bulk of the Laboratory's architectural design for the past 20 years and was responsible for the DNALC's major renovation in 1993. Centerbrook received the coveted 1998 Architectural Firm Award presented by the American Institute of Architects. So we were not surprised when architect Jim Childress came up with a brilliantly simple solution to our problem of how to make a two-story addition look like only one. He simply continued the ground floor partially below grade, so that the second floor merges with the natural break in the hill behind our building. The net effect—the elevation at the back of the facility—gives the impression of a single-story ranch house!

The centerpiece of the addition will be an octagonal computer laboratory to support hands-on instruction in bioinformatics, explorations using interactive and virtual reality technologies, and distance learning programs. To allow us to fully explore the "convergence" of CD/ROM, video, broadcast, and cable media, the upper level will feature a video studio/production suite and a multimedia conference room. A lunchroom and rest rooms are practical elements needed to deal with our ever-increasing number of young visitors. Staff offices will be located on the upper and lower levels, according to function.

The *BioMedia* addition, and reconstruction of existing space, will create a suite of three teaching laboratories and a student research laboratory. Believing that sequencing will become a substantial part of future laboratory programs, we have included a dedicated DNA sequencer in the capital budget for the *BioMedia* addition. A *Visible DNA Sequencing Laboratory* will perform double duty as an exhibit to showcase DNA technology and as a working laboratory. On a daily basis, high school interns will process DNA samples submitted by high school and college classes around the nation. Visitors can watch all phases of sequencing, culminating with a real-time output of finished sequence on a scrolling ticker. The DNA sequences processed in the visible laboratory will be returned via the Internet to participating schools, where they will be the starting points for a series of online exercises.

Shedding Light on an Important Lesson from the Past

Early in the year, we received a 2-year grant of $404,000 from the Ethical, Legal, Social Issues (ELSI) Program of the National Human Genome Research Institute to establish a *Digital Image Archive on the American Eugenics Movement*. We considered this award an important vote of confidence in our work, since it is the first grant the ELSI Program has made to explore this sensitive issue.

The *Archive* will be drawn from the corpus of the Eugenics Record Office at Cold Spring Harbor, which was the primary repository of American eugenical thought and activity from 1910 to 1940. Some documents remain in the CSHL Archives, but the bulk have come to rest at the American Philosophical Society (APS) Library in Philadelphia and the Harry Laughlin Archives at Truman State University in Kirksville, Missouri. Nearly 700 images—toward our goal of 1000—were collected at Cold Spring Harbor and during two trips to APS in Philadelphia. Collected items included photographs, family records, personal correspondence, quotations, clippings, pedigrees, tables, and sample publications.

The American Philosophical Society Library in Philadelphia.

The major aim of the *Digital Image Archive* is to provide materials that will stimulate independent, critical thinking about the parallels between eugenics and modern genetics research. The *Archive* is intended as an educational tool to allow individuals to learn about society's past involvement in genetics by exploring primary materials that heretofore have been inaccessible to the layperson. By basing the *Archive* on primary materials, users assume the role of historian/researcher, finding materials according to their own preferences and drawing inferences based on their own syn-

West Elevation

Site Plan

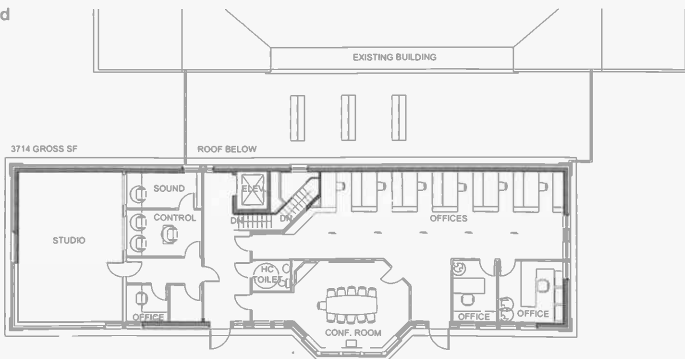
WEST MAIN STREET
S.R. 25A
CONC. CURB
CONC. SIDEWALK
DRIVEWAY
60.7'
STONE RET. WALL
CONC. WALK
SLATE SIDEWALK
WOOD RAMP
SLATE
ENT.
ASPHALT
P/O WHALING AVE.
EDGE OF PAVEMENT
EXISTING PARKING
19
20
21
22
23
24
DIRT ROAD
1 2 3 4 5 6 7
8
9
10
11
12
13
14 15 16 17 18
ASPHALT PARKING LOT
EXISTING DNA LEARNING CENTER
CONC. RAMP
ASPHALT
21.6'
LINE OF WHALING AVENUE
EXTENT OF EXISTING PARKING LOT
ADDITION
30' SETBACK LINE
(NOT OPEN) WHALING AVENUE (HIGH STREET)

DNA Learning Center *BioMedia* Addition elevation and site plan.

First Floor Plan

EXISTING LAB
PREP. AREA
EXISTING OFFICE
EXISTING EXHIBIT 1
EXISTING AUDITORIUM
DN
EXISTING EXHIBIT 2
CLOSET
CLOSET
CLOSET
TELE
EXISTING LAB
TABLE STORAGE
TABLE STORAGE
EXISTING EXHIBIT 3
STOR
EL.
EXISTING EXHIBIT 4
LUNCH ROOM
GROSS SQUARE FEET: 5230 SF
30' SETBACK
ELEV.
UP
UP
ELEV. MACHINE ROOM
PHONES
VENDING
LAB
INSTRUCTOR'S OFFICE
INSTRUCTOR'S OFFICE
COMPUTER CLASSROOM
GIRLS
BOYS

Second Floor Plan



DNA learning Center *BioMedia* floor plans.

thesis. By focusing primarily on visual documents, we hope to engage young people who are accustomed to visual media and others who would not normally access a scholarly collection.

While we want to allow users to form their own opinions about the eugenics movement and its parallels to modern genetics research, we also recognize that people need to have some sense of the historical, social, and ethical context in which the eugenics movement flourished. Thus, construction of the *Archive* involves formal review by an Editorial Advisory Panel, which includes historians, clinical geneticists, educators, bioethicists, philosophers of science, and healthcare advocates:

Garland E. Allen, Washington University of St. Louis
Elof Carlson, State University of New York, Stony Brook
Patricia Colbert-Cormier, NASA
Nancy L. Fisher, Washington State Department of Social and Health Services
Henry Friedlander, Department of Justice
Daniel J. Kevles, California Institute of Technology
Philip Kitcher, University of California, San Diego
Martin L. Levitt, American Philosophical Society
Paul Lombardo, University of Virginia, Charlottesville
Nancy Press, Oregon Health Sciences University
Philip R. Reilly, Shriver Center for Mental Retardation, Inc.
Pat Ryan, Hopkins High School
Marsha Saxton, World Institute on Disability
Steven Selden, University of Maryland, College Park
G. Terry Sharrer, National Museum of American History

The first of four EAP meetings took place at CSHL Banbury Center in September. Lengthy discussions resulted in a policy that takes every reasonable effort to protect people's privacy and confidentiality, without sacrificing the historical integrity of documents. Panel discussion also led to development of the site's narrative outline, consisting of 11 context topics. Several panel members took on the challenge of writing 750–1000-word "theme essays" to introduce key persons and events in the context of social and historical events. We hope that this rigorous, participatory review process will provide a model for other ELSI educational efforts that demand cultural, racial, and ethnic sensitivity.

Dan Kevles (gesturing, near center) addresses the Editorial Advisory Panel.

(*Left*) Henry Friedlander, Elizabeth Thompson, and Jan Witkowski during a break in the meeting.

(*Right*) Dave Micklos talks with Garland Allen.

Bringing the Human Genome Project into the Science Classroom

During the year, we continued our work to involve students and teachers in human molecular genetics—through grants from the Howard Hughes Medical Institute, the National Science Foundation's Advanced Technological Education Program, and the Department of Energy's ELSI program. We are striving to develop a robust and accurate analog of human genome research that allows students to use their own chromosomal and mitochondrial DNA polymorphisms as the basis of explorations into contemporary genomic biology.

The DOE Program introduces high school biology faculty to a laboratory-based unit on human DNA polymorphisms, which provides a uniquely personal perspective on the science and ELSI aspects of the Human Genome Project. We conducted three training workshops, drawing a total of 65 teachers, at the Mills Godwin Specialty Center (Richmond), Eccles Institute of Human Genetics (Salt Lake City), and the University of Denver. Each workshop mixed theoretical, laboratory, and computer work with practical and ethical implications. Program participants learned simplified lab techniques for amplifying two types of chromosomal polymorphisms: an *Alu* insertion and a VNTR. These polymorphisms illustrate the use of DNA variations in disease diagnosis, forensic biology, and identity testing and provide a starting point for discussion of the uses and potential abuses of genetic technology.

DOE workshop participants also learned how to use their *Alu* insertion data as an *entrée* to human population genetics and evolution, using our *Student Allele Database*. This online facility, developed with Howard Hughes funding, contains more than 3000 student DNA types, as well as archival data from populations around the world. Several statistical functions are available: testing Hardy-Weinberg equilibrium within a single population, measuring genetic distance between two populations, and comparing two populations using contingency Chi square. A Hardy-Weinberg simulator shows the effect of genetic drift and gene fixation in small populations. Working with the *Student Allele Database* stresses the shared ancestry of all human beings and sets the stage for further investigations of human origins using mitochondrial DNA.

Under the NSF program, we are developing a hands-on curriculum to bring students up to the minute with the techniques and applications of genomic biology. The curriculum merges the DNALC's content expertise with the vocational-technical expertise of project collaborator CORD (Center for Occupational Research and Development) of Waco, Texas. The project targets participation by leading high school and community college faculty interested in technology education and instructional innovation.

As part of this program, we began development of a new PCR (polymerase chain reaction) experiment of extraordinary promise. The object is to visualize the insertion polymorphism responsible for wrinkled pea seeds—one of the seven original traits investigated by Gregor Mendel. This experiment provides a bridge between the "old" classical genetics and the "new" molecular genetics, and emphasizes the connection between genotype and phenotype. The transposon responsible for the wrinkled phenotype appears related to the *Ac/Ds* system discovered at Cold Spring Harbor Laboratory by Barbara McClintock. Although this pea experiment illustrates a natural mutation caused by a transposon, a parallel experiment shows the use of transposon mutagenesis as a research tool for exploring the plant genome. The transposon insertion in peas is also analogous to the human *Alu* insertion used in the DOE Workshop.

The most novel aspect of the NSF program is a *Sequencing Service* to provide low-cost sequencing of mitochondrial DNA samples. Over the past year, we have worked closely with Dick McCombie at the *Arabidopsis* Genome Sequencing Center of CSHL to provide proof of concept for processing DNA samples submitted from around the country. During each DOE workshop, teacher participants amplified the mitochondrial control region from DNA prepared from their hair roots or cheek cells. The amplified DNA was returned to the DNALC, where dye terminator reactions were performed; the samples were then passed to the Genome Center for sequencing. The completed sequences were then posted at the DNALC's WWW site on the DNA Sequence Server, which currently contains 350 sequences. This process was further replicated with 60 student samples submitted, by mail, from teachers in New York, Maryland, Utah, and Virginia.

Bioinformatics on the World Wide Web

During the year, we developed case materials that use sequence and polymorphism data to illustrate key principles of biology, including evolutionary relatedness and conservation of function. In addition, we introduced several step-by-step Internet templates that allow teachers and students to analyze their own mitochondrial DNA sequences, including similarity searches and multiple sequence alignments.

To support these investigations, we developed two novel computer tools: *Bioforms* and *DNA Sequence Server*. *Bioforms* let students explore realistic, meaningful biology problems in a guided environment. By "wrapping" difficult-to-use genome resources within an easy-to-use contextual guide, the innovative *Bioform* interface lets participants easily select and submit data sets for analysis to Internet genome servers. Results returned by the remote servers are intercepted by the *Bioform* data processor and reformatted to a simplified display. In this manner, *Bioforms* allow students to focus on the biological question at hand, rather than being overwhelmed by navigating their way through complex research sites.

Our first *Bioform* allows students to solve the "mystery of the Romanovs" by using mitochondrial DNA sequence polymorphisms to determine whether a set of bones found in a Russian mass grave were those of the murdered Russian royal family. A second *Bioform* improves on an existing unit where students' mitochondrial DNA sequences are compared with reference sequences to determine whether Neanderthal hominids were our direct ancestors.

In contrast to the guided framework used in *Bioforms*, *DNA Sequence Server* is an open-ended "exploratorium" that puts powerful computational biology tools in the hands of the beginning learner. *DNA Sequence Server* is a full-featured database and sequence analysis application built upon Microsoft SQL Server database technology. The application lets users access DNALC sequence data sets, search external databases, and directly manipulate personal sequence collections. In addition, *DNA Sequence Server* lets users easily analyze sequences using a number of built-in resources, including BLAST and CLUSTALW. Additional analysis tools—including evolutionary tree drawing, restriction mapping, and open reading frame determination—will be added over time.

Corporate Advisory Board

The Corporate Advisory Board (CAB) provides a crucial link to local businesses that serve the same population base as the DNALC. Represented on the board are companies of all kinds—from family-owned to multinational and from biotechnology to banking. A major goal of the Board is to raise awareness of the DNALC among opinion leaders on Long Island and to involve others in our work.

Under the chairmanship of Jack Leahy, the CAB raised $200,000 in support of the DNALC and the Partners for the Future Program, a 20% increase over 1997. Key to this huge success was the 5th Annual Golf Tournament, held on June 18 at Piping Rock Club. Organized by John Kean, the tournament netted $125,000, a 28% increase over 1997. The tournament's founding sponsor is J.P. Morgan & Co., Cablevision and Luitpold Pharmaceuticals, Inc. were major corporate sponsors. Other corporate sponsors were Badge Agency, Inc.; Conde Nast Publications; Festo Corporation; Kean Development Company; KPMG Peat Marwick LLP; Motors and Armatures, Inc.; Price Waterhouse Coopers; RMC Industrial Supply; and The Rosyln Savings Foundation.

Staff and Interns

Malissa Hewitt went on maternity leave in the summer and, after giving birth to Charles Fowler Lindsay, decided to become a full-time mom. Malissa came to the DNALC in 1994, becoming our first full-time staff member devoted to middle-school instruction. On short notice, she gamely stepped in to manage

Chairman
John J. Leahy, Citibank
Vice Chairman
Gary E. Frashier, OSI Pharmaceuticals, Inc.

Members

Michael Aboff, Aboff's Inc.
Andrew D. Ackerman, Chase Manhattan Bank
Rocco S. Barrese, Esq., Dilworth & Barrese
Howard M. Blankman, The Blankman Group
Jane S. Block, Pall Corporation
Thomas J. Calabrese, Daniel Gale Real Estate
Richard Catalano, KPMG Peat Marwick, LLP
Edward A. Chernoff, Motors & Armatures, Inc.
Paul Czeladnicki, Rivkin, Radler & Kremer
Robert E. Diller, Brinkmann Instruments
Candido E. Fuentes-Felix, M.D.
Arthur D. Herman, Herman Development Corp.
Richard Kalenka, Price Waterhouse Coopers
John Kean, Kean Development Co., Inc.
Laurie J. Landeau, V.M.D.
Ralf Lange, Luitpold Pharmaceuticals, Inc.
Lilo Leeds, CMP Publications
James Mattutat, Shore Pharmaceutical Providers, Inc.
Patricia Petersen, Daniel Gale Agency
William Porter, U.S. Trust Company
William Roche, Republic New York Securities Corp.
Wendy Vander Poel Russell, CSHL Trustee
Horst Saalbach, Festo Corporation
Peter G. Schiff, Northwood Ventures LLC
Charles R. Schueler, Cablevision
James Shaw, Newsday
Paul A. Vermylen, Jr., Meenan Oil Company, L.P.
Lawrence J. Waldman, KPMG Peat Marwick LLP
Raymond A. Walters, Ph.D., CSH Central School District

the burgeoning program in 1997 and set the stage for this year's large expansion. The DNALC will miss her can-do attitude, and the students will miss her attentive and caring style.

Then, in the fall, we bid farewell to Assistant Director Mark Bloom, as he prepared to take a new position at the Biological Sciences Curriculum Study in Colorado Springs. Mark joined CSHL in 1987, when our educational programs shared offices (and staff) with the Public Affairs and Development Departments. Mark was there when we embarked on the initial renovation of the DNALC facility, and he initiated the student lab field trip program. Over the years, he personally instructed more than 1000 biology faculty at workshops sponsored by the National Science Foundation and the Howard Hughes Medical Institute. Students and teachers alike responded to his friendly and down-to-earth way of presenting science. On many occasions, he helped the DNALC go through the "eye of the needle"—a pressing grant or other crisis—to survive to become the institution we now are. For that, we owe him a large debt of gratitude.

Mark Bloom in a photo from 1989.

The departures of Malissa and Mark provided opportunities for expanded responsibility for Trisha Harrison and Scott Bronson, who were each promoted to the rank of Education Manager. Trish maintained the momentum of the middle school program, while Scott assumed responsibility for managing the laboratory program and our growing cadre of student interns. Working together, they fostered a collaborative management style that has provided a common vision for our education programs at all levels.

The educational staff was bolstered by the arrival of two new laboratory instructors—Amanda Broege and Martha Mullally. Having come from a family of dog breeders, Amanda started her biology degree at SUNY, Stony Brook with an eye toward a career in veterinary medicine. It is our gain that she later decided to become NYS-certified in secondary science education. A native of Halifax, Nova Scotia, Martha became our second Canadian staff member. While working on her bachelor's degree in

biochemistry at Dalhousie University, Martha conceived of the *World Weavers*, a province-wide environmental education program for precollege students.

In recognition of his leadership in establishing the *BioMedia* Group, John Kruper was named assistant director of the DNALC. Three new staff members, programmer Matt Christensen and media designers Gisella Walter and Mark Feingold, joined *BioMedia* group members Sue Lauter and Shirley Chan. Born in Chile and raised in San Francisco, Gisella received a fine arts degree from Alfred University. Several years in the New York art world and working as a free-lance Web designer have made her a superb computer animator. Mark also received a fine arts degree from Alfred University, but got his start in art as a graffiti artist on the streets of Manhattan.

The expansion of the staff put added pressures on core administrators Dave Micklos and Judy Cumella-Korabik, so we were pleased when Janeen Russo joined the administrative group as secretary. A native of Brooklyn, Janeen provides the DNALC with needed clerical and administrative support. She assumed responsibility for scheduling and introducing Cablevision's multimedia presentation *Long Island Discovery*.

An intern program funded by the Howard Hughes Medical Institute allows high school students to assist the instructional staff in preparing the teaching laboratories and to undertake independent research projects. The independent work allows interns to push themselves intellectually while gaining technical expertise they can carry to the university level. Intern research projects also contribute to new lab experiences offered as student field trips or as part of summer workshops.

Hana Mizuno, of Cold Spring Harbor High School, received highest honors in the Long Island Science Congress, as well as a cash award from the Suffolk County Science Teachers Association, for her work using green fluorescent protein (GFP) as a reporter molecule in subcloning experiments. Much of her work was incorporated into a special summer workshop entitled "Green Genes" and also helped to update lab field trips on bacterial transformation. Hana later began research on programmed cell death (apoptosis) with CSHL scientist Yuri Lazebnik. Rachael Neumann, of Syosset High School, was one of only six students selected to participate in the *Partners for the Future* Program, working under the tutelage of CSHL scientist Grigori Enikolopov.

The instructional staff was ably assisted by veteran interns Jermel Watkins (New York Institute of Technology), Karin Glaizer (Portledge High School), and Gerry DeGregoris (Chaminade High School). Salley Anne Gibney returned from her freshman year at Johns Hopkins University to assist with the summer workshops. Hetty Ashton, a native of Australia, visited the DNALC in July to volunteer and partcipate in the middle school summer workshops. Using her education background, Hetty worked closely with the instructors and students to aptly assist in the laboratories and activities. She has retured to Australia to pursue her interest in education. In August, we bid farewell to Trevor Sammis, who began his freshman year at the University of Virginia at Richmond, and to Mera Goldman, who started at Barnard College. In the fall, we welcomed newcomers Gina Conenello (John Glenn High School), Sunjay Kelkar (Syosset High School), Stephen Mak (Syosset High School), Laura Roche (Cold Spring Harbor High School), and Rebecca Yee (Huntington High School).

Hetty Ashton talks with a parent of a *Fun With DNA* participant.

January 10	Laboratory for Town of Huntington Senior Citizens Center
January 17	Laboratory for Queens Bridge to Medicine High School
February 7	Laboratory for Rampart and Sierra High Schools, Colorado Springs, Colorado
February 18–19	National Human Genome Research Institute ELSI Project, *Eugenics Image Archive*, image collection visit to American Philosophical Society Library, Philadelphia, Pennsylvania
March 9–11	National Science Foundation Grant Review Site Visit, City College of San Francisco, California
March 20	Site visit by Linda Conlon, International Center for Life, Newcastle, Great Britain
March 26–28	Department of Energy Workshop, *The Science and Issues of Human DNA Polymorphisms*, University of Utah, Salt Lake City, Utah
April 3	Seminar at Trudeau Institute, Saranac Lake, New York
April 3–5	National Science Foundation Workshop, *Human Genome Diversity–Student Allele Database* Workshop, Pierce College, Woodland Hills, California
April 10	Site visit by Ray Gladden and Maria Rapoza, Carolina Biological Supply Company, Burlington, North Carolina
April 16	Site visit by National Science Foundation National Visiting Committee
April 21	*Great Moments in DNA Science* Honors Students Seminar, CSHL
April 22	Seminar for National Institute of Social Sciences, Harvard Club, New York, New York
April 23	Site visit by Topaz Conway, Garvan Research Foundation, Sydney, Australia
April 23–25	Visiting Scholar Program, Mills Godwin Specialty Center, Richmond, Virginia
April 28	Site visit by Simon Collier, University of Virginia, Charlottesville, Virginia
May 1–3	National Science Foundation Workshop, *Human Genome Diversity–Student Allele Database* Workshop, University of Washington, Seattle, Washington
May 5	*Great Moments in DNA Science* Honors Students Seminar, CSHL
May 8–10	National Science Foundation Workshop, *Human Genome Diversity–Student Allele Database* Workshop, Kingsborough Community College, Brooklyn, New York
May 12	*Great Moments in DNA Science* Honors Students Seminar, CSHL
May 18–19	Site visit by Sylvia Metcalfe, University of Melbourne, Australia
June 4	National Human Genome Research Institute ELSI Review Panel, Washington, D.C.
June 9	Site visit by June Osborn, Josiah Macy, Jr. Foundation, New York, New York
June 10-11	Gateway Summer Teachers Institute Reception, New York, New York
June 29–July 2	*World of Enzymes* Workshop, DNALC
	DNA Science Minority Workshop, Central Islip, New York
June 29–July 8	*Advanced DNA Science* Workshop, DNALC
June 29–July 29	Gateway Summer Teachers Institute, Stuyvesant High School, New York, New York
July 6–10	*Fun With DNA* Workshop, DNALC
	Fun With DNA Minority Workshop, Intermediate School 109, Queens, New York
July 6–17	*Advanced DNA Science* Minority Workshop, Central Islip, New York
July 13–17	*Fun With DNA* Minority Workshop, Intermediate School 109, Queens, New York
	Green Genes Workshop, DNALC
July 20–24	*World of Enzymes* Minority Workshop, Intermediate School 109, Queens, New York
	World of Enzymes Workshop, DNALC
	DNA Science Minority Workshop, John F. Kennedy High School, Bronx, New York
	DNA Science Workshop, DNALC
July 27–31	*Fun With DNA* Workshop, DNALC
	DNA Science Workshop, DNALC
August 3–7	*Fun With DNA* Workshop, DNALC
	DNA Science Minority Workshop, Stuyvesant High School, New York, New York
	DNA Science Workshop, DNALC
August 10–14	*Fun With DNA* Workshop, DNALC
	DNA Science Minority Workshop, Mt. Sinai School of Medicine, New York, New York
August 17–19	National Human Genome Research Institute ELSI Project, *Eugenics Image Archive* image collection visit to American Philosophical Library Society, Philadelphia, Pennsylvania
	Genomic Biology & PCR Workshop, DNALC

August 17–21	*Fun With DNA* Workshop, DNALC
	Reading the Code of Life Workshop, DNALC
August 20–28	*Advanced DNA Science* Workshop, DNALC
August 24–28	*World of Enzymes* Workshop, DNALC
	DNA Science Workshop, DNALC
September 21–22	National Human Genome Research Institute ELSI Project, *Eugenics Image Archive*, Advisory Panel Meeting, Banbury Center, CSHL
October 7	Site visit by Bronwyn Terrill, Museum Victoria, Australia
October 9–12	National Science Foundation ATE Project, *Genomic Biology*, Editorial Advisory Board Meeting, DNALC
October 20–24	*Western States Courts and Genetic Testing* Conference, Snowbird, Utah
October 21–23	Howard Hughes Medical Institute Directors Meeting, Rockville, Maryland
October 29	Site visit and laboratory for participants in *The Art of Judging: Perspectives of Science* Banbury Center Meeting
October 30	*Mid-Atlantic Judicial* Conference, Ocean City, Maryland
November 3	Laboratory for science faculty from High School for the Humanities, New York, New York
November 3	National Institutes of Health ELSI Grant Review
November 12–14	Department of Energy Workshop, *The Science & Issues of Human DNA Polymorphisms*, Mills Godwin Specialty Center, Richmond, Virginia
November 20–21	National Science Foundation ATE Director's Meeting, Washington, D.C.
November 22–24	Macy Foundation Meeting, *Genes, Teens, and the World Wide Web*, Banbury Center, CSHL
December 3–5	Department of Energy Workshop, *The Science & Issues of Human DNA Polymorphisms*, University of Denver, Colorado

Sites of Major Faculty Workshops 1985–1998

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**
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
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**

IINDIANA	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*
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
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**
	DNA Learning Center	*1990–1992*
	Fostertown School, Newburgh	*1991*
	Huntington High School	1986
	Irvington High School	1986
	Junior High School 263, Brooklyn	*1991*
	Lindenhurst Junior High School	*1991*
	Mt. Sinai School of Medicine, New York	1997
	Orchard Park Junior High School	*1991*
	Plainview-Old Bethpage Middle School	*1991*
	State University of New York, Purchase	1989
	State University of New York, Stony Brook	1987–1990
	Titusville Middle School, Poughkeepsie	*1991,1993*
	Wheatley School, Old Westbury	1985
	US Military Academy, West Point	**1996**
	Stuyvesant High School, New York	1998
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
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**
UTAH	University of Utah, Salt Lake City	1993
	University of Utah, Salt Lake City	**1998**
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**
WEST VIRGINIA	Bethany College	1989
WISCONSIN	Marquette University, Milwaukee	1986,1987
	University of Wisconsin, Madison	1988,1989
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	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

1998 PUBLICATIONS

Laboratory Manuals

Detecting Genes: A Laboratory Manual
Genome Analysis, Volume 2
Bruce Birren, Eric D. Green, Sue Klapholz, Richard M. Myers, and Jane Roskams (eds.)

Cloning Systems: A Laboratory Manual
Genome Analysis, Volume 3
Bruce Birren, Eric D. Green, Sue Klapholz, Richard M. Myers, Harold Reithman, and Jane Roskams (eds.)

Mapping Genomes: A Laboratory Manual
Genome Analysis, Volume 4
Bruce Birren, Eric D. Green, Philip Hieter, Sue Klapholz, Richard M. Myers, Harold Reithman, and Jane Roskams (eds.)

Using Antibodies
Ed Harlow and David Lane

Crystallization of Biological Macromolecules
Alexander McPherson

Concepts in Eukaryotic DNA Replication
Melvin L. DePamphilis (ed.)

CSHL Monograph Series

The Development of Human Gene Therapy
Theodore Friedmann (ed.)

The RNA World, Second Edition
Raymond F. Gesteland, Thomas R. Cech, and John F. Atkins (eds.)

General Books

Pattern Formation during Development
Cold Spring Harbor Symposia on Quantitative Biology LXII

I Wish I'd Made You Angry Earlier
Max Perutz

At the Bench: A Laboratory Navigator
Kathy Barker

The Structure and Reproduction of Corn
T.A. Kiesselbach

Cancer Surveys Series

Vol. 31: *Bladder Cancer*
R.T.D. Oliver (ed.)

Vol. 32: *Precancer*
J. Ponten (ed.)

Journals

Genes & Development (Volume 12, 24 issues)
T. Grodzicker and N. Hastie (eds.)

Genome Research (Volume 8, 12 issues)
L. Goodman, A. Chakravarti, R. Gibbs, E. Green, R. Myers, and M. Boguski (eds.)

Learning & Memory (Volume 5, 6 issues)
J.H. Byrne (ed.)

Slides

Cells: Basic Principles of Microscopy
David Spector and Robert Goldman (eds.)

Other

CSHL Annual Report 1997

Banbury Center Annual Report 1997

Administration and Financial Annual Report 1997

COLD SPRING HARBOR LABORATORY PRESS

The 1998 results for Cold Spring Harbor Laboratory Press owed much to the previous year's gains in productivity through staff restructuring and investment in technology. Fourteen new books were released, all produced digitally. All three journals were published for the first time in both electronic and print editions and all made gains in quality, influence, advertising pages, and circulation. Financially, there was a substantial improvement in operating margin on increased sales of $6.34 million.

Books for Scientists

The complete list of new books published is shown opposite. The most eagerly anticipated was the laboratory manual *Using Antibodies*, by Ed Harlow and David Lane, released in December. This handsome book with its innovative design features is an update of material from Harlow and Lane's classic manual *Antibodies*, which has been a mainstay of our publishing program since its appearance in 1988. Entirely rethought and rewritten in the light of a further decade's experience, this new book is essential for molecular and cell biologists who want to use antibodies with insight as well as accuracy.

The year also saw the culmination of the series of four lab manuals entitled *Genome Analysis*. Conceived when the human genome project was only just getting under way, this series was an enormous undertaking, encompassing and at times overwhelming a team of 7 editors and more than 50 contributors, whose goals were continually challenged by the rapid evolution of genomic science. Now, their efforts have created a set of techniques with lasting value as the era of mapping and sequencing gives way to functional genomic approaches that are destined to change how experimental biology is done.

The celebrated series of Cold Spring Harbor Laboratory monographs continued to expand with the appearance of two new titles. *The Development of Human Gene Therapy* is a volume with a particularly well-defined mission. Its editor, Ted Friedmann, undertook the work with the aim of celebrating the remarkable scientific achievements that have helped found the brand-new medical discipline of gene science. But he and the contributors have ensured that the book provides a clear-eyed view of the improvements still required to make the technology of gene transfer stable as well as a careful and critical assessment of the medical benefits that have been achieved so far—results that have been oversold to an eager public. As a result, the book has provoked vigorous comment and some bruised feelings.

The public became aware of another new monograph, thanks to a prominent story in the Science section of *The New York Times* that spoke to continuing popular fascination with the origins of life. *The RNA World,* edited by Ray Gesteland, Tom Cech, and John Atkins, is devoted to the possibility that prebiotic evolution depended on RNA replication, but it also provides a valuable perspective on the modern world of RNA. A second edition of one of our most successful recent books, this volume is being appreciated by research scientists and educators alike.

Our decision last year to develop a number of books with appeal to an audience beyond professional science was vindicated with the extraordinarily warm response to the appearance of a new collection of essays by the distinguished protein chemist Max Perutz. *I Wish I'd Made You Angry Earlier* consists of articles written by Max at the invitation of several well-known magazines such as *New York Review of Books*. Reviews of the biographies of scientists, many of whom were friends and acquaintances, become in Max's hands wonderful reflections on science and society as a whole, often illuminated by autobiographical fragments that make fascinating reading. This essay collection has been enthusiastically received by a scientific community which cherishes the author; but reviews in a number of international literary magazines introduced the book to a wider public and prompted a distribution agreement for a European edition through Oxford University Press and translation into five languages.

Widespread praise also greeted the appearance of an introduction to bench science for neophytes entitled *At the Bench: A Laboratory Navigator,* by Kathy Barker. Written in a light-hearted but thoroughly practical tone, this user-friendly manual offers advice, moral support, and social etiquette as well as detailed descriptions of basic laboratory procedures that are seldom explained clearly enough to novices by experienced colleagues. Based on the author's own observations of life in teaching and research institutions, the book has found a responsive audience in graduate students, lab technicians, and doctors rotating through research labs, as well as the senior staff who have to shepherd them.

Our front-list was rounded out by two books notable for the beauty of their illustrations as well as the excellence of their text. The 1997 Symposium volume, *Pattern Formation during Development*, reviewed the remarkable convergence in the mechanisms of embryonic development across vertebrate and invertebrate species, its 60 authoritative chapters embellished with the results of a battery of new techniques for visualizing biological processes. On the other hand, an imaging technique with a long and honorable history, X-ray crystallography, is the target of a new work by an expert in the art, Alexander McPherson. *Crystallization of Biological Macromolecules* presents the physical and chemical principles of crystal growth in an approachable way with the aid of splendid photographs, helping investigators prepare their favorite molecules for X-ray analysis.

Books for Students

As the year came to a close, a decision was made with the potential to transform the future shape of book publishing at Cold Spring Harbor. Jim Watson has been the visionary and driving force behind the Laboratory's role in scholarly publishing since his appointment as Director in 1968. Already a highly successful author, with the pioneering textbook *Molecular Biology of the Gene* and the extraordinary, best-selling memoir *The Double Helix* to his credit, Jim devoted part of his formidable energies to building upon the already prestigious reputation established by the annual volumes in the *Symposia for Quantitative Biology* series. From his ideas and recruitment of influential editors and authors came the beginnings of the classic monographs and lab manuals that form the mainstay of our professional publishing program today.

During the seventies and eighties, Jim and teams of talented colleagues created two additional innovative, influential, and highly successful textbooks for undergraduate teaching, *Recombinant DNA,* and *Molecular Biology of the Cell*, while nurturing MBOG through several editions of increasing size and scope as molecular biology entered the teaching mainstream. All these books were published by commercial publishers with the resources to gamble on breaking new ground and the marketing muscle to back up that investment—not the characteristics of a small publishing program at an institution such as the Laboratory. Nevertheless, Jim never lost sight of the possibility, and appropriateness, of expanding the publishing program here and bringing to the development of books for undergraduates the reputation for quality and outstanding scholarship earned by the books for working scientists.

Jim's vision became a reality this year when the Trustees approved investment in the development of a series of innovative books for teaching undergraduate biology. Half a dozen titles are proposed to begin with, books aimed at college juniors and seniors that reflect both new facts and new approaches to the way research is done. The editorial development of the books will be in the hands of Cold Spring Harbor Laboratory Press, particularly those of Alexander Gann, a writing collaborator of Mark Ptashne's and an experienced undergraduate teacher, who joined us from the University of Lancaster as the editor spearheading the program. But the funding and final publication of the books are planned to be the responsibility of a separate organization set up in partnership with a commercial publisher. Initial conversations with prospective partners have been highly encouraging. Even more gratifying has been the reaction of prospective authors of the books concerned, who have unanimously applauded our decision to get involved in this important aspect of teaching and are delighted to be participants. Central to the success of the program will be the use of the newly acquired and splendidly refurbished

Meier House at Banbury, as a writing center where authors can retreat from the pressures of other academic activities.

The prospects for this leap forward in the book publishing program are truly exciting and have a pleasing symmetry with the Laboratory's intiation this year of its own graduate school. Our goal for the textbook program is an ambitious combination of first-class publishing, a novel educational approach, and substantial financial success.

Journal Publishing

The three Cold Spring Harbor journals, *Genes & Development*, *Genome Research*, and *Learning & Memory,* were offered more manuscripts for publication this year and the number of pages published by each rose substantially. Their contents diversified with the addition of short papers and regular reviews to *Genes & Development,* website reviews to *Genome Research,* and the publication of special issues of *Learning & Memory* devoted to single topics such as mushroom bodies and transgenic techniques.

Online editions of *Genes & Development* and *Genome Research*, initiated in 1997 in a partnership with the HighWire Press of Stanford University, were renewed for the first time. Print editions of journals have an elaborate infrastructure for such renewal, particularly at an institutional level, but subscription management is one of many ways in which the business of electronic journals is still evolving, and we were pleased when this milestone was passed with only minor difficulties.

Learning & Memory was published online for the first time in November, aided by a grant from the Oliver S. and Jennie R. Donaldson Charitable Trust, and an electronic subscription was made available starting January 1999. All three journals are currently offered as a combination of print and online editions, which provides users with the optimum blend of paper's portability and software's flexibility. One of the many benefits of the collaboration with HighWire Press is our membership of the community of publishers participating in the Stanford initiative. Respectful of the different agendas of these organizations, largely nonprofit but with often quite divergent goals, HighWire has enabled open discussion of many publishers' experiments, so that real information is available on which to base business decisions in this emerging marketplace. Several organizations have decided that print issues are a liability quickly to be disposed of. For the moment, encouraged by our readers, we take a different view.

All three journals were buoyed by increasing subscriptions, particularly from institutions, higher advertising sales, and increasing influence as measured by the Institute for Scientific Information. The impact factor of *Genes & Development* increased to 18.86 in 1998, ranking it, as last year, in the top ten of all primary research journals. The impact factor for *Genome Research* rose from 3.67 last year to 5.42, placing it second among genome science journals. And *Learning & Memory* acquired an impact factor for the first time, at 3.67, positioning it 13th among the enormous number and diversity of neuroscience journals. This measure is likely to improve since the journal's acceptance this year for inclusion in Medline and Index Medicus.

As always, we are grateful to the scientists who edit our journals from locations beyond Cold Spring Harbor: Davor Solter (Freiburg) at *Genes & Development*; Mark Boguski (Bethesda), Aravinda Chakravarti (Cleveland), Richard Gibbs (Houston), Eric Green (Bethesda), and Richard Myers (Palo Alto) at *Genome Research*; and Jack Byrne (Houston) at *Learning & Memory*.

Marketing and Distribution

Marketing activities in 1998, which centered on direct mail, meeting exhibits, space advertising, and the Web, produced increased income. A new catalog of front and mid-list titles and the fall *New Book Titles* newsletter were widely circulated. Striking, large-format four-color brochures advertising the *Cells* and *Genome Analysis* manuals successfully drew sales for these new and important titles.

Exhibits were mounted at 14 major scientific society meetings, twice the number attended last year. Our largest presence was at the ASCB meeting held in December, where the newly published *Using Antibodies* was launched. The thriving Cold Spring Harbor meetings and courses provide further promotional opportunities and John Coffin and Steve Hughes, two of the editors of the *Retroviruses* volume, signed numerous copies of the book during the Retroviruses meeting in May.

A gratifyingly high proportion of our books received favorable reviews in prominent locations such as *Nature*, *Science*, and the *Trends* journals. In addition, the 1997 title *Mutants of Maize* won honorable mention in the biological sciences category at the annual awards of the Professional and Scholarly Publishing Division of the American Association of Publishers.

The Press website, consistently updated with new announcements and descriptions of new releases, is a marketing channel of growing importance and has become the definitive statement of our book program. During the year, the site was redesigned and book covers for 1998 and 1997 titles were added. Electronic advertisements on other websites added to the online visibility of our publications.

In the Fulfillment Department, a new proprietary package for order fulfillment was fully implemented and has already demonstrated its effectiveness and flexibility. It permits major improvements in the speed and accuracy of attention to customer requirements and the subsequent analysis of sales and costs.

Staff

The staff members of the Press as of December 1998 are listed elsewhere in this volume. Gratitude and thanks are extended to each one of them for the hard work, commitment, and participation in the processes of change that led to this successful year. It should be noted that we are now a truly networked organization, with staff members Judy Cuddihy and Kaaren Janssen making important contributions as book editors from their home offices in New Mexico and Connecticut, respectively; Ingrid Benirschke managing many of our marketing activities from San Diego; and several freelance associates of the editorial development department telecommuting from locations as far apart as Seattle, Washington, and Malvern, U.K.

The heart of any publishing program is the quality of its output and in this respect we are fortunate to be able to work with some of the world's most outstanding scientists. But to create the vehicle for that work and find an audience for it, many additional kinds of craft must be applied. For the careful, intelligent application of this expertise, we are grateful for the leadership of the senior staff of the Press: the editors of our journals, Terri Grodzicker at *Genes & Development* and Laurie Goodman at *Genome Research*; Jan Argentine, Editorial Development Manager; Denise Weiss, Production Manager; Ingrid Benirschke, Marketing Manager; Marcie Ebenstein, Advertising Manager; Nancy Hodson, Business Manager; and Guy Keyes, Fulfillment and Distribution Manager.

John R. Inglis

FINANCE

FINANCIAL STATEMENTS

CONSOLIDATED STATEMENTS OF FINANCIAL POSITION
December 31, 1998 and 1997

	1998	1997
Assets:		
Cash and cash equivalents	$ 16,258,527	16,542,395
Investments	158,108,972	140,573,553
Accounts receivable:		
Publications	782,598	829,653
Other	172,405	158,235
Grants receivable	2,384,707	3,038,893
Contributions receivable	2,657,748	1,707,605
Publications inventory	1,923,784	1,991,707
Prepaid expenses and other assets	1,482,543	1,377,264
Investment in employee residences	2,464,260	2,269,345
Land, buildings and equipment:		
Land and land improvements	12,557,726	9,980,071
Buildings	69,897,882	66,519,640
Furniture, fixtures, and equipment	5,037,823	4,950,130
Laboratory equipment	12,626,629	12,655,257
Library books and periodicals	365,630	365,630
Construction in progress	4,256,539	626,728
	104,742,229	95,097,456
Less accumulated depreciation and amortization	(32,455,453)	(30,500,090)
Land, buildings, and equipment, net	72,286,776	64,597,366
Total assets	$ 258,522,320	233,086,016
Liabilities and Net Assets:		
Liabilities:		
Accounts payable and accrued expenses	$ 2,809,788	2,599,761
Notes payable	276,163	645,839
Bonds payable	30,000,000	30,000,000
Deferred revenue	3,358,722	5,554,037
Total liabilities	36,444,673	38,799,637
Net assets:		
Unrestricted		
General operating	10,598,288	5,760,936
Designated by board for:		
Research programs	1,900,000	1,150,000
Capital expenditures	1,207,889	3,709,145
Endowment	71,296,987	70,713,010
Net investment in plant	42,010,613	33,951,527
Total unrestricted	127,013,777	115,284,618
Temporarily restricted	4,185,424	4,723,413
Permanently restricted	90,878,446	74,278,348
Total net assets	222,077,647	194,286,379
Total liabilities and net assets	$ 258,522,320	233,086,016

CONSOLIDATED STATEMENT OF ACTIVITIES
Year Ended December 31, 1998
With comparative totals for the year ended December 31, 1997

	Unrestricted	*Temporarily Restricted*	*Permanently Restricted*	*1998 Total*	*1997 Total*
Revenue, gains, and other support:					
Public support (contributions and nongovernment grant awards)	$ 9,069,659	4,155,424	9,342,949	22,568,032	14,010,944
Government grant awards	15,929,141	–	–	15,929,141	15,392,415
Indirect cost allowances	11,230,797	–	–	11,230,797	10,000,564
Other revenue:					
Program fees	2,388,531	–	–	2,388,531	2,117,904
Rental income	333,196	–	–	333,196	389,658
Publications sales	6,341,142	–	–	6,341,142	5,237,814
Dining services	2,444,384	–	–	2,444,384	2,172,196
Rooms and apartments	1,877,794	–	–	1,877,794	1,712,294
Royalty and licensing fees	754,705	–	–	754,705	679,082
Net appreciation in fair value of investments	4,686,928	–	7,257,149	11,944,077	17,183,813
Investment income (interest and dividends)	6,053,220	–	–	6,053,220	5,677,213
Miscellaneous	124,984	–	–	124,984	167,324
Total other revenue	25,004,884	–	7,257,149	32,262,033	35,337,298
Net assets released from restrictions	4,693,413	(4,693,413)	–	–	–
Total revenue, gains, and other support	65,927,894	(537,989)	16,600,098	81,990,003	74,741,221
Expenses:					
Research	28,718,081	–	–	28,718,081	27,715,449
Summer programs (meetings and courses)	7,834,447	–	–	7,834,447	6,848,437
Publications	6,219,895	–	–	6,219,895	5,400,006
Banbury Center conferences	877,834	–	–	877,834	953,562
DNA Learning Center programs	1,034,538	–	–	1,034,538	627,763
General and administrative	6,663,226	–	–	6,663,226	6,091,915
Dining services	2,850,714	–	–	2,850,714	2,617,705
Total expenses	54,198,735	–	–	54,198,735	50,254,837
Increase (decrease) in net assets	11,729,159	(537,989)	16,600,098	27,791,268	24,486,384
Net assets at beginning of year	115,284,618	4,723,413	74,278,348	194,286,379	169,799,995
Net assets at end of year	$ 127,013,777	4,185,424	90,878,446	222,077,647	194,286,379

CONSOLIDATED STATEMENTS OF CASH FLOWS
Year ended December 31, 1998 and 1997

	1998	1997
Cash flows from operating activities:		
Increase in net assets	$ 27,791,268	24,486,384
Adjustments to reconcile increase in net assets to net cash provided by operating activities:		
Depreciation and amortization	3,443,290	3,370,584
Net appreciation in fair value of investments	(11,944,077)	(17,183,813)
Contributions restricted for long-term investment	(10,762,186)	(4,998,910)
Changes in assets and liabilities:		
Decrease (increase) in accounts receivable	32,885	(306,794)
Decrease (increase) in grants receivable	654,186	(787,248)
(Increase) decrease in contributions receivable	(950,143)	1,810,560
Decrease (increase) in publications inventory	67,923	(565,589)
Increase in prepaid expenses and other assets	(105,279)	(68,803)
Increase in accounts payable and accrued expenses	210,027	1,129,754
Decrease in deferred revenue	(2,195,315)	(161,473)
Net cash provided by operating activities	6,242,579	6,724,652
Cash flows from investing activities:		
Capital expenditures	(11,132,700)	(8,316,421)
Proceeds from sales and maturities of investments	25,341,828	100,940,803
Purchases of investments	(30,933,170)	(100,647,823)
Net change in investments in employee residences	(194,915)	101,598
Net cash used in investing activities	(16,918,957)	(7,921,843)
Cash flows from financing activities:		
Permanently restricted contributions	9,342,949	335,498
Contributions restricted for investment in land, buildings, and equipment	1,419,237	4,663,412
Repayment of notes payable	(369,676)	(570,122)
Issuance of notes payable	—	351,000
Net cash provided by financing activities	10,392,510	4,779,788
Net (decrease) increase in cash and cash equivalents	(283,868)	3,582,597
Cash and cash equivalents at beginning of year	16,542,395	12,959,798
Cash and cash equivalents at end of year	$ 16,258,527	16,542,395
Supplemental disclosures:		
Interest paid	$ 1,254,036	1,239,341

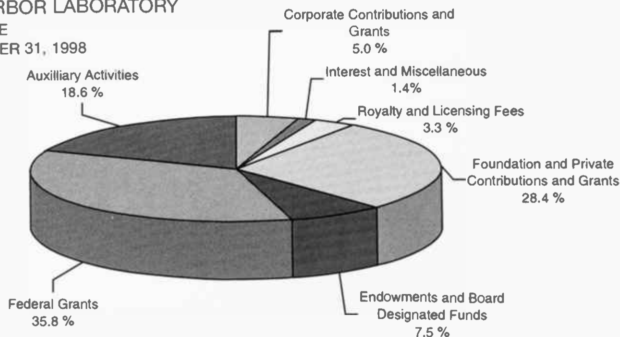
COMPARATIVE OPERATING HISTORY 1994–1998
(Dollars in Thousands)

	1994	1995	1996	1997	1998
Revenue:					
Main Lab:					
Grants and contracts	$ 19,293	19,653	20,879	22,743	24,025
Indirect cost reimbursement	8,460	8,881	9,704	9,910	11,054
Other	6,808	7,461	7,859	8,472	9,441
CSHL Press	4,390	5,119	4,805	5,238	6,341
Banbury Center	1,569	1,732	1,214	1,495	1,444
DNA Learning Center	824	954	754	875	1,334
Total income	41,344	43,800	45,215	48,733	53,639
Expenses:					
Main Lab:					
Research and training	19,293	19,653	20,879	22,743	24,025
Operation and maintenance of plant	5,141	5,266	5,446	5,274	5,549
General and administrative	2,909	3,329	3,438	3,625	3,378
Other	4,847	4,959	5,367	5,759	7,328
CSHL Press	4,309	5,079	5,032	5,320	6,141
Banbury Center	1,498	1,643	1,225	1,437	1,321
DNA Learning Center	798	958	781	887	1,228
Total expenses, excluding depreciation	38,795	40,887	42,168	45,045	48,970
Excess before depreciation, amortization, and release of designated funds	2,549	2,913	3,047	3,688	4,669
Depreciation and amortization	(2,668)	(2,821)	(2,988)	(3,371)	(3,443)
Release (designation) of funds (1)	200	–	–	–	(750)
Net operating excess	$ 81	92	59	317	476

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.

COLD SPRING HARBOR LABORATORY
SOURCES OF REVENUE
YEAR ENDED DECEMBER 31, 1998



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 1998.

GRANTS January 1, 1998–December 31, 1998

COLD SPRING HARBOR LABORATORY

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*1998 Funding+*	
FEDERAL GRANTS				
NATIONAL INSTITUTES OF HEALTH				
Program Projects	Dr. Herr	1/92–12/01	$ 4,005,655	
	Dr. McCombie	9/98–7/99	1,100,000	*
	Dr. Stillman	8/90–7/00	3,104,389	
	Dr. Tully	5/96–4/99	726,521	
Research Support	Dr. Beach	5/94–2/99	328,573	
	Dr. Beach	8/95–5/00	275,338	
	Dr. Cline	12/95–11/98	292,524	
	Dr. Cline	3/98–2/01	279,341	*
	Dr. Futcher	4/93–3/01	267,507	
	Dr. Futcher	1/91–12/99	175,093	
	Dr. Hengartner	5/95–4/00	242,477	
	Dr. Hernandez	7/87–6/00	152,992	
	Dr. Herr	8/96–7/00	212,883	
	Dr. Hirano	5/96–4/00	214,855	
	Dr. Joshua-Tor	8/96–7/99	234,063	
	Dr. Krainer	7/89–6/02	418,728	
	Dr. Malinow	5/92–4/00	252,839	
	Dr. Malinow	4/95–2/03	357,330	
	Dr. Nedivi	7/97–8/98	22,393	
	Dr. Neuwald	10/98–8/01	360,556	*
	Dr. Skowronski	4/98–3/03	452,942	*
	Dr. Spector	4/95–3/99	338,280	
	Dr. Stillman	7/91–5/00	518,706	
	Dr. Svoboda	12/98–11/03	433,635	*
	Dr. Tonks	8/91–3/01	495,885	
	Dr. Tonks	5/97–4/01	282,203	
	Dr. Tully	4/94–3/02	332,224	
	Dr. Tully	8/96–6/00	288,400	
	Dr. Tully	5/97–4/99	84,500	
	Dr. Van Aelst	12/97–11/01	361,040	
	Dr. Wigler	7/95–4/99	1,465,194	
	Dr. Wigler	7/98–4/02	949,501	*
	Dr. Xu	12/97–11/02	285,815	
	Dr. Yin	9/96–8/99	275,374	
	Dr. Yin	10/97–9/01	100,000	
	Dr. Zhang	9/97–8/00	354,253	
	Dr. Zhong	2/96–1/00	250,385	

*New Grants Awarded in 1998
+Includes Direct and Indirect Cost

Grantor	Program/Principal Investigator	Duration of Grant	1998 Funding+
Fellowships	Dr. Dickinson	7/97–11/98	10,743
	Dr. Dubnau	7/96–6/99	28,600
	Dr. Haas	7/98–6/01	26,176 *
	Dr. Horiuchi	10/98–9/00	26,176 *
	Dr. Kogan	1/97–12/98	29,160
	Dr. Nimchinsky	9/98–8/00	30,160 *
Training Support	Training in Cancer Cell Biology and Tumor Virology	7/94–2/99	176,841
Course Support	Advanced Bacterial Genetics	5/93–4/99	62,089
	Cancer Research Center Workshops	4/92–3/00	268,388
	Neurobiology Short-term Training	5/82–4/01	143,129
	Analysis of Large DNA Molecules	4/91–3/01	54,469
	Computational Genomics	9/91–8/01	41,224
	In Situ Hybridization and Immuno-cytochemistry	7/98–6/03	61,929 *
	Automated Genome Sequencing	4/95–3/01	77,960
	Molecular Biology and Development of *Xenopus laevis*	4/96–3/00	10,000
	Caenorhabditis elegans	8/98–6/01	36,101 *
Meeting Support	Genome Mapping and Sequencing	4/90–3/99	37,871
	The Cell Cycle	4/98–3/99	11,000 *
	Zebrafish Development and Genetics	4/98–3/99	18,090 *
	Genetics of Aging	4/98–3/00	25,732 *
	Heat Shock Proteins and Molecular Chaperones	5/98–4/99	5,000 *
	63rd Symposium: Mechanisms of Transcription	6/98–5/01	10,000 *
	Cancer Genetics and Tumor Suppressor	7/98–6/99	10,000 *
	Mouse Molecular Genetics	7/98–6/99	15,000 *
	Gametogenesis	7/98–6/99	6,000 *
	NCI Cancer Workshop	1/98–12/98	10,075 *
	Gene Therapy	8/98–7/99	24,000 *
	Conditional Genetic Technologies in the Mouse Workshop	9/98–8/99	26,000 *
Equipment	Automated DNA Sequencer	5/98–5/99	128,500 *
NATIONAL SCIENCE FOUNDATION			
Cooperative Agreement	Drs. Martienssen/McCombie	9/96–8/99	1,335,000
Research Support	Dr. Cline	9/96–8/99	108,750
	Dr. Grossniklaus	8/97–7/00	125,000
	Dr. Jackson	1/98–12/00	100,000 *
	Dr. Ma	1/98–8/99	87,292 *
	Dr. Ma	4/98–8/99	22,490
	Dr. Martienssen	11/98–10/99	736,538 *
	Dr. Nestler	4/98–3/00	100,000 *
Training Support	Undergraduate Research Program	6/91–5/00	50,000
Course Support	*Arabidopsis* Molecular Genetics	6/94–5/00	60,000
	Early Development of *Xenopus laevis*	9/98–8/02	19,311 *
	Computational Neuroscience: Vision	7/98–6/99	46,710 *

*New Grants Awarded in 1998
+Includes Direct and Indirect Cost

Grantor	Program/Principal Investigator	Duration of Grant	1998 Funding+
Meeting Support	The Cell Cycle	4/98–3/99	4,000 *
	Zebrafish Development and Genetics	2/98–3/99	9,000 *
	Mouse Molecular Genetics	5/98–4/99	4,000 *
	Gametogenesis	9/98–8/99	12,776 *
	The Molecular Genetics of Bacteria and Phages	9/98–8/99	5,000 *
Equipment	Genomic Computing Resource	9/98–8/01	113,615 *
DEPARTMENT OF ENERGY			
Research Support	Dr. Martienssen	8/91–2/01	98,000
UNITED STATES DEPARTMENT OF AGRICULTURE			
Research Support	Drs. McCombie/Martienssen	9/97–8/00	122,773
	Dr. Martienssen	9/97–8/00	64,915
	Drs. McCombie/Martienssen	7/98–8/01	98,456 *
UNITED STATES DEPARTMENT OF THE ARMY			
Research Support	Dr. Futcher	7/97–6/00	99,802
	Dr. Hannon	9/96–8/00	150,000
	Dr. Hengartner	7/97–6/00	99,698
Fellowship Support	Dr. Liu	12/96–11/99	39,474
	J. Polyakova	7/97–6/00	20,000
	Dr. Schneider	8/97–7/00	41,000
	Dr. Sherlock/Donovan	7/97–6/00	41,000
MISCELLANEOUS GRANTS			
Research Support			
American Cancer Society	Dr. Wigler, Professorship	1986–2012	50,000
	Dr. Wigler, Supply Allowance	1986–1998	10,000
Calbiochem-Novabiochem Corporation	Dr. Krainer	2/96–1/00	60,000
Council for Tobacco Research	Dr. Futcher	1/97–12/99	67,500
	Dr. Skowronski	7/97–6/00	78,751
Devgen N.V.	Dr. Hengartner	4/98–3/01	200,000 *
The Lillian Goldman Charitable Trust through The Breast Cancer Research Foundation	Dr. Wigler	9/98–8/99	300,000 *
Lita Annenberg Hazen Foundation	Neurobiology Support	12/98–11/99	200,000 *
The John A. Hartford Foundation, Inc.	Dr. Tully	1/97–12/99	302,082
Helicon Therapeutics	Drs. Tully/Yin	9/97–8/00	1,400,000
Human Frontier Science Program	Dr. Hirano	7/98–6/01	55,000 *
	Dr. Svoboda	9/98–8/01	64,484 *
Huntington Breast Cancer Action Coalition	Dr. Wigler	10/98–9/99	25,000 *
1 in 9: L.I. Breast Cancer Action Coalition	Dr. Wigler	1998	100,000 *

*New Grants Awarded in 1998
+Includes Direct and Indirect Cost

Grantor	Program/Principal Investigator	Duration of Grant	1998 Funding[+]
L.I.F.E.	Dr. Wigler	1998	4,590 *
March of Dimes	Dr. Enikolopov	6/98–5/00	66,048 *
The McKnight Endowment Fund for Neuroscience	Dr. Yin	7/96–6/99	50,000
Mellam Family Foundation	Dr. Tonks	12/96–11/00	50,000
Maxfield Foundation	Dr. Lazebnik	12/98–11/99	5,000 *
Merck Genome Research Institute	Dr. Zhang	11/97–10/99	90,000
Monsanto, Inc.–Plant Consortium Program	Dr. Martienssen	1/98–12/02	135,000 *
NIH/The Jackson Laboratory Consortium Agreement	Dr. Stein	1/98–12/98	44,253 *
NIH/Sloan Kettering Consortium Agreement	Dr. Wigler	9/95–8/00	399,261
NIH/Sloan Kettering Consortium Agreement	Drs. Tonks/Van Aelst	8/97–5/01	444,152
NIH/Nanoprobes, Inc.	Dr. Spector	9/96–8/00	31,439
Consortium Agreement	Dr. Spector	9/96–8/00	12,724
NIH/University of Pennsylvania Consortium Agreement	Dr. Yin	10/97–9/01	100,000
NIH/Washington University Consortium Agreement	Dr. Stein	1/98–6/99	182,762 *
N.A.T.O.	Dr. Hannon	1/98–4/99	5,080 *
Novartis–Plant Consortium Program	Dr. Martienssen	1/98–12/02	135,000 *
Offin Charitable Trust	Dr. Hengartner	8/98–7/99	25,000 *
Perkin Fund	Dr. Zhong	6/97–5/99	25,000
Pioneer Hi-Bred International	Dr. Grossniklaus	10/97–9/99	35,000
Michael Rankowitz and Shelia Heffron	Dr. Zhong	4/98–3/99	25,000 *
Roche Discovery Welwyn	Dr. Stenlund	1/98–12/98	50,000 *
The Kelly Rich Rett Research Foundation	Dr. Hatchwell	12/98–11/99	10,000 *
Robin and Enrique Senior Philanthropic Fund of The Jewish Communal Fund	Dr. Helfman	12/97–11/99	10,000
Seraph Foundation	Dr. Enikolopov	12/98–11/99	27,000 *
	Dr. Lazebnik	12/98–11/99	23,000 *
Lauri Strauss Leukemia Foundation, Felix Schnyder Memorial Fund	Dr. Tonks	12/97–11/98	15,000
Alexander and Margaret Stewart Trust	Dr. Hannon Dr. Hengartner Dr. Stenlund Dr. Tonks	1/98–12/98	150,000 *
St. Giles Foundation	Dr. Wigler	4/98–3/00	251,000 *
Tularik, Inc.	Dr. Wigler	10/97–10/03	660,000
Westvaco–Plant Consortium Program	Dr. Martienssen	1/98–12/02	135,000 *
The Whitaker Foundation	Dr. Svoboda	6/98–7/01	87,380 *

Fellowships

Grantor	Program/Principal Investigator	Duration of Grant	1998 Funding[+]
Academy of Finland	Dr. Vaahtoka	12/97–12/98	8,368 *
Rita Allen Foundation	Dr. Hengartner	9/94–8/99	30,000
American Cancer Society	Dr. Kass-Eisler	11/96–10/99	28,000
Burroughs Wellcome Fund	Dr. Mainen	9/97–8/99	58,300

*New Grants Awarded in 1998
+Includes Direct and Indirect Cost

Grantor	Program/Principal Investigator	Duration of Grant	1998 Funding+
CSHL Association	Dr. Ding	4/95–3/99	181,559
	Dr. Fraser		
	Dr. Jackson		
	Dr. Kidner		
	Dr. Shen		
	Dr. Spillane		
	Dr. Tansey		
European Molecular Biology Organization	Dr. Gartner	6/97–3/99	36,000
Fonds National Suiss de la Recherche Scientifique	Dr. Vielle-Calzada	1/98–3/00	12,000 *
Joseph G. Goldring Foundation	Dr. Stillman	7/98–6/99	50,000 *
Human Frontier Science Program	Dr. Cartegni	10/97–9/99	35,400
	Dr. Chong	10/97–9/99	35,400
	Dr. Mendez	10/97–9/99	35,400
	Dr. Poncer	4/97–3/99	29,400
	Dr. Soengas	6/97–6/99	35,400
IBRO Fellowship	Dr. Filipowski	3/98–3/99	25,000 *
	Dr. Lendvai	3/98–2/99	25,000 *
Japan Society for Promotion of Science	Dr. Hayashi	1/98–3/98	7,500 *
	Dr. Shibahara	4/97–3/99	40,000
Sidney Kimmel Foundation	Dr. Lowe	7/97–6/99	100,000
	Dr. Van Aelst	7/97–6/99	100,000
Esther A. and Joseph Klingenstein Fund, Inc.	Dr. Svoboda	7/98–6/01	40,000 *
Charles Henry Leach II Foundation	Dr. Enikolopov	1/97–12/97	25,000
Leukemia Society of America, Inc.	Dr. Kimura	7/97–6/00	36,700
	Dr. Pendergrast	7/98–6/01	39,700 *
	Dr. Tansey	7/96–6/99	35,640
	Dr. Weinreich	7/97–6/00	36,700
New Zealand Foundation for Research Science and Technology	Dr. Packer	12/97–11/99	59,940
Pew Charitable Trust	Dr. Hannon	7/97–6/01	50,000
	Dr. Hirano	7/96–6/00	50,000
	Dr. Lazebnik	7/95–6/99	50,000
	Dr. Svoboda	7/98–6/02	50,000 *
Rotary Foundation	Dr. Shibahara	9/97–9/98	19,400
Searle Scholars Program	Dr. Grossniklaus	7/98–6/01	60,000 *
Andrew Seligson Memorial Fellowship	Dr. Fearnhead	1/97–12/98	35,000
Mildres Scheel-Stiftung Krebshilfe, The German Cancer Aid	Dr. Schmitt	2/98–2/00	26,000 *
Tularik, Inc.	Fellowships	1/98–12/03	100,000 *
Uehara Memorial Foundation	Dr. Hayashi	4/98–12/98	18,000 *
The V Foundation	Dr. Van Aelst	8/97–7/99	50,000
Helen Hay Whitney Foundation	Dr. Sun	1/97–12/99	30,000
The Wellcome Trust	Dr. Chew	2/98–1/99	15,000 *
	Dr. Connolly	6/97–5/99	10,990
Henry Wendt	Dr. Nakaya	1998	56,450
	Dr. Wang		
Training Support			
Howard Hughes Medical Institute	Graduate Student Support	1994–1999	28,000

*New Grants Awarded in 1998
+Includes Direct and Indirect Cost

Grantor	Program/Principal Investigator	Duration of Grant	1998 Funding+
Course Support			
Applied Spectral Imaging	Advanced Molecular Cytogenics	1998	10,000 *
Chroma Technology Corporation	Advanced Molecular Cytogenics	1998	5,000 *
Cyberlab, Inc.	Crystallography Workshop	1998	1,000 *
Grass Foundation	Scholarships	5/94–4/99	15,000
Hampton Research Corporation	Crystallography Workshop	1998	500 *
Hoffmann-La Roche Inc.	Crystallography Workshop	1998	400 *
Howard Hughes Medical Institute	Advanced Neurobiology Courses	1991–1999	275,000
Esther A. and Joseph Klingenstein Fund, Inc.	Advanced Neurobiology Courses	3/98–2/99	60,000 *
Labscientific, Inc.	Crystallography Workshop	1998	250 *
Leica Imaging Systems, Inc.	Advanced Molecular Cytogenics	1998	5,000 *
Molecular Structure Corporation	Crystallography Workshop	1998	1,000 *
Nonius	Crystallography Workshop	1998	500 *
Protein Solutions, Inc.	Crystallography Workshop	1998	500 *
Vysis	Advanced Molecular Cytogenics	1998	5,000 *
Carl Zeiss	Advanced Molecular Cytogenics	1998	5,000 *
Meeting Support			
Affinity Bioreagents, Inc.	Heat Shock	1998	1,000 *
Alhzeimer's Association	Heat Shock	1998	2,500 *
Berlex Laboratories	Vector Targeting Strategies for Therapeutic Gene Delivery	1998	1,000 *
Bristol-Myers Squibb Co.	Heat Shock	1998	500 *
Cereon Genomics	The *Arabidopsis* Genome: From Model to Crops	1998	1,000 *
Cystic Fibrosis Foundation	Heat Shock	1998	2,000 *
DeKalb Genetics Corporation	The *Arabidopsis* Genome: From Model to Crops	1998	1,500 *
GenMedicine, Inc.	Vector Targetting Strategies for Therapeutic Gene Delivery	1998	2,000 *
Gen Vec, Inc.	Vector Targeting Strategies for Therapeutic Gene Delivery	1998	1,000 *
Genzyme	Vector Targeting Strategies for Therapeutic Gene Delivery	1998	1,000 *
Glaxo Wellcome Inc.	Vector Targeting Strategies for Therapeutic Gene Delivery	1998	500 *
Irvine Scientific	Translational Control	1998	3,500 *
Johnson & Johnson Corporation	Vector Targeting Strategies for Therapeutic Gene Delivery	1998	500 *
Key Gene N.V.	The *Arabidopsis* Genome: From Model to Crops	1998	2,000 *
Lalor Foundation	Gametogenesis	1998	5,000 *
Leica Imaging Systems, Inc.	Zebrafish	1998	1,200 *
Lexicon Genetics, Inc.	Corporate Contribution Program	1998	3,000 *
Lilly Research Laboratories	Vector Targeting Strategies for Therapeutic Gene Delivery	1998	500 *
March of Dimes	Gametogenesis	1998	6,000 *
Marine Biotech, Inc.	Zebrafish	1998	1,000 *
Medical & Biological Laboratories Co., Ltd.	Heat Shock	1998	5,000 *

*New Grants Awarded in 1998
+Includes Direct and Indirect Cost

Grantor	Program/Principal Investigator	Duration of Grant	1998 Funding+
Megabios Corporation	Vector Targeting Strategies for Therapeutic Gene Delivery	1998	1,000 *
Merck Research Laboratories	Heat Shock	1998	2,000 *
Novartis Crop Protection, Inc.	The *Arabidopsis* Genome: From Model to Crops	1998	1,000 *
Pioneer Hi-Bred International, Inc.	The *Arabidopsis* Genome: From Model to Crops	1998	500 *
Promega Corporation	Translational Control	1998	1,000 *
RiboGene, Inc.	Translational Control	1998	10,000 *
Selective Genetics Inc.	Vector Targeting Strategies for Therapeutic Gene Delivery	1998	1,000 *
Systemix	Vector Targeting Strategies for Therapeutic Gene Delivery	1998	2,000 *
StressGen Biotechnologies Corporation	Heat Shock	1998	7,500 *
Transgene S.A.	Vector Targeting Strategies for Therapeutic Gene Delivery	1998	1,000 *
Carl Zeiss	Zebrafish	1998	1,200 *
Zeneca Pharmaceuticals	Vector Targeting Strategies for Therapeutic Gene Delivery	1998	500 *

*New Grants Awarded in 1998
+Includes Direct and Indirect Cost

BANBURY CENTER

Grantor	Program/Principal Investigator	Duration of Grant	1998 Funding+
FEDERAL SUPPORT			
The Federal Judicial Center, Judiciary Leadership Development Council	The Art of Judging: Perspectives of Science	1998	$15,855 *
NIH–National Heart, Lung, and Blood Institute	The Molecular Basis of Asthma: Fundamental Processes with Potential Genetic and Therapeutic Targets	1998	5,842 *
NIH–National Human Genome Research Institute	Large-scale Discovery and Genetic Applications of SNPs	1998	14,240 *
NIH–National Institute of Allergy and Infectious Diseases	Laboratory Methods for the Diagnosis of Lyme Disease	1998	11,393 *
NONFEDERAL SUPPORT			
Meeting Support			
ALS Association	Superoxide Dismutase and Motor Neuron Disease	1998	32,940 *
The William Theodore Denslow Foundation	The Molecular Basis of Asthma: Fundamental Processes with Potential Genetic and Therapeutic Targets	1998	27,331 *
Glaxo Wellcome Inc.	Large-scale Discovery and Genetic Applications of SNPs	1998	23,773 *
The Dorothy Russell Havemeyer Foundation, Inc.	Horse Genomics and the Genetics of Factors Affecting Horse Performance	1998	25,000 *
Immunetics	Laboratory Methods for the Diagnosis of Lyme Disease	1998	5,000 *
The Merck Genome Research Institute, Inc.	Full-length cDNA Cloning: A Workshop on Problems and Solutions	1998	24,861 *
National Marfan Foundation	Critical Issues in Marfan Research	1998	11,563 *
Oxnard Foundation	Muscle Gene Regulation and Its Therapeutic Potential	1998	47,826 *
Private Contributions	The Molecular Basis of Asthma: Fundamental Processes with Potential Genetic and Therapeutic Targets	1998	2,000 *
Research Genetics, Inc.	Full-length cDNA Cloning: A Workshop on Problems and Solutions	1998	2,000 *
Albert B. Sabin Vaccine Institute, Inc. at Georgetown University	From Bench to Bedside: Colloquium on Translational Vaccine Research	1998	13,785 *
Shriners Hospitals for Children	Critical Issues in Marfan Research	1998	5,000 *
SmithKline Beecham Pharmaceuticals	Laboratory Methods for the Diagnosis of Lyme Disease	1998	5,000 *
The Swartz Initiative for Computational Neuroscience	Neurocomputational Strategies: From Synapses to Behavior	1998	40,655 *
Tularik Inc.	The Molecular Basis of Asthma: Fundamental Processes with Potential Genetic and Therapeutic Targets	1998	1,000 *

*New Grants Awarded in 1998
+Includes Direct and Indirect Cost

DNA LEARNING CENTER

Grantor	Program/Principal Investigator	Duration of Grant	1998 Funding+
FEDERAL SUPPORT			
NATIONAL INSTITUTES OF HEALTH National Human Genome Research Institute	Creation of a Digital Archive on the American Eugenics Movement	2/98–1/00	$148,358
NATIONAL SCIENCE FOUNDATION	A Novel Mechanism for Introducing Human Genome Research in Freshman Biology Classes, Mark Bloom	4/95–4/98	16,245
	A Partnership to Develop Advanced Technology Units on Genomic Biology	8/97–7/00	138,856
DEPARTMENT OF ENERGY	The Science and Issues of Human DNA Polymorphisms: An ELSI Training Program for High School Biology Teachers	1/97–1/00	91,073
NONFEDERAL GRANTS			
Hearst Foundation	Genetics as a Model for Whole Learning	7/98-6/99	22,234
Howard Hughes Medical Institute	Precollege Science Education Initiative for Biomedical Research Institutions	7/94-8/99	77,169
Josiah Macy, Jr. Foundation	Gene Almanac	10/97-9/00	264,418
NYS Education Department		1/98-12/98	48,954*

The following schools each awarded a grant for the *Genetics as a Model for Whole Learning Program:*

School	Amount
China Town School District 1	4,950
Community School District 29	18,400
East Meadow Union Free School District	3,150
Elwood Union Free School District	2,725
Friends Academy	8,024
Garden City Public School	5,725
Great Neck Union Free School District	14,515
Green Vale School	3,550
Harborfields Central School District	8,800
Jericho Union Free School District	6,700
Mamaroneck Union Free School District	500
Massapequa Union Free School District	950
Oceanside Union Free School District	1,050
Plainedge Union Free School District	1,075
Port Washington Union Free School District	4,875
Old Westbury School of the Holy Child	3,896
South Huntington Union Free School District	10,150
St. Dominic Elementary School	3,175
Syosset Central School District	17,125

The following schools each awarded a grant for *Curriculum Study* of 1,100:

Commack Union Free School District
East Meadow Union Free School District
Elwood Union Free School District
Garden City Union Free School District
Great Neck Union Free School District
Herricks Union Free School District
Island Trees Union Free School District
South Huntington Union Free School District
Syosset Central School District
West Hempstead Union Free School District
Locust Valley Central School District
Massapequa Union Free School District
North Shore Central School District
Oyster Bay-East Norwich Central School District
Plainedge Union Free School District
Portledge School
Port Washington Union Free School District
Roslyn Union Free School District
Sachem Central School District

of 1,500:

Green Vale School
Hicksville Union Free School District
Plainview-Old Bethpage Central School District

*New Grants Awarded in 1998
+Includes Direct and Indirect Cost

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 (44.1%) of our annual support is 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, 1998–December 31, 1998

Contributions of $5,000 and above, exclusive of Annual Fund

In 1998, the Laboratory received significant support in the form of capital and program contributions from individuals, foundations, and corporations.

Rita Allen Foundation, Inc.
Banbury Fund
The Breast Cancer Research Foundation
Gladys Brooks Foundation
Burroughs Wellcome Fund
The Eppley Foundation for Research
Goldring Family Foundation
The Grass Foundation
Irving A. Hansen Memorial Foundation
The John A. Hartford Foundation
Lita Annenberg Hazen Charitable Trust
The Helen Hoffritz Foundation
Huntington Breast Cancer Action Coalition
Sidney Kimmel Foundation for Cancer Research
The Esther A. and Joseph Klingenstein Fund, Inc.
Charles Henry Leach II Foundation
Marjorie A. and William L. Matheson Charitable Trust
The Maxfield Foundation
The Merck Genome Research Institute
Estate of Vernon Merrill
Offin Charitable Trust
1 in 9: The Long Island Breast Cancer Action Coalition
The Perkin Fund
The Pew Charitable Trusts
The William E. and Maude S. Pritchard Charitable Trust
Michael L. Rankowitz and Sheila Heffron
John R. Reese
Roche Discovery Welwyn
Mr. and Mrs. Alan Seligson
The Seraph Foundation
St. Giles Foundation
The Stone Foundation, Inc.
The V Foundation
Westvaco
The Whitaker Foundation
Helen Hay Whitney Foundation

Total **$2,881,806**

NANCY AND EDWIN MARKS IMAGING CENTER CAPITAL CAMPAIGN

January 1, 1998–December 31, 1998

Contributions of $20,000 and above, exclusive of Annual Fund

The Nancy and Edwin Marks Advanced Neuroscience Imaging Center will employ a revolutionary, new imaging technique called two-photon laser optical scanning that uses tremendous pulses of light to image individual brain cells and networks of such cells in the intact brains of living animals. In 1998, the Laboratory received significant support from individuals, trusts, foundations, and corporations.

Arrow Electronics
Booth Ferris Foundation
Fairchild Martindale Foundation
David H. Koch Charitable Foundation
Mary D. Lindsay
Marks Family Foundation
The William E. and Maude S. Pritchard Charitable Trust
Estate of Sophie Rubinfeld
Mary G. Turner
The Weezie Foundation

Total **$2,816,410**

THE SCHOOL OF BIOLOGICAL SCIENCES CAPITAL CAMPAIGN

January 1, 1998–December 31, 1998

Contributions of $50,000 and above, exclusive of Annual Fund

The Watson School of Biological Sciences at Cold Spring Harbor Laboratory has been established for the purpose of granting the Ph.D. degree. Students who complete this intensive, accelerated, 4 to 4.5 year doctoral degree will have a solid foundation of basic biological processes and disease mechanisms to use as they enter the highly competitive research field, academia, or corporate realm. In 1998, the Laboratory received significant support from individuals, foundations, and trusts.

Dean's Chair	The Lita Annenberg Foundation Lita Annenberg Hazen Charitable Trust
Fellowships	William Stamps Farish Fund Mr. and Mrs. David L. Luke III Mr. and Mrs. David H. Koch Mrs. and Mrs. William R. Miller Mr. and Mrs. Leslie C. Quick, Jr.
Founders Endowment	Anonymous (provides five fellowships)
Knight House	Dr. and Mrs. Mark A. Ptashne
Graduate Residence	The Seraph Foundation

Total $11,954,000

ANNUAL CONTRIBUTIONS

Corporate Sponsor Program

The Cold Spring Harbor Laboratory Corporate Sponsor Program continues to make a very substantial contribution to the meetings programs in Grace Auditorium on the main campus and at Banbury Center. This funding enables us to bring specially invited speakers to the meetings; to help underwrite the expenses of young scientists attending meetings; and to ensure that the meetings at Banbury Center are on cutting-edge research. More than 6000 scientists attended meetings at Cold Spring Harbor Laboratory and Banbury Center in 1998. Full details of these meetings are found elsewhere in this Annual Report.

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. Three of these scientists 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 & Development, Learning & Memory*, and *Genome Research*. Special meetings held in Grace Auditorium in the Spring and Fall are also available to Corporate Sponsor Program members.

In addition, we acknowledge our Sponsors in all relevant publications, including the abstract books given to every participant. The names of the sponsoring companies are listed on the poster describing each meeting. This is mailed to approximately 17,000 scientists throughout the world. There were 37 members of the Program in 1998, including virtually all of the pharmaceutical companies and major players in biotechnology. We welcomed our first Foundation contributor, Albert B. Sabin Vaccine Institute, Inc., at Georgetown University. We are grateful to all members of the Program for their generosity.

COLD SPRING HARBOR LABORATORY CORPORATE SPONSORS

Alafi Capital Company
Amgen Inc.
BASF Bioresearch Corporation
Bayer Corporation
Bristol-Myers Squibb Company
Chiron Corporation
Chugai Research Institute for Molecular Medicine, Inc.
Diagnostic Products Corporation
The DuPont Merck Pharmaceuticals Company
Forest Laboratories, Inc.
Genentech, Inc.
Genetics Institute
Glaxo Wellcome Inc.
Hoechst Marion Roussel
Hoffmann-La Roche Inc.
Johnson & Johnson
Kyowa Hakko Kogyo Co., Ltd.
Eli Lilly and Company
Merck Research Laboratories
Novartis Pharma Research
Novo Nordisk Biotech, Inc./ZymoGenetics, Inc.
OSI Pharmaceuticals, Inc.
Pall Corporation
Parke-Davis Pharmaceutical Research
PE Biosystems
Pfizer Inc.
Pharmacia & Upjohn, Inc.
Research Genetics, Inc.
Schering-Plough Corporation
SmithKline Beecham Pharmaceuticals
Wyeth-Ayerst Research
Zeneca Group PLC

COLD SPRING HARBOR LABORATORY PLANT CORPORATE ASSOCIATES

American Cyanamid Company
Monsanto Company
Pioneer Hi-Bred International, Inc.
Westvaco Corporation

FOUNDATIONS

Albert B. Sabin Vaccine Institute, Inc., at Georgetown University

Total	**$858,000**

DNALC Corporate Advisory Board Annual Fund

An important objective of the Corporate Advisory Board is to provide a sustainable level of annual funding for the DNA Learning Center's programs and to increase visibility in the local business community. As a means of reaching this objective, the Board conducts an Annual Fund and an Annual Golf Tournament with proceeds benefiting the DNA Learning Center.

Chairman: John J. Leahy, Citibank
Vice Chairman: Gary E. Frashier, OSI Pharmaceuticals, Inc.

Michael Aboff, Aboff's Inc.
Andrew D. Ackerman, Chase Manhattan Bank
Rocco S. Barrese, Esq., Dilworth & Barrese
Howard M. Blankman, The Blankman Group
Jane S. Block, Pall Corporation
Thomas J. Calabrese
Edward Chernoff, Motors & Armatures, Inc.
Paul Czeladnicki, Rivkin, Radler & Kremer
Robert Diller, Brinkmann Instruments
Candido E. Fuentes, M.D.
Arthur D. Herman, Herman Development Corp.
Richard Kalenka, PricewaterhouseCoopers
John Kean III, Kean Development Company
Laurie J. Landeau, V.M.D.
Ralf Lange, Luitpold Pharmaceuticals, Inc.
Lilo Leeds, CMP Publications
James Mattutat, Shore Pharmaceutical Providers, Inc.
Patricia Petersen, Daniel Gale Agency
William Porter, U.S. Trust Company
William D. Roche, Republic New York Securities Corp.
Wendy Vander Poel Russell, CSHL Honorary Trustee
Horst Saalbach, Festo Corporation
Peter G. Schiff, Northwood Ventures LLC
Charles R. Schueler, Cablevision Systems Corp.
James D. Shaw, Newsday
Paul A. Vermylen, Jr., Meenan Oil Co., LLP
Lawrence J. Waldman, KPMG Peat Marwick LLP
Raymond A. Walters, Ph.D. CSH Central School District

ANNUAL FUND CONTRIBUTORS

CORPORATE BENEFACTORS
Contributions of $10,000 or more

Luitpold Pharmaceuticals, Inc.
Edward A. Chernoff, Motors & Armatures, Inc.
Pall Family Fund

CORPORATE PATRONS
Contributions of $5,000 or more

Laurie J. Landeau, V.M.D.
Newsday
Pall Corporation

CORPORATE ASSOCIATES
Contributions of $2,500 or more

Anonymous
Citibank
Louis and Virginia Clemente Foundation, Inc.
KPMG Peat Marwick LLP
Meenan Oil Co., LP
OSI Pharmaceuticals, Inc.

CORPORATE MEMBERS
Contributions of $1,000 or more

Aboff's Inc.
Adchem Corporation
The Blankman Group
Brinkmann Instruments, Inc.
Daniel Gale Agency
Dilworth & Barrese
Festo Corporation
Harweb Foundation
Herman Development Corp.
Gerard and Lilo Leeds
John and Katherine Naudin Foundation
Rivkin, Radler & Kremer
William D. Roche
The Schiff Foundation
Donald J. Sutherland
Robert and Patrick Wong

FRIENDS OF THE DNALC
Contributions of $50 or more

American Biogenetic Sciences, Inc.
John W. Andrews
Anonymous
Charles A. and Elizabeth M. Beach
Carl J. Becker
George A. Bourne
Rosemary Bourne
Thomas and Marilyn Chengot
J. and L. Conenello
Mr. and Mrs. Gerald R. Davino
Gerard and Ann E. DeGregoris, Jr.
John DeLalio
Marilyn DeLalio
Dr. and Mrs. Steven Eisner
Mr. Robert and Dr. Cynthia Hoey
Beatrice W. Ingham
J. M. R. Concrete Corp.
Dr. and Mrs. Alexander Kofinas
Mr. and Mrs. Joseph Leondis
Richard and Anita MacDougall
James E. Mattutat
Joseph P. O'Brien Agency
John G. Peterkin
Joseph C. Pickard
George A. Roche
John G. Roche
Roslyn Eye Centre
Wendy Vander Poel Russell
Alan Shamoon
Tom Singh
Lydia T. Smith
Jeff Stern
Paige C. Sutherland
Dr. and Mrs. S.V. Tanguturi
Thomson Industries, Inc.
M. & G. Tumminio
Mr. and Mrs. Joseph C. Viviani
Raymond A. Walters, Ph.D.
Windemere Development Corporation
Zurich Centre Group

OTHER CONTRIBUTORS

James P. Canner
Mr. and Mrs. C. Eisler
Philip Lichtenstein
Mr. and Mrs. Anthony Sabino

MATCHING GIFT CONTRIBUTIONS

Republic New York Securities
Zurich Centre Group

Total	**$93,525**

FIFTH ANNUAL GOLF TOURNAMENT SPONSORS

Golf Tournament Committee

Tournament Chairman
John C. Kean, Kean Development Company

Vice Chairmen
Vincent L. Adimando, Badge Insurance Agency
Howard M. Blankman, Blankman Cunningham Group
G. Morgan Browne, Cold Spring Harbor Laboratory
Richard A. Catalano, KPMG Peat Marwick, LLP
Edward A. Chernoff, Motors & Armatures, Inc.
Frank J. Ciardullo, Ciardullo Landscape and Designs, Inc.
Paul Czeladnicki, Rivkin, Radler & Kremer
Steven Florio, Condé Nast Publications
Arthur D. Herman, Herman Development Company
William Keen, Cold Spring Harbor Laboratory
John J. Leahy, Grant Thornton
James S. Margolin, Margolin & Margolin
James E. Mattuat, Omnicare, Inc.
Patricia Petersen, Daniel Gale Agency
Robert Ricciuti, Diamond Mortgage
James Rojas, Diamond Mortgage
John F. Romeo, Price Waterhouse LLP
Horst Saalbach, Festo Corporation

Founding Sponsor
J.P. Morgan and Co. Inc.

Major Corporate Sponsors
Cablevision
Luitpold Pharmaceuticals, Inc.

Corporate Sponsors
Badge Agency, Inc.
Condé Nast Publications
Festo Corporation
Kean Development Company, Inc.
KPMG Peat Marwick LLP
Motors & Armatures, Inc.
Price Waterhouse LLP
The Roslyn Savings Foundation
RMC Industrial Supply

Prize Sponsor
Gracewood Properties

Auction Prize
Lufthansa

Brunch Sponsor
Zeluck Brothers, Inc.

Cocktail Reception Sponsor
Centerbrook Architects and Planner

$30,000 Hole-In-One Sponsor
Badge Insurance Agency

Golf Cart Sponsor
Diamond Mortgage

Driving Range Sponsor
Chase Manhattan Bank

Refreshments Sponsor
Herman Development Company

Putting Green Sponsor
Daniel Gale Real Estate

Gold Sponsors
All-Pro Carpet Co., Inc.
Arthur Andersen LLP
Barnes & Noble Bookstores
Brinkmann Instruments, Inc.
Mr. Thomas Calabrese
Chase Manhattan Bank
Ciardullo Landscapes and Designs, Inc.
Daniel Gale Real Estate
Dilworth & Barrese
Ferran Brothers, Inc.
Grant Thornton
J.P. Morgan & Co. Inc.
Luitpold Pharmaceuticals, Inc.
Northwood Ventures Inc.
OSI Pharmaceuticals, Inc.
Rivkin, Radler & Kremer
Sprint
U.S. Trust Company of N.Y.

Silver Sponsors
ADT Security Services
Brinkmann Instruments, Inc.
J&H Marsh & McLennan, Inc.
Omnicare, Inc.
Sprint PCS
Suffolk Bookstore, Inc.
The Hartford
Village Green Service Center

Bronze Sponsors
Aboff's Inc.
Advantage Title
Bank of Smithtown
Callan, Regenstreich, Koster & Brady
Margolin & Margolin, Esqs.
James Van de Walle

Commemorative Journal Sponsors
Alarm Guard Security Services
Alarm-Tech Security Systems, Inc.
The Albicocco Family
A Partymaker
Berger's Wholesale Meats, Inc.
Blackman-Hicksville Company
D. Reis Furniture
Essig Electrical Services Inc.
Fala Direct Marketing, Inc.
Farrell, Fritz, Caemmerer, Cleary, Barnosky & Armentano
Farmingdale Iron Works, Inc.
Feraco, Inc.
Fisher Scientific
G.A. Feddersen, Inc.
HRH/A.D. Herman Construction Co. Inc.
Huntington Business Products Centre
Jack Berleth & Sons
Jeff's Seafood
J&M Bakery Products
L&N Prochilo, Inc.
La Forge Francaise Ltd.
Lawrence & Lawrence
New York Biotechnology Association, Inc.
Nortech Laboratories
Oyster Bay Appliance World Inc.
Palmetto, Mollo & Company
Paul Rosenvold Inc.
Riverhead Building Supply
Roman Concrete Corp.
Sammis, Smith & Brush
Super Millwork Inc.
Sunrise Medical Laboratories
To-Jo Provisions

Tee/Green Sponsors
Alliant Foodservice, Inc.
Blankman Cunningham Group, LLC
De Lalio Sod Farms, Inc.
Dvirka & Bartilucci
Fala Direct Marketing, Inc.
Stirling Creek Associates

Raffle Prize Contributors
Aboff's Inc.
All-Pro Carpet Co., Inc.
Aromatherapy, Cold Spring Harbor
Arthur D. Herman
Audio Command Systems
Audio Interiors
Mr. Peter Barmonde
Barney's Restaurant
Capriccio's Restaurant
Carl Zeiss Inc.
Cineplex Odeon Cinemas
Clare Rose
Coca Cola Bottling Co.
Crossroads
Custom Shirts by Tarlow
Daniel Gale Real Etate
Distinctive Products Co.
Ecco Environmental
Ecology Tree, Inc.
Enterprise Rent-A-Car
Fala Direct Marketing
Halesite Deli
Arthur D. Herman
Huntington Business Products Centre
Huntington Indoor Tennis
Industrial Art & Sculpture
The Inn on the Harbor
Kean Development Company
Lemongrass
L&L Camera
Main Street Nursery
Mill River Inn
National Arts Club Dining Room
Newsday
Nickels North
107 Ocean Bistro
OC Tanner
Olympus America
Oyster Bay Frame Shop
Piccolo's
Rain Man Irrigation
Rivkin, Radler & Kremer
Scarsella's Florist
Sharp
Sunderland of Scotland
Spinnaker Travel
Texas Instruments
Tiffany & Co.
Trattoria Grasso, Cold Spring Harbor
Tupelo Honey
Winebow Inc., Ltd.
Zip Limousine

Other Contributors
Bumble Ingham-The Worth Collection
CompuMail Business Services
Country Printer
Dvirka & Bartilucci
Mr. Timothy T. Kruszewski, Wallace Business Forms
Parker, Chapin, Flattau & Klimpl
Mr. William D. Roche
Sunrise Medical Laboratories
Tony's Fish & Seafood Corp.
Vittorio Auto Body, Inc.
Roy J. Zuckerberg Family Foundation

Beverage Donations
Arizona Beverage

Gift Bags
Torneau
Price Waterhouse

Total $119,910

Total DNA Learning Center Annual Fund $213,435

Cold Spring Harbor Laboratory Association (CSHLA)

Officers

Vernon L. Merrill, President*
James L. Spingarn, Vice President/Acting President
Cathy Cyphers Soref, Secretary/Treasurer

Directors

W. Dillaway Ayres, Jr.
Nicholas J. Bartilucci
Trudy H. Calabrese
Mary Ann Charlston
David C. Clark
David H. Deming
Joseph T. Donohue
Frances N. Elder
Linda R. Franciscovich
Robert A. Gay
John S. Grace
Antoine C. Kemper, Jr.
E. Coe Kerr
Carol E. Large
Mary D. Lindsay
Gilbert R. Ott, Jr.
Phillip M. Satow
Jordan Saunders
Lisa Schiff
Ann C. Seifert
Cynthia R. Stebbins
Bruce Stillman, Ph.D.
Marjorie M. von Stade
James D. Watson, Ph.D.

President's Report

Succeeding many years of past accomplishments, 1998 was a year of notable achievement for the Association and also a year tinged with great sadness. On July 13, our dynamic and highly esteemed Association president, Vernon Merrill, passed away after a long battle with breast cancer. Her death was both a shock and an inestimable loss. Vernon's short tenure had been one of sparkling vitality—an attribute that those of us who follow her will surely attempt to emulate. Her direction, leadership, wisdom, and warmth will be sorely missed. Shortly after her death, the Association directors decided to make a contribution as a Board to the Laboratory in Vernon Merrill's memory; the funds will be used specifically for breast cancer research.

During the course of 1998, our directors and members helped to create a memorable year. Events, lectures, and fundraising were all very successful. The year started with the Next Generation Initiative lecture. CSHL investigator Nicholas Tonks, Ph.D., spoke on "Drug Tests for Cancer and Diabetes." It was a fascinating and extremely informative talk on the great progress being made in this important field and the considerations that such tests have raised. Afterward, Lola and John Grace hosted a lovely cocktail buffet.

Dr. Judah Folkman at 1998 CSHLA Annual Meeting.

The Association's Annual Meeting held on February 1 began with election of officers and new directors. New directors elected were Joseph Donohue, Franny Elder, Robert Gay, Ann Seifert, and Marjorie von Stade. The following talented, devoted directors retired after years of great contributions to the Association: Holly Brooks, our exceptional past president John P. Cleary, Laurie Nesi, and Gerald D. Olin. Many thanks to them for their fine service.

*deceased July 13, 1998

Co-Chair Bob Gay, CSHLA President Vernon Merrill, and Co-Chair Nancy Gay

After the meeting, Association members were treated to a not to be forgotten lecture by Dr. Judah Folkman, Senior Associate in Surgery and Director of the Surgical Research Laboratory at Children's Hospital and Harvard Medical School in Boston. Dr. Folkman spoke of his twenty-plus years of investigation into anti-angiogenic therapy and his recent success in tumor eradication in mice. His research involves interrupting the growth of tumors by curtailing the blood supply through the revolutionary application of two compounds developed in his laboratory—angiostatin and endostatin. He told the audience that if eventual clinical trials in humans prove to be as successful as his work in mice, then radiation and chemotherapy treatment might eventually become unneeded in the war on cancer. The audience was awestruck, and you could literally have heard a pin drop!

On March 8, we were fortunate to have Dr. Patrick Cunningham, Professor of Animal Genetics of Trinity College, Dublin, give a public lecture on the "Genetics of the Thoroughbred." For those who have wondered about the role of genetics in horse breeding and performance, myself included, this lecture was fascinating. Dr. Cunningham made a complicated subject understandable and enjoyable.

April 18 was the night of the ever-special Blue Hill Troupe. This Gilbert and Sullivan performance was a sell-out smash hit, and the audience thoroughly enjoyed the group's repertoire, which included selections from many operettas. This was Blue Hill Troupe's second performance at the Laboratory, and the event, expertly chaired by Bob and Nancy Gay and their enthusiastic committee, raised a spectacular $23,000. The money provided support for start-up costs of CSHL assistant investigator Bill Tansey, Ph.D., who is doing research into the very important cell division cycle and its role in cancer.

Association director Phillip Satow hosted a very successful reception in New York City on April 22. Both Dr. Watson and Dr. Stillman addressed the attendees.

June brought one of the biggest events of the year, the Dorcas Cummings Memorial Lecture and its attendant dinner parties. This year's lecture on June 7 featured Dr. Ronald Evans of the Salk Institute, who spoke on "The Molecular Biology of Fat: Weighing the Risks." All I know is that it made me feel very guilty. I headed home for the treadmill and a more sensible diet. Actually, the genetic discussion on fat and health was fascinating and very thought-provoking. The lecture was followed by 23 very successful dinner parties for visiting scientists, CSHL staff, and many interested friends. Cynthia Stebbins was the organizer with the adept assistance of the Association's most capable staff. The following individuals deserve our special thanks for hosting dinner parties:

Mr. and Mrs. W. Dillaway Ayres, Jr.
Ms. Mary Lenore Blair
Mr. and Mrs. Thomas J. Calabrese
Mr. and Mrs. John P. Cleary
Mr. and Mrs. Norris Darrell, Jr.
Mr. and Mrs. Joseph T. Donohue
Mr. and Mrs. Duncan Elder
Mr. and Mrs. Robert A. Gay
Mr. and Mrs. John S. Grace
Mr. and Mrs. Antoine C. Kemper, Jr.
Mr. and Mrs. E. Coe Kerr
Mr. and Mrs. Townsend J. Knight
Mr. and Mrs. Robert D. Lindsay
Mr. and Mrs. David L. Luke III
Mr. and Mrs. Robert G. Merrill
Mr. and Mrs. Robert O'Connor
Mr. and Mrs. Gilbert R. Ott, Jr.
Mr. and Mrs. John J. Phelan
Mr. and Mrs. Whitney D. Pidot
Mr. and Mrs. Luis E. Rinaldini
Mrs. William F. Russell
Mr. and Mrs. Douglas S. Soref
Mr. and Mrs. James L. Spingarn
Mr. and Mrs. James F. Stebbins
Mr. and Mrs. John C. Stevenson
Mr. and Mrs. Hugh Wallace
Mr. and Mrs. Robert P. Walton

The annual Volunteer Appreciation Reception held at the Laboratory on September 28 was occasioned by an enthusiastic turnout for a pleasant evening. This event honors all those who volunteer their time and efforts on behalf of CSHLA.

A wonderful time was had by all at Carol and Jim Large's beautiful home for the October 18 Major Donor Cocktail Party. Carol and Jim, thanks again for your always wonderful, and delicious, hospitality.

Last but not least, through the extraordinary dedication and efforts of our board, we have exceeded our 1998 Annual Appeal goal of $675,000 by raising $701,664. The number of 1998 gifts rose to 1032 (from 684 in 1997), and the number of Association members rose to 848 (from 571 in 1997). Wonderful participation! My special thanks and deep appreciation for all the time each Association member contributed. Those great efforts and their generosity made this year's Annual Appeal an unqualified success.

Let us keep up the good work and growth. It can only lead to bigger, more stunning successes and greater support for the marvelous Cold Spring Harbor Laboratory and its dedicated scientists.

February 1999 **James L. Spingarn**

Members of the Cold Spring Harbor Laboratory Association

ANGELS
Contributions of $10,000 or more

The Albert C. Bostwick Foundation
Mr. and Mrs. John P. Cleary
Mr. and Mrs. Roderick H. Cushman
Mr. Robert B. Gardner, Jr.
Mrs. Oliver R. Grace
Mrs. George N. Lindsay
Mr. and Mrs. David L. Luke III
Dr. and Mrs. Walter C. Meier
Mr. Arthur C. Merrill
The Miller Family Foundation
Mr. and Mrs. Douglas P. Morris
Dr. David B. Pall
Quick & Reilly, Inc.
The Dorothy Schiff Foundation
Dr. and Mrs. James D. Watson

BENEFACTORS
Contributions of $5,000 or more

Mr. and Mrs. Roger L. Bahnik
Mr. and Mrs. Harry A. Brooks
Mr. and Mrs. G. Morgan Browne
Mr. and Mrs. Peter B. Cannell
Mr. and Mrs. Norris Darrell, Jr.
The Samuel Freeman Charitable Trust
Mrs. Robin T. Hadley
Mrs. John H. Livingston
Mr. and Mrs. Robert L. Marcus
Samuel I. Newhouse Foundation, Inc.
Mr. and Mrs. Gerald D. Olin
Mr. and Mrs. Edward L. Palmer
Mr. and Mrs. John J. Phelan, Jr.
The Howard Phipps Foundation
Mr. and Mrs. John R. Reese
Mr. and Mrs. Phillip M. Satow
Mr. and Mrs. James L. Spingarn
The Ruth and Oliver Stanton Foundation
Mr. and Mrs. Philip R. von Stade
The Alex J. Weinstein Foundation, Inc.
H. G. Wellington & Co., Inc.

PATRONS
Contributions of $2,500 or more

Mr. and Mrs. W. Dillaway Ayres, Jr.
Mr. and Mrs. Thomas M. Bancroft, Jr.
Mr. and Mrs. Nicholas J. Bartilucci
Cheryl and Rod Brayman
Dr. and Mrs. C. Thomas Caskey
Craigmyle Foundation
The Arthur & Barbara Crocker Charitable Trust
Mr. and Mrs. David H. Deming
Mr. and Mrs. Edward E. Fessenden
Mr. and Mrs. Robert A. Gay
Mr. and Mrs. Edward H. Gerry
Grey Healthcare Group
Mr. and Mrs. Barcley G. Jones
The Ada Howe Kent Foundation
Mr. and Mrs. Townsend J. Knight
Dr. Laurie J. Landeau
Mr. and Mrs. James M. Large, Jr.
Mrs. Cynthia R. Mead
Ruth Nussdorf/Quality King Dist.
Mrs. William H. Osborn
Mrs. Jessie E. Phillips
Mrs. H. Irving Pratt
Mr. and Mrs. Richardson Pratt, Jr.
Mr. and Mrs. Lawrence J. Schecter
Alexandra White Smith
Mr. and Mrs. Douglas S. Soref
Mr. and Mrs. James F. Stebbins
Frederick and Lotte Stern Philanthropic Fund
The Tilles Family Foundation
Mr. and Mrs. Douglas A. Warner III

ASSOCIATES
Contributions of $1,000 or more

Mr. Warren S. Adams II
Mr. and Mrs. Robert F. Albertson
Mrs. Harriet W. Bailey
Mr. Robert H.B. Baldwin
Mrs. Robert H.B. Baldwin
Mr. and Mrs. David L. Banker
Mr. and Mrs. Edmund Bartlett
Mr. and Mrs. Rodney B. Berens
Mr. and Mrs. Allen L. Boorstein
Dr. and Mrs. Hans Bosch
David Botstein and Renee Fitts
Mr. Lester E. Brion, Jr.
Mr. and Mrs. F. Sedgwick Browne
Mrs. Jackson Burke
Centerbrook Architects
Mr. and Mrs. Harry G. Charlston
Mrs. Jane H. Choate
Dr. and Mrs. Bayard D. Clarkson
Mrs. Robert L. Clarkson, Jr.
Mr. James W. Cleary
Mr. and Mrs. John H. Coleman
Cord Meyer Development Company
The Peter O. Crisp Fund
Dr. and Mrs. Burke A. Cunha
Mr. and Mrs. Richard T. Cunniff
Dr. and Mrs. Paul Cushman, Jr.
Mr. and Mrs. George W. Cutting, Jr.
Mr. and Mrs. Donald L. Deming
The Dess Group, Inc.
Dr. Lester Dubnick
Mr. and Mrs. William T. Dunn, Jr.
Duplex Electrical Supply Corp.
Mr. and Mrs. James A. Eisenman
Mr. and Mrs. Duncan Elder
Mr. and Mrs. William Everdell
Farrell, Fritz, Caemmerer, Cleary, Barnosky & Armentano, P.C.
Mr. and Mrs. E. Maxwell Geddes, Jr.
Mr. Jonathan Gold and Dr. Sari H. Gold
Mr. Peter C. Gould
Mr. and Mrs. John S. Grace
Mr. and Mrs. Oliver R. Grace, Jr.
Mr. and Mrs. Austen T. Gray, Jr.
Mr. Henry U. Harris, Jr.
Mr. and Mrs. Philip R. Herzig
Mr. Lindsay C. Herkness III
Mr. and Mrs. Waldo Hutchins III
Mr. Frederic B. Ingraham
Mr. George E. Isaacs
Walter B. James Fund #2
The Howard Johnson Foundation
Mr. Jeffrey Keller and Ms. Dolores Fredrich
Mr. and Mrs. E. Coe Kerr
Mr. and Mrs. David M. Knott
Mr. and Mrs. Ragnar M. Knutsen
Mr. and Mrs. Gordon E. Lamb
Mr. and Mrs. Edward M. Lamont
Mrs. Lois A. Lenahan
Eugene and Shirley Levitt
Mrs. Henry Lewis III
Dr. Robert V. Lewis
Mr. and Mrs. Robert V. Lindsay
The Fay J. Lindner Foundation
Mrs. Vladimir S. Littauer
Mr. and Mrs. Luke D. Lynch, Jr.
Mr. John P. Maguire
David & Hildegarde Mahoney Foundation
Dr. Thomas Maniatis
Mr. and Mrs. Gerald M. Mayer, Jr.
Mr. and Mrs. John E. Merow
Mr. and Mrs. Robert G. Merrill
Mr. and Mrs. Clarence F. Michalis
Leo Model Foundation, Inc.
Mr. and Mrs. Keith M. Moffat
Mr. and Mrs. Francis W. Murray III
Mr. and Mrs. William J. Nammack
Mr. and Mrs. Edmond J. Nouri
Mrs. Carol R. Noyes
Mr. and Mrs. Michael S. O'Brien
Ms. Nina Ogen
Abby & George O'Neill Trust
Mr. and Mrs. George D. O'Neill
The Anthony & Claire Pace Foundation, Inc.
Mrs. Arthur W. Page, Jr.
Dr. and Mrs. Philip F. Palmedo
Mr. and Mrs. Nicholas B. Paumgarten
Pegalis & Wachsman, PC
Mr. and Mrs. Whitney D. Pidot
Mr. and Mrs. Robert A. Pilkington
Mr. and Mrs. Richard F. Powers III
Mrs. Frances S. Reese
Mr. and Mrs. William S. Robertson
Mr. and Mrs. Arthur M. Rogers, Jr.
Mr. Harold L. Rosenberg and Dr. Laurie Sayet
Mr. and Mrs. J. Wright Rumbough, Jr.
Mrs. Wendy Russell
Mr. and Mrs. Peter G. Schiff
Mr. and Mrs. Thomas Seifert
Mr. and Mrs. Enrique F. Senior
Mr. and Mrs. Owen T. Smith
Mr. and Mrs. Peter O. A. Solbert
Mr. and Mrs. Paul Stallings
Dr. and Mrs. Bruce Stillman
Mrs. Richard Storrs
Mr. and Mrs. Donald Taylor
Mr. and Mrs. Warren I. Titus, Jr.
Town of Brookhaven
Helene P. Victor
WLNY TV55
Mr. and Mrs. Thomas J. Walsh
Townsend Weekes and Diana Collins
Mrs. Taggart Whipple
Mr. Gerald I. White
Mr. Mayer Wolf

FRIENDS
Contributions of $300 or more

Joan and Alan M. Ades
Albrecht, Viggiano, Zureck & Company, P.C.
Mr. and Mrs. Charles O. Ames
Anonymous
Mr. William R. Appleby
James J. Arsenault, Medi Media Inc.
Mrs. Jules W. Aubry
Mr. and Mrs. Edward H. Auchincloss
Mr. and Mrs. Richard Aurelio
Diane and Joseph Azznara
Henry and Erica Babcock
Bancker Construction Corp.
Dr. and Mrs. Henry H. Bard
Dr. and Mrs. Douglas Barkin
Mrs. George DeB. Bell
Dr. and Mrs. Paul Berg
Mr. and Mrs. Rynn Berry
Dr. William Bloom
Mr. and Mrs. William Braden
Miranda Brooks
Buck Consultants, Inc.
Mr. and Mrs. Thomas J. Calabrese, Jr.
Mr. and Mrs. Samuel R. Callaway
Mr. and Mrs. Ward C. Campbell
Mrs. Jean Cattier
Eleanor Chernick
Dr. and Mrs. Joseph B. Conolly, Jr.
Richard L. Cosnotti and The Rev. Sharon C. Bledsoe
Reverend Mario Costa
Mr. and Mrs. Miner D. Crary, Jr.
Mr. Robert L. Cummings III
Mr. Robert L. Cummings
Mr. Theodore N. Danforth
Richard S. Davis
Mr. and Mrs. Daniel P. Davison
Mr. and Mrs. Daniel C. de Roulet
Ms. Lorinda de Roulet
Mr. and Mrs. William Tucker Dean
Elizabeth and Robert Deans
Philip F. DiLeo and Virginia K. DiLeo
James and Sophie Donaldson
Mr. and Mrs. Joseph T. Donohue
Mr. and Mrs. Chuck Dubroff
Mr. and Mrs. Anthony D. Duke, Jr.
Mr. and Mrs. John E. Eckelberry
Julie C. Eichenberger
Mr. Martin Elias
Dr. and Mrs. Jerry L. Ellstein
Stephen Ely
Mr. and Mrs. Stephen C. Eyre
Mr. and Mrs. Joel M. Fairman

Mr. and Mrs. George J. Farrell, Jr.
Dr. and Mrs. Robert James Feeney
Mr. J. Barry Ferguson
Mr. and Mrs. Dugald A. Fletcher
Joan F. French
Mr. and Mrs. Guy A. Fritts
Clarence E. Galston
William C. Gay, Jr.
Joyce M. Goldring
Lola and Allen Goldring
Joseph L. Goldstein, M.D.
Dr. and Mrs. Maurice Green
GreenPoint Financial
Mr. and Mrs. John P. Havens
Mr. and Mrs. Harold W. Hawkey
Mrs. Albert L. Hoffman
Mrs. Bruce Duff Hooton
Mr. and Mrs. James B. Hoover
Mr. John H. Humpstone, Jr.
Thomas Iovino
Mr. and Mrs. John F. Johnston II
Kathleen and Scott Katzman
Mr. and Mrs. John M. Keese IV
Mr. and Mrs. Francis E. Kelly, Jr.
Mr. and Mrs. Antoine C. Kemper, Jr.
Andrea B. and Peter D. Klein
John Klingenstein
Virginia C. Knott
Mr. and Mrs. Michael L. Kolodner
Mort and Deborah Kunstler
Mr. and Mrs. John W. Lapsley
Mr. and Mrs. Peter M. Lee
Mrs. Richard Leeds
Mr. and Mrs. Anthony V. Leness
Mr. and Mrs. Thomas Leon
Liddell, Sapp, Zivley, Hill and LaBoon, L.L.P.
George N. Lindsay, Jr. and Nancy Metz
Mr. and Mrs. Robert D. Lindsay
Mr. and Mrs. Robert V. Lindsay
Muriel and Tom Losee
Mr. Henry Luce III
Mr. and Mrs. Patrick H. Mackay
Mrs. Doris Magowan
Mr. and Mrs. Norton W. Mailman
The Chase Manhattan Foundation
Bart D. Marksohn and Jan M. Lockett
Mr. and Mrs. William H. Mathers
Mr. and Mrs. William Matheson
Mr. and Mrs. Robert P. May
Malcolm Scott McClintock
Mr. and Mrs. Randall P. McIntyre
Mrs. Linda H. McLaughlin
Mr. and Mrs. David B. Miller
Kay Miller
Lindley G. Miller
Mr. and Mrs. Jeffery H. Miller
Mr. and Mrs. Gordon Mills
Mrs. Gloria S. Mooney
Mr. and Mrs. John P. Morgan II
Barbara Mosbacher
Mr. Charles A. Riggs and Ms. Sheila Mahony
Larry Nathanson, M.D.
Peter and Marcy Neumann and Family
Mr. and Mrs. Adams Nickerson
Mr. and Mrs. Richard M. Nicklas
Joseph P. O'Brien
Mr. and Mrs. Denis J. O'Kane
Mr. and Mrs. Peter O'Neill
Innis O'Rourke, Jr.
Gilbert R. Ott, Jr.
Mrs. John H. Page
Mr. and Mrs. Walter H. Page
Mr. and Mrs. Howard Phipps, Jr.
Mr. and Mrs. Sterling Pile, Jr.
Mr. and Mrs. James Pirtle
Mr. and Mrs. Richard C. Pisano, Jr.
Ann and Graham L. Platt
Grant and Christina Porter
J.D. Posillico, Inc.
Mr. and Mrs. Nicholas C. Potter
Barbara S. Powers
Dr. and Mrs. Richard M. Powers
Mr. and Mrs. Clayton A. Prugh
Mr. and Mrs. Richard I. Purnell
Rita and Bill Quinlan
William Rand
Random House, Inc.
Curtis and Louise Read
Mr. and Mrs. Samuel B. Rogers
Charlotte and Bob Rubin
Richard and Pamela Rubinstein
Sabin, Bermant & Gould LLP
Mr. Henry Salzhauer
Patricia P. Sands
Mr. and Mrs. R. Sheldon Scherer
Drs. Joseph and Laura Schor
Bill and Susan Sheeline
Dr. and Mrs. Edward M. Shepard
Mr. and Mrs. Edward Shorin
Dr. and Mrs. Walter Shropshire, Jr.
Mary L. and Robert Sibert
Mr. and Mrs. Richard Simon, Jr.
Mr. and Mrs. Howard Simpson
Dr. Maxine F. Singer
Herbert L. Smith III
Sotheby's
Georgette A. Spingarn
Dr. and Mrs. David Stacy
Mr. and Mrs. Erwin P. Staller
Mr. and Mrs. James F. Stebbins
Stebel & Paseltiner, P.C.
Drs. Joan and Thomas Steitz
Mr. and Mrs. Jeffrey Stevenson
Dr. Charles and Jame Strom
Carol and Jim Swiggett
Jill and Stuart Tane
Mrs. David S. Taylor
Anne M. Thomas
James and Mary Gay Townsend
Dr. and Mrs. Henry J. Travis
Mr. and Mrs. Stanley S. Trotman, Jr.
U. S. Trust Company
Mr. and Mrs. Eugene D. Wadsworth
Mrs. Em Lee Walker
Dr. and Mrs. Peter Walker
Mr. and Mrs. William E. Walsh
Mr. and Mrs. Henry G. Walter, Jr.
Mr. and Mrs. Robert P. Walton
Mr. Charles Wellington
Mr. and Mrs. John E. Welsh III
Mr. and Mrs. Douglas Williams
Ms. Natalie Wolcott Williams
Mr. and Mrs. Rivington R. Winant
Mrs. T. Scudder Winslow
Mr. and Mrs. Samuel Wolcott
Mr. Hughlier Wyatt
Diane and Bernie Yatauro
Diane and Irwin Young
Dr. Takashsi Yura
Dr. and Mrs. Norton D. Zinder

BASIC MEMBERSHIP
Contributions of $50 or more

Mr. and Mrs. Thomas H. Ackerman
Adalsan, Inc.
John L. Adams
Mr. and Mrs. Stirling S. Adams
Mr. Frank Adduci
Bruce and Betty Alberts
Dr. and Mrs. Thomas M. Alderson
Mr. and Mrs. Prescott Ammarell
Drs. H. W. and S. R. Andersen
Carl W. and Mary E. Anderson
Mr. and Mrs. J. Bryan Anthony
Mrs. S. Reed Anthony
George D. Baker
Mr. and Mrs. Benjamin H. Balkind
Daniel P. Barbiero
Mrs. Laura H. Barr
Mr. and Mrs. Anthony B. Barton
Mr. and Mrs. Robert E. Benson
Mr. John Berejka and Dr. Diane Moriarty
Dr. Barbara S. Bergman
Dr. Gergory A. Beroza
Mr. and Mrs. Ashok Bhavnani
Sheila M. Bidwell
Mr. and Mrs. William Binnian
Georgina Bissell
Mary Lenore Blair
Betsy and Jonathan Blattmachr
Dr. Gunter Blobel
Mr. and Mrs. Peter H. Blohm
Martin Blumenthal
Dorothy Blumner
Mr. George H. Bostwick, Jr.
Mari Brady
Cathy and Kevin Broderick
Mrs. Catherine D. Brown
Mr. and Mrs. Douglas Brown
Betty A. Brunauer
Mrs. Julian G. Buckley
Mr. and Mrs. Robert Carlson
Mr. and Mrs. Douglas T. Carpenter
Dr. and Mrs. Walter T. Carpenter
Mrs. Clifford M. Carver
Mr. and Mrs. William M. Casler
Wendy and Larry Chamberlin
Mr. and Mrs. Gilbert W. Chapman, Jr.
Chase Bank of Texas, N.A.
The Chase Manhattan Private Bank
Dr. and Mrs. Donald Chiareilo
Mr. and Mrs. William H. Chisholm
Chubb Group of Insurance Companies
Mr. and Mrs. Henry C. Clark
Kathryn J. Clark
Waleta H. Clark
Ira Cohen
Mr. Michael Cohen
Mr. and Mrs. Payson C. Coleman
Mrs. Gertrude Coletti-Perucca
Peter B. Colgrove
Maryda S. Colowick
Mr. Cornelius P. Connors
Mrs. Alan L. Corey, Jr.
Mr. and Mrs. Ronald M. Craigmyle, Jr.
Mr. and Mrs. Peter O. Crisp
Mrs. Howard H. Dana
Daniel Gale Agency, Inc.
Dr. E. William Davis
Mary and Chuck DeBare
Rick and Jenice Delano
Edward F. DelFoe III
Mr. and Mrs. John Deming
Mr. and Mrs. Andrew P. DeNatale
Mark and Lisa DeNatale
Robert E. DeNatale
Constance and William R. Denslow, Jr.
Sydney Hatch Di Villarosa
Mr. and Mrs. Sidney G. Dillon
Robert Ditkoff and Andi Emerson-Ditkoff
Mr. William Doremus
E. Richard Droesch
Don Durgin
Mrs. William S. Eakins
Frederick Eberstadt
Dr. and Mrs. James D. Ebert
Michael and Clarissa Elgarten
Gretchen Elkus
Alexander T. Ercklentz
Dr. and Mrs. N. Dennis Eryou
Mrs. James M. Estabrook
Frances C. Fennebresque
Mr. and Mrs. James C. Ferrer
Annette and Anthony Ferruggio
Mr. and Mrs. Peter L. Fetterolf
Neil Fidel, M.D.
Dr. Noah and Risa Finkel
Mr. and Mrs. William Floyd-Jones, Jr.
Rosemary Foehl
Mr. and Mrs. Henry L. Fox
Freddy and Seth French
Joan F. French
Norman Friedman
Arline B. Frish
Mr. and Mrs. Robert Fusco
Ellen Futter and John Shutkin
Charles R. Gabalis
Mr. and Mrs. Richard Gachot
Stanley C. Gale
Mr. and Mrs. John W. Galston
Mr. and Mrs. M. Dozier Gardner
Helena Gaviola
Mr. and Mrs. Charles S. Gay
Dolores G. Gebhardt
Dr. Martin Gellert
Ms. Jeanne Genovese
Mr. and Mrs. Roger M. Gerber
Mr. and Mrs. Henry A. Gerry
William F. Gerry
Hugh Jay Gershon
Helen and Monte Getler
Dr. and Mrs. Stephen A. Gettinger
Mr. and Mrs. Stephen E. Gilhuley
William and Maxine Glenn
Harold Glick
Dr. and Mrs. Lewis A. Goldfinger
Andrew and Susan Goldstein
Hope T. Goodwin
Mr. and Mrs. Michael M. Gottsegen
Janet and Charles Goulding
Mr. and Mrs. Edward R. Greeff
Mr. and Mrs. Stephen Ridgely Green
Mr. and Mrs. Scott H. Greenfield
Mr. and Mrs. Stanley Greenman
Dr. and Mrs. Raymond B. Grifiths
Ellen Grossman

Frank and Lynn Gundersen
Susan Gunduz, M.D.
John L. Gwydir Company, Inc. Employees
Mr. and Mrs. Michael J. Hambrook
Clifford and Sherrie Hammond
Virginia H. Hanson
Louisa Hargrave
Mr. and Mrs. Gordon S. Hargraves
Matthew N. Harris
Dr. and Mrs. Chester Hartenstein
Mr. and Mrs. Charles L. Hatton
Thomas S. and Mary Jo Heckman
Mr. Huyler C. Held
Debbie and Michael Held
Mr. Anthony S. Hendrick
Jean Hermann
Larry Hertz
Catherine G. Heyes
Mr. and Mrs. Charles A. Hickmann
Mr. and Mrs. Lawrence P. Hilbert
Tom and Judy Hogan
Mr. and Mrs. Charles R. Holcomb
Irving and Edna Holtzman
Mary Jo and George Hossfeld
Mr. and Mrs. George H. Howard III
George H. Howard
Dr. Shiu-Lok Hu
Charles and Elizabeth Hubbard
Hubbinette-Cowell Associates, Inc.
Philip and Gretchen Hull
Mr. and Mrs. Rudolf Hunermund
Frank and Patricia Huntington
Mrs. Eugene E. Husting
Robert Hutchings
Beatrice W. Ingham
Mr. and Mrs. Herbert S. Ingham
Dr. and Mrs. John R. Inglis
Mrs. Laura Ingraham
Institute of Ecosystem Studies
Fred and Janet Irwin
Mr. and Mrs. Howard S. Jacobs
Mr. and Mrs. Valdemar F. Jacobsen
Mr. and Mrs. Raymond A. Jansen
Mr. and Mrs. Allen D. Jebsen
Mr. and Mrs. J. William Johnson
Ruth B. Jordan
Mrs. Gilbert W. Kahn
Howard and Sandra Kane
Ms. Bonnie J. Kansler
Yoshiro Kawano
Mrs. Norman Keith
Mr. and Mrs. Spencer Kellogg II
Thomas J. and Joan T. Kelly
Mr. and Mrs. A. Claude Kemper
Arnold Keppler
Jane and Jim Keresey
Doris Ketcham
Heather M. Kirby
Mary and Gordon Kirwan
Ricahrd Kleinknecht
Richard and Constance Knies
Angela Koenig
Peter and Margot Kohler
Peggy Lehman Korn
Edward Kramer
Mr. and Mrs. Z. Jerry Kuliga
Mrs. Mitsuko T. Kurahara
William and Evelyn Kurtz
Liz and Mark Ladner
David Lamb
Betty Dods Lasek
Mr. and Mrs. Charles Lassen
Ms. Sue Lathrop
Lois K. Learned
Lorraine P. Lee
Isabel and Jack Leib
Mr. and Mrs. Daniel S. Leon
Mr. and Mrs. William LeSuer
Robert V. Lewis, M.D.
Mr. and Mrs. Thomas K. Lieber
Dr. Irene Llovera and Mr. Candido Llovera
Brian and Ann Lonegan
Alan J. and Joan Long
Long Island Old Car Club, Inc.
L.I. Region Horseless Carriage Club
M.M.G. Agency Inc.
Ralph L. MacDonald, Jr.
Richard and Anita MacDougall
Mr. Wilfred G. Mackey
Mrs. Henry R. Macy
Mary Louise Magee
Mr. and Mrs. Scott Marcus
Paula Marglin
Mrs. Lawrence M. Marks
Mildred Marmur
Mr. and Mrs. Henry C. Marshall, Jr.
Ernst Mayr
Mr. and Mrs. Gregory McMahon
Charles McCurdy and Lisa Maddox
Mrs. Martha McDuffie
Diane McGaw
D. McIlvaine
Robert M. McLane
Jeanne L. McRoberts
Mrs. Bayard H. Megear
Ronald Menzin, M.D.
Carolyn and Norman Mernoff
Elizabeth Meyer
Richard W. Meyer
Mr. and Mrs. William A. Milanese
William H. Miller
Ms. Mary Misiorek
Carolyn R. Moomaw
Marjorie K. Moore
Richard Moore
Claire and Joe Moosbrugger
Mr. and Mrs. John W. Morrison
Gisela Mosig
Mr. and Mrs. Brent M. Mowery, Jr.
The Mobile Press Register, Inc.
Theresa Mall Mullarkey
Mr. and Mrs. G. Robert Muller
Mrs. Alfred E. Munier
Mrs. George Murnane, Jr.
Mr. and Mrs. Yale Murov
Mr. and Mrs. Vincent R. Nesi
Mr. and Mrs. Barry P. Newburger
Benny K.C. Ng, M.D.
Mrs. William S. Niven
Ann L. Nolte
North Country Garden Club
North Side Sunshine Fund
Mr. and Mrs. David C. Noyes
John and Theresa O'Brien
Mr. and Mrs. Matthew M. O'Connell
Robert and Noelle O'Connor
Mr. and Mrs. Frank J. Olt, Jr.
Martha M. Osterhus
Samuel D. Parkinson
Mr. and Mrs. Scott L. Paterson
Paul and Cecily Pennoyer
Mr. and Mrs. John R. Pflug
J. Gardiner Pieper
Judge and Mrs. Thomas C. Platt
Sarah Plimpton
Mr. and Mrs. Martin M. Pollak
Mr. and Mrs. Edward Everett Post
Mr. and Mrs. R. Scott Powers
Mr. and Mrs. Michael S. Press
Mr. and Mrs. Millard B. Prisant
Sherry T. Pryor
Mr. and Mrs. Preston V. Pumphrey
Mr. and Mrs. John J. Randall IV
Edward R. Raskin, D.D.S.
Mr. and Mrs. Daren A. Rathkopf
Miss Sandra Read
Jacqueline and Mark Rechler
Geraldyn L. Redmond
Mr. Arthur G. Reid
Mr. Cornelius J. Reid, Jr.
Robert J. Reidy
Mrs. Ewing W. Reilley
Mr. and Mrs. Charles J. Reina
Dr. and Mrs. Robert M. Reiss
Mr. Bernard J. Reverdin
Mr. and Mrs. James A. Reynolds
The Richard Foundation
Mr. and Mrs. Bernard Richards
Elizabeth L. Ricks
Mr. and Mrs. Joseph M. Rizza, Jr.
Francis and Patricia Roberts
Linda L. Roll
Sarane H. Ross
Harriet and Stanley Roth
Jerry and Brigitte Rounds
Robert and Lois Rubenstein
Cynthia F. Rupp
Leon A. Rushmore
Charlene Russert
Mr. Robert Sabin
Selig D. Sacks
Dr. Marilyn Moffat Salant
Caroline W. Salas
Mrs. Harold P. Salembier
Dr. Kay Hutchins Sato
Phyllis Satz
Paul Scherer & Company LLP
Mr. and Mrs. T.V. Shiavoni
Harvey A. Schneier, M.D.
Mr. and Mrs. Richard R. Schnier, Jr.
Mr. and Mrs. William J. Scholan
Jane R. Schreuder
Carol and Norm Schulman
Melinda Straus Schwartz
Mr. and Mrs. John E. Schwartzberg
Mr. and Mrs. Steven Schwartzreich
Mr. and Mrs. Alfred J. Seaman
Mr. and Mrs. Frederick E. Seymour
Ellen and Dan Shapiro
Gloria and Marvin J. Sharfin
Lisa and David Simek
Hope G. Simpson
Mr. John D. Simpson
William C. Simpson
Bernard and Natalie Sklar
Mr. and Mrs. Alexander B. Slater
Mr. and Mrs. John E. Slater
Mr. and Mrs. Alexander J. Smith
C. Arthur Smith, Jr.
Mr. and Mrs. John C. Smith
Mr. and Mrs. William S. Smoot
Drs. Carl and Madelon Soloway
James and Joan Sommermeyer
Stephen W. Spencer
Spinnaker Travel
John and Margaret Starkes
Mr. Lawrence Starr
Judith Steers
Mr. and Mrs. B. Albert Stern, Jr.
Mr. William M. Stiger
Barbara H. Stout
Laura and Brian Swiggett
Judith and Irwin Tautleff
Missie and Zack Taylor
Mr. and Mrs. John W. Taylor
Mr. Jon L. Ten Haagen
Dudley B. Tenney
The Chase Manhattan Private Bank
The Mobile Press Register, Inc.
The Richard Foundation
Mr. and Mrs. Edward M. Thomas
Mr. George M. Thomas
Charles O. Thompson
Mr. and Mrs. Henry B. Thompson
Mr. and Mrs. William D. Thompson
Three Harbors Garden Club
Stanford W. Ting
Alexander C. Tomlinson
Elizabeth and David Townsend
Mr. and Mrs. Bronson Trevor
Edward D. Truslow
Mrs. Mary G. Turner
Mr. and Mrs. Cornelius M. Ulman
Mr. and Mrs. Stephen van R. Ulman
Mr. Nicholas Valkenburg
Mrs. B.F. VanderPoel
Dr. and Mrs. Thornton Vandersall
Paul and Robin Vermylen
Mr. and Mrs. Niklaus Vetsch
Anna Glen Vietor
Mr. and Mrs. Colton P. Wagner
Mrs. Sherwood Waldron
Mr. and Mrs. Robert Walker
The Hugh Wallace Family
Dr. Heidi Wang
Mr. Jin Wang
Cathie B. Wardell
Charles W.B. Wardell, Jr.
Mrs. David E. Warden
Lewis and Lisa Warren
Mr. and Mrs. Kenneth W. Washburn
Mr. and Mrs. Richard K. Webel
Mrs. Bradford G. Weekes
Mr. and Mrs. L.B. Wehle, Jr.
Mr. and Mrs. Gary Wehren
Mr. and Mrs. Richard Weir, III
Mr. and Mrs. W. Perry Welch
Edward C.R. Whitcraft
Mrs. John R. Wierdsma
Mrs. John C. Wilmerding
Catherine Bostwick Wilson
Dr. and Mrs. Philip D. Wilson, Jr.
Roy L. Wilson
Eckard Wimmer, Ph.D.
Dr. Jan and Fiona Witkowski
Mr. and Mrs. Frederick C. Witsell, Jr.
Sam and Nora Wolcott
Mr. and Mrs. Bertrand Wolff
Mrs. Ethel M. Woolverton
Mrs. Gordon R. Wright
Dr. Keith R. Yamamoto
Drs. Moshe and Josette Yaniv
YPO ETA Forum Education Fund
Bob and Melissa Zakarian
Ms. Joan C. Zulch

CSHLA CORPORATE AND FOUNDATION MATCHING CONTRIBUTIONS AND OTHER GIFTS

The Bristol-Myers Squibb Foundation, Inc.
W. P. Carey Foundation, Inc.
Citicorp Foundation
Computer Associates International, Inc.
Corning Incorporated Foundation
Florence Corporation
The Fluor Foundation
GE Fund
Greenpoint Financial
The John A. Hartford Foundation, Inc.
Kemper National Insurance Companies
J.P. Morgan Charitable Trust
Morgan Stanley Dean Witter & Co.
Newsweek
Old Westbury Gardens
Oppenheimer Funds, Inc.
The Pechter Foundation
Pfizer Inc.
Phelps Dodge Foundation
The Prudential Foundation Matching Gifts
H. G. Wellington & Co., Inc.
Union Pacific Corporation

CSHLA Annual Fund

CSHLA Membership (848) and Other Support

Angels, Benefactors, Patrons, Associates	$567,191.52
Friends and Basic Members	133,092.00
Other	1,231.25
Total	**$701,514.77**

MEMORIAL AND GIFT IN HONOR CONTRIBUTORS

Adalsan, Inc.
Mr. John L. Adams
Mr. Frank Adduci
Mr. and Mrs. Charles O. Ames
Dr. and Mrs. C. E. Anagnostopoulos
Mr. David T. Andrews
Mrs. S. Reed Anthony
Mr. and Mrs. Edward H. Auchincloss
Mr. George D. Baker
Dr. and Mrs. Henry H. Bard
Mr. and Mrs. Edmund Bartlett
Mr. and Mrs. Rynn Berry
Mrs. Georgina M. Bissell
Dr. Gunter Blobel
Mr. and Mrs. James C. Bostwick
Ms. Mari Brady
Mr. and Mrs. Kevin P. Broderick
Mrs. Helen M. Brooks
Mrs. Anne C. Brown
Mrs. Linda H. Calabrese
Dr. C. Thomas Caskey
Chase Bank of Texas, N.A.
The Chase Manhattan Private Bank
Mrs. Thomas H. Choate
Chubb Group of Insurance Companies
Ms. Kathryn J. Clark
Dr. Bayard D. Clarkson
Mrs. Waleta H. Clark
John P. Cleary, Esq.
Mr. Ira Cohen
Mr. Michael Cohen
Mr. and Mrs. C. Payson Coleman
Mrs. Gertrude Coletti-Perucca
Dr. and Mrs. Joseph Conolly, Jr.
Mrs. Susan L. Cooper
Mrs. Alan L. Corey, Jr.
Mr. and Mrs. Robert M. Craigmyle, Jr.
The Peter Crisp Fund
Mr. Raymond V. Damadian
Mr. Charles A. DeBare
Mrs. Vincent de Roulet
Mrs. Robert B. Deans, Jr.
Mr. and Mrs. Donald L. Deming
Mr. Sydney Hatch Di Villarosa
Mr. Sidney G. Dillon
Mr. and Mrs. Charles F. Dolan
Mr. and Mrs. William T. Dunn, Jr.
Mr. David L. Einsidler
Mr. Stephen Ely
Mr. Alexander T. Ercklentz
Mr. and Mrs. William Everdell
Mr. and Mrs. James C. Ferrer
Mr. Anthony C. Ferruggio
Mr. and Mrs. Dugald A. Fletcher
Ms. Meryl B. Fordin
Mrs. Ann Kerr Franklin
The Samuel Freeman Charitable Trust
Mr. and Mrs. Seth B. French, Jr.
Mr. and Mrs. Raymond French
Mr. Norman Friedman
Mrs. Arline B. Frish
Daniel Gale Agency, Inc.
Mr. and Mrs. M. Dozier Gardner
Ms. Helena Gaviola
Ms. Dolores G. Gebhardt
Mr. Henry A. Gerry
Mrs. Martha Farish Gerry
Mr. and Mrs. Stephen E. Gilhuley
Mr. and Mrs. Allen A. Goldring
Dr. and Mrs. Andrew Goldstein
Mr. Peter C. Gould
Mr. and Mrs. Austen T. Gray, Jr.
Mr. and Mrs. Frank Gundersen
John L. Gwydir Company, Inc. Employees
Mr. Gordon S. Hargraves
Mr. and Mrs. David Harrison
Mr. and Mrs. Thomas S. Heckman
Miss Jean E. Hermann
Mrs. L. Shepley Hermann
Mrs. Frederick L. Heyes
Mrs. Bruce Duff Hooton
Ms. Jean L. Houghton
Mr. and Mrs. Michael J. Hrinyak
Mr. and Mrs. Waldo Hutchins III
The George E. Isaacs Foundation
Ms. Susan B. Jaye
Mr. and Mrs. Allen D. Jebsen
Mr. and Mrs. Francis E. Kelly, Jr.
Kemper National Insurance Companies
Mr. and Mrs. James F. Keresey
Mr. C. Gordon Kirwan
Mr. and Richard A. Knies
Ms. Virginia Commander Knott

John Kuriyan, Ph.D.
Mr. William Kurtz
Mr. Mark P. Ladner
Mrs. Carol E. Large
Mr. Joel Lehman
Mr. and Mrs. John H. Leib
Mrs. Lois A. Lenahan
Liddell, Sapp, Zivley, Hill & LaBoon, L.L.P.
Mrs. Mary D. Lindsay
Long Island Old Car Club
Long Island Region Horseless Carriage Club
M.M.G. Agency Inc.
Mr. Ralph L. MacDonald, Jr.
Mrs. Robert A. Magowan
David & Hildegarde Mahoney Foundation
Mr. and Mrs. William L. Matheson
Mrs. Rachel May
Ms. Elizabeth M. McIntosh
Mr. Robert M. McLane
Mrs. Linda H. McLaughlin
Mrs. Jeanne L. McRoberts
Dr. Lorne Mendell
Mr. Donald G. Meyer
Mr. Richard W. Meyer
Mr. Lindley G. Miller
Mr. William R. Miller
Mr. and Mrs. Gordon Mills
The Mobile Press Register, Inc.
Mr. and Mrs. James D. Mooney, Jr.
Ms. Marjorie K. Moore
Mr. and Mrs. John W. Morrison
Ms. Mary R. Moser
Mr. and Mrs. Francis W. Murray III
Samuel I. Newhouse Foundation, Inc.
Mr. and Mrs. Richard M. Nicklas
North Side Sunshine Fund
Mr. and Mrs. Edmond J. Nouri
Mr. and Mrs. John P. O'Brien
Mr. and Mrs. George D. O'Neill
Mrs. William Osborn
Mr. and Mrs. John B. Pace
Mr. and Mrs. Edward L. Palmer
Mr. John J. Phelan, Jr.
Mr. and Mrs. J. Gardiner Pieper
Mr. and Mrs. Sterling Pile, Jr.
Mr. and Mrs. James J. Pirtle, Jr.
Mr. and Mrs. Grant A. Porter
Mrs. Barbara Powers
Mr. and Mrs. Tom Press
Mrs. Sherry T. Pryor
Dr. Mark Ptashne
Mr. and Mrs. Richard I. Purnell
Mrs. Barbara B. Ramsey
Mr. and Mrs. William Rand
Random House, Inc.
Miss Sandra Read
Mr. John R. Reese
Mr. and Mrs. Cornelius J. Reid, Jr.
Mr. and Mrs. James A. Reynolds
Mr. William S. Robertson
Mrs. Linda L. Roll
Mrs. Sarane H. Ross
Mr. and Mrs. Robert Rubenstein
Ms. Cynthia Foy Rupp
Mrs. Wendy Russell
Mr. and Mrs. Charles Sabin
Mr. Thomas A. Saunders III
Sabin, Bermant & Gould LLP
Paul Scherer & Company LLP
Mr. and Mrs. Norman Schulman
Ms. Melinda Straus Schwartz
Mr. and Mrs. William C. Simpson
Mrs. Hope G. Simpson
Mr. John E. Slater
Mr. and Mrs. Herbert L. Smith III
Ms. Margaret O'Brien Smith
Mrs. Cathy Syphers Soref
Sotheby's
Mr. Stephen W. Spencer
Mr. and Mrs. James L. Spingarn
Mr. John Starkes
Mrs. Cynthia R. Stebbins
Ms. Judith Steers
Joan Steitz, Ph.D.
Mr. and Mrs. Jeffrey T. Stevenson
Dr. Bruce Stillman
Missy and Zach Taylor
Mr. Donald Taylor
Mr. Jon L. Ten Haagen
Mr. and Mrs. Warren I. Titus, Jr.
Mr. and Mrs. Bronson Trevor
U. S. Trust Company
Mr. and Mrs. Colton P. Wagner
Mrs. Sherwood Waldron
The Hugh Wallace Family
Mr. and Mrs. Henry G. Walter, Jr.
Mrs. Cathie B. Wardell
Mr. and Mrs. Kenneth W. Washburn
Dr. James D. Watson
Mrs. Bradford G. Weekes
Mr. and Mrs. Gary Wehren
H. G. Wellington & Co., Inc.
Mr. and Mrs. Caleb Whitaker
Mrs. John R. Wierdsma
Mr. and Mrs. Clarence Wilson
Eckard Wimmer, Ph.D.
Mrs. Eunice Johnson Winslow
Mr. and Mrs. Samuel H. Wolcott III
Mrs. Gordon R. Wright
Mr. and Mrs. John Yaworski
YPO ETA Forum Education Fund

IN MEMORY OF

Barbara Altman
Morris Fensterstock
Dorothy Kornfeld
Helen M. Kraft
William Leibfried
Vernon L. Merrill
George Mutterperl
Martha Papalcure
Helen Roppelt
Joseph Rosenberg
Pearl Sale
Adelaide Simpson
Rose Stetson
Marvin ThomasHower
Robert Wagner

IN HONOR OF

Allen A. Goldring
Ralph Guild
Mr. and Mrs. Frank Hain
Louis Lester
David L. Luke III
Leslie C. Quick, Jr.
James D. Watson

Memorials and In Honor Totals	**$49,421.39**

President's Council

The President's Council was formed five years ago in an effort to bring together a small group of individuals interested in science and the work at Cold Spring Harbor Laboratory. Members of the President's Council contribute $25,000 or more in any given year in support of the research and educational programs of the Laboratory. This funding is critical to the Laboratory's continued initiative to attract top young scientists fresh from their Ph.D. or M.D. studies. These fellowships support new researchers who have already demonstrated a capacity to pursue their own high-level, independent research, rather than assisting in the laboratory of an established scientist.

A major feature of the Council is an annual meeting for this select group of leaders from business, research, and biotechnology. The Council's 1998 meeting, held May 15–16, focused on the topic of human evolution and began with lunch on Friday at the President's House and was followed by an afternoon lecture by Dr. Karel Svoboda on imaging neuronal function on the intact brain. The keynote speaker, Dr. Roger Lewin, a collaborator with Richard Leakey, addressed the issue of what archaeology is able to tell us about evolution. The speakers on Saturday were Dr. Sean Carroll from the University of Wisconsin, Dr. Mark Stoneking from Pennsylvania State University, and Dr. Michael Hammer from the University of Arizona. These presentations evoked interesting insights as well as provocative discussions. The following are members of the 1998 President's Council.

Abraham Appel, Appel Consultants, Inc.
Peter Bloom, General Atlantic Partners, LLC
James T. Conneen, A.T. Hudson & Co.
Theodore N. Danforth, Oxford Bioscience
Michel David-Weill, Lazard Freres & Co.
Stefan Englehorn
Leo A. Guthart, ADEMCO
Charles E. Harris, Harris & Harris Group, Inc.
Walter B. Kissinger, WBK Associates
Thomas J. McGrath, Simpson Thacher & Bartlett
Donald A. Pels, Pelsco, Inc.
George B. Rathmann, ICOS Corporation
Hubert J.P. Schoemaker, Centocor, Inc.
James H. Simons, Renaissance Technologies Corporation
Sigi Ziering, Diagnostic Products Corporation

Total	**$258,750**

The Harbor Society

One of the most personal, and meaningful, ways of saying, "I believe in what the Laboratory does" is to make a Planned Gift. A Planned Gift can take many forms: a bequest in your will, a charitable remainder trust, a gift of real estate, securities, life insurance, retirement plan, or other assets.

The Harbor Society is a distinguished group of individuals, currently numbering 44, who have made Planned Gifts to the Laboratory and have given us permission to list their names. In 1998, several more individuals pledged their support to Cold Spring Harbor Laboratory, either with a new gift or by adding to an existing gift.

CONTRIBUTORS

Anonymous
Jim and Jan Eisenman
Lois Learned
Ed and Betty Palmer
Sam and Ann Parkinson
John and Joyce Phelan
John and Hope Reese

Undergraduate Research Program/Partners for the Future

The Undergraduate Research Program introduces college students to the skills they need to become research scientists. Approximately 20 participants work under the guidance of staff scientists for 10 weeks each summer on projects of mutual interest. The Partners for the Future Program teaches the principles and methods of basic scientific research to six Long Island high school seniors, who work with a scientist 10 hours per week after school, from October through March.

CONTRIBUTORS

Cornelius N. Bliss Memorial Fund
Dorcas Cummings Memorial Lecture
Reverend Linda P. Hancock
Jephson Educational Trust
Joan P. Tilney

ENDOWMENTS

Burroughs Wellcome Fund
Garfield Fellowship Emanuel Ax Fund
H. Bentley Glass Fellowship
Libby Fellowship
Olney Fund
Joan Redmond Read Fund
William Shakespeare Fellowship
Frederica von Stade Fund

Total $113,930

Total All Annual Funds $19,797,596.00

LABORATORY STAFF

James D. Watson, President
Bruce W. Stillman, Director
Winship Herr, Asst. Director/Dean, Watson School of Biological Sciences
G. Morgan Browne, Administrative Director
W. Dillaway Ayres, Jr., Assoc. Administrative Director
John P. Maroney, Asst. Administrative Director
William D. Keen, Comptroller
Terri I. Grodzicker, Asst. 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

Investigators

Hollis Cline
David Helfman
Nouria Hernandez*
Adrian Krainer
Roberto Malinow
Robert Martienssen
David Spector
Nicholas Tonks
Timothy Tully
Michael Wigler

Associate Investigators

Grigori Enikolopov
Bruce Futcher
Michael Hengartner
Tatsuya Hirano
Ryuji Kobayashi
Yuri Lazebnik
Scott Lowe
W. Richard McCombie
Jacek Skowronski
Arne Stenlund
Linda Van Aelst
Yi Zhong

Assistant Investigators

Shivinder Grewal
Ueli Grossniklaus
Masaaki Hamaguchi
Gregory Hannon
David Jackson
Leemor Joshua-Tor
H. Peter Nestler
Andrew Neuwald
Lincoln Stein
Karel Svoboda
William Tansey
Rui Ming Xu
Chi-Ping (Jerry) Yin
Michael Zhang

CSHL Fellow

Marja Timmermans

Research Investigators

Neilay Dedhia
Robert Lucito
Natalia Peunova
Andrew Reiner
Clifford Yen

Adjunct Investigator

David Beach

Adjunct Associate Investigator

Scott Powers

Adjunct Assistant Investigator

Craig Ogata*

Visiting Scientists

Jiaxin An
Shern Chew
Tatyana Michurina
Jyoti Raychaudhuri
Vladimir Scheinker
Imran Siddiqi
Toshiro Tsukamoto

Senior Fellows

Douglas Conklin
Edward Stern

Postdoctoral Fellows

Andres Barria-Roman
Christine Berthier
Guy Birkenmeier
Benjamin Bottner
Katherine Braun
Mary Byrne
Isabel Cantallops
Luca Cartegni
James Chong
John Connolly
Robert Del Vecchio
Serge Desnoyers
James Dezazzo
Dennis Dong
Joseph Donovan
Eric Drier
Joshua Dubnau
Nicholas Edgington
Viola Ellison
Jose Esteban
Howard Fearnhead
Gerardo Ferbeyre
Francisco Ferrezuelo
Robert Filipkowski
Lisa Foa
Andrew Fraser
Anton Gartner
Michael Greenberg
Andrew Groover
Hui-Fu Guo
Kurt Haas
Frances Hannan
Michelle Hastings
Mariko Hayashi
Yasunori Hayashi
Andreas Herbst
Sangeet Honey
Junjiro Horiuchi
Ilya Ioschikhes
Nobuhiro Kashige
Alyson Kass-Eisler
Catherine Kidner
Keiji Kimura
Peter Krasnov
Balazs Lendvai
Wen-Chuan (Athena) Lin
Zhixin Lin
Dehua Liu
Hong-Xiang Liu
Qiong Liu
Ana Losada
Zachary Mainen
Mireya Marin
Juan Mendez
Thomas Misteli
Vivek Mittal*
Michael Murray
Michael Myers
Naoki Nakaya
Esther Nimchinsky
Paul O'Farrell
Michael Packer
Helena Palka
Laurence Parnell
Shannon Pendergrast
Antonella Piccini
Jean-Christophe Poncer
Pablo Rabinowicz
Baskar Ramamurthy
Michael Regulski
Edward Ruthazer
Minoru Saito
Noriko Saitoh
Cyril Sanders
Clemens Schmitt
Arndt Schmitz
Lidia Serina
Andrew (Mark) Settles
Yu Shen
Keiichi Shibahara
Kaetrin Simpson
Wun Chey Sin
Maria Soengas
Mona Spector
Charles Spillane
Yuri Stasiv
Peiqing Sun
Jack Tabaska

*Funding provided by Howard Hughes Medical Institute

Anne Vaahtokari
Daan van Aalten
Daniel Vaughn
Jean-Philippe Vielle-Calzada
Erik Vollbrecht
Jing (Jenny) Wang
Yalin Wang
Michael Weinreich
Shouzhen Xia
Tomoki Yokochi
Shahid Zaman
Lifang Zhang
Shaohui Zhang
Zhiguo Zhang
Xinyang Zhao*
Xuemei Zhao
Jianlong (Jim) Zhou
Jian Zhu
Dong-Jing Zou

Graduate Students

Robert Babb
Kenneth Chang
Grace Chen
Jeffrey Christensen
Elisa de Stanchina
Yi Chieh (Nancy) Du
Lavina Faleiro
Eric Gillitzer
Eve-Ellen Govek
Tina Gumienny
Jill Hemish
Brian Hill
Daniel Hoeppner
Ashkan Javaherian
Jianzhong Jiang
Flavio Kamenetz
So Young Kim
Tracy Kuhlman
Arlinda Lee
So Young Lee
Zheng Li
Miguel Maravall
Michele Mc Donough
Troy Messick
John Mignone
Stuart Milstein
Paul Mintz
James Moore
Shirly Pinto
Julia Polyakova
Alex Rai
Patrick Reilly
Jose Rodriguez*
Ahmed Samatar
Andrew Samuelson
Laura Schramm
Setareh Sepehri
Hang Shi
Songhai Shi
Michael Stebbins
Javor Stolarov
Tomasz (Tomek) Swigut
Marcela Tello-Ruiz
Suzanne Tharin
Jiayuan (James) Tong
Beth Trumbull
Tzu-Ling Tseng
Thomas Tubon
Thomas Volpe
Joseph West
Herman Wijnen
Joanna Wysocka
Xiaohong (Helena) Yang
Ming Ming Zhao
Jun Zhu
Lee Zou

Research Programmers

John Healy
Zhanna Ioschikhes
Aleksandar Poleksic

Research Associates

Carmelita Bautista
Robert Gasperini
Lisa Manche
Linda Rodgers

Lab Facility Managers

Diane Esposito
Timothy Mulligan
Spencer Teplin
Ming Wang
Regina Whitaker

Laboratory Manager

Martha Daddario

Secretaries

Sadie Arana
Patricia Bird
Idee Mallardi
Carol Marcincuk
Barbara Matusik
Lorraine McInerny
Jane Reader
Judith Smith
Madeline Wisnewski

Research Assistants

Robin Holden
Indraneil Mahapatra

Laboratory Technicians

Caroline Alexander
Joan Alexander
Juana Arroyo
Deborah Aufiero
Kimberly Bronson
Anthony Buglino
Barry Burbach
Eileen Cahill
Xiocun (Joan) Chen
Jodi Coblentz
Alison Coluccio
Maureen Cowan
Catherine Cronin
Nancy Dawkins-Pisani
Melissa de la Bastide
Joseph Di Leo
Ping Dong
Margaret Falkowski
Eugenia Friedman
Scott Gossweiler
Kristina Habermann
Michiko Hirano
Tamara Howard
Emily Huang
Wei Jiang
Santosh John
Rachel Jones
Susan Kourpanidis
Robert Krimper
Hui Li
Jianping Liu
Anthony Matero
Mila McCurrach
Robert McGuirk
Joshua Nagel
Lidia Nascimento
Aigoul Nourjanova
Peter O'Brien
Andrew O'Shaughnessy
Ximena Opitz-Araya
Ronnie Packer
Kimberly Pennino
Nora Poppito
Raymond Preston
Lori Pyzocha
Michael Riggs
Milka Rodriguez
Julie Rosenbaum
Simone Salghetti
Kristin Schutz
Lei-Hoon See
Ravi Shah
Monica Shekher
Nadim Shohdy
Joseph Simorowski
Patrick Smith
Lori Spiegel
I'kyori Swaby
Julie Thomas
Jennifer Troge
Philip Vahab
Klara Velinzon
Marie Vil
Kimberly Wanat
Kimberly Weinreich
Patricia Wendel
Jeanne Wiggins
Priscilla Wu
Cristina Yordan
Zhuang Yuan
Li Zhang
Ying Zhang
Hong Zhou

Media Makers/ Laboratory Aides

Esteban Araya
June Blanchford
Sabrina Boettcher
Patricia Brady
Jack Brodsky
Florinda Chaves
Rodney Chisum
Janice Douglas
Salvador Henriquez
Robin Kirchner
Vincent Meschan
Rulan Shen
Margaret Wallace
Xiu-Xia Zhang

Howard Hughes Medical Institute Employees

Support Staff

Beicong Ma
Carol Sekunda
Yuling Sun

DEPARTMENTS

Art

Michael Ockler
James Duffy
Irwin Mendlinger
Philip Renna

Information Services

Gerald Latter
James Bense
Derek Johnson
Ardavan Kanani

*Funding provided by Howard Hughes Medical Institute

Matthew Labush
Robert Pace
Barbara Peters
Salvatore Serafino
John Uhl
Lee Wilbur

CSHL Press

Acquisition

Elizabeth Powers

Books: Editorial

Janet Argentine
Mary Cozza
Judith Cuddihy
Maryliz Dickerson
Joan Ebert
Kaaren Janssen
Inez Sialiano

Journals: Editorial

Laurie Goodman
Margaret Calicchia
Kimberly Gavin
Valerie Pakaluk

Production

Denise Weiss
Patricia Barker
Dorothy Brown
Daniel de Bruin
Nadine Dumser
Barbara Elliott
Cynthia Grimm
Kristin Kraus
Laura Mintz
Susan Schaefer

Fulfillment/Distribution

Guy Keyes
Kathleen Cirone
Gabriella Horvath
Jacqueline Matura
Michael McInerny
Llywelyn Passmore
Sharon Story
Joanne Thomas

Finance

Nancy Hodson

Marketing

Ingrid Benirschke
Cynthia Weidman

Advertising Sales

Marcie Ebenstein

Public Affairs

Deborah Barnes
Margot Bennett
Marlena Emmons
Wendy Goldstein
Lynn Hardin
Laura Hundt

Development

Richard Cosnotti
Gordon Hargraves
Kelly MacKay
Katherine Ruffolo
Julie Sutherland
Kathleen Waisane
Cathie Wardell

Library

Margaret Henderson
Clare Bunce
Ellen de Bruin
Laura Hyman
Leigh Johnson
Wanda Stolen
Claudia Zago

Accounting

Jennifer Blovsky
Guy Cozza
Mary Ellen Goldstein
Elizabeth Janow
Patricia Maroney
Alison McDermott
Carlos Mendez
Mary Ann Miceli
Elizabeth Panagot
Lari Russo
Amy Stiso
Patricia Urena
Noreen Vance
Barbara Wang

Grants

Susan Schultz
Sharon Bense

Wendy Crowley
Maureen Davies
Carol DuPree
Patricia Kurfess
William Mahoney
Dee McKeon

Human Resources

Cheryl Sinclair
Marjorie Berko
Jan Eisenman
Kim Gronachan
Barbara Purcell
Merilyn Simkins
Anastasia Velar-Stephen

Food Service

James Hope
Jaime Bonilla
Francis Bowdren
Guiditta Carino
William Carmona
Michael Corral
Joan Doxey
Julie Ehrlich
Lesley Finnegan
Barbara Gordon
Jennifer Gordon
Oscar Lastra
Jesus Magana
Thomas McIlvaine
Maureen Morrow
Ronald Padden
Jorge Ramirez
Mauricio Rodriguez
Susana Roman
David Sarinick
Cathy Van Deurs
Leslie Wenzel
Clarke Williams
Javier Zepeda

Meetings/Courses

Edward Campodonico
William Dickerson
Donna Dykeman
Michael Glaessgen
Michela McBride
Andrew Mendelsohn
Andrea Newell
James (Herb) Parsons
Mary Smith
Margaret Stellabotte
Andrea Stephenson
Diane Tighe

Nancy Weeks
Barbara Zane

Purchasing

Philip Lembo
Margaret Brock
Carol Brower
Lorraine Byers
Susan De Angelo
Bruce Fahlbusch
Patricia Hinton-Stenko
Christopher Oravitz
Susan Rose
Leonard Sposato

Central Administration

Maureen Berejka
Barry Braunstein
Carol Dempster
Delia King
Roberta Salant

Banbury Center

Katya Davey
Eleanor Sidorenko
Beatrice Toliver

DNA Learning Center

Joan Alexander
Amanda Broege
Scott Bronson
Shirley Chan
Matthew Christensen
Judy Cumella-Korabik
Patricia Harrison
Malissa Hewitt
John Kruper
Susan Lauter
Michele McDonough
Vivek Mittal
Andrew Morotti
Martha Mullally
Janeen Russo
Joel Stern
James Tong
Thomas Volpe
Gisella Walter

Facilities

Arthur Brings
Christopher Hubert
Jack Richards

Peter Stahl
Leslie Allen
Margaret Chellis
Marlene Rubino
Frank Russo
Charles Schneider

Carpentry and Painting

Peter Schwind
Edwin Bennett
Michael Demers
Paul Edwards
John Meyer
Joseph Pirnak
Wilson Ramones
Benjamin Veneable
Harry Wozniak

Custodial/Housekeeping

Steven Tang
Dessie Carter
Ming-Kai Chan
Joseph Houser
Louis Hunter
Susanne MacPherson
Patricia McAdams
Dora Merlino
Guido Mosquera
Maria Mosquera
Mary Muno
Wayne Pav
Ronald Romani
Claudia Schmid
Danuta Slodkowska
Yew Teo
Curtis Williams

Electrical

Louis Jagoda
Jeffrey Goldblum

Environmental Health and Safety

Randal Jones
John Pisciotta

Equipment Design Fabrication and Repair

Cliff Sutkevich
Robert Eifert
George Newell
James Robey

Grounds

Daniel Miller
Drew Comer
Joseph Ellis
Lawrence Giordano
Jeffrey Haefling
Edward Haab
Christopher McEvoy
Jose Perez
Andrew Sauer
Stanley Schwarz
Nelson Zepeda

Harris Research Support Facility

Lisa Bianco
Kelly Brings
Jodi Coblentz
Charlene De Poto
Christine Maurel
Halina Swidzinski
Ryszard Swidzinski

Mechanical Services

Robert Collins
Russell Allen
Greg Beal
William Bishop
Robert Dickerson
Paul Draser
Jeffrey Klaverweiden
Lane Smith

Security

Robert Gensel
Frank Carberry
Randy Wilfong

Shipping and Receiving

Daniel Jusino
Renee LeStrange
Joseph Marcello
Charles Ranaudo

Research Staff Departures during 1998

Faculty

Hong Ma
Akila Mayeda
Elly Nedivi
Alcino Silva
Shou Waga

Visiting Scientists

Yoshitaka Azumi
Eli Hatchwell
Mirjana Maletic-Savatic
Keiko Mizuno
Mariko Nakamura
Zuoping Xie

Postdoctoral Fellows

Marcia Belvin
Anton Bennett
Mark Curtis
Pedro De Campos-Lima
Jeffrey Dickinson
Jianzhong Ding
Andrea Doseff
Ype Elgersma
Nikolai Federov
Paul Frankland
Fei Gao
Andrew Garton
Karl Giese
Cameron Gray
Michael Gutch
Ronald (Bill) Henry
Craig Hinkley
Jeffrey Kogan
Chun Liang
Ruiping Liu
Yu Liu
Martin Lock
Hiroyasu Onaka
Masuo Ono
Indrani Rajan
Brandt Schneider
Gavin Sherlock
Hua Tu
Alain Verreault
Jonathan Wallach
Yixing Wang
Yung-Chih (Judy) Wang
Nicholas Wright
Ming Yang

Graduate Students

Ofelia Carvalho
Degui (Charlie) Chen
Pin (Adele) Chen
Ethan Ford
Richard Freiman
Anthony (John) Iafrate
Kenneth LaMontagne
Debra Morrison
Peter Rubinelli
Meenakshi Selvakumar
Rachel Wallace-Brodeur
Qiang Wu