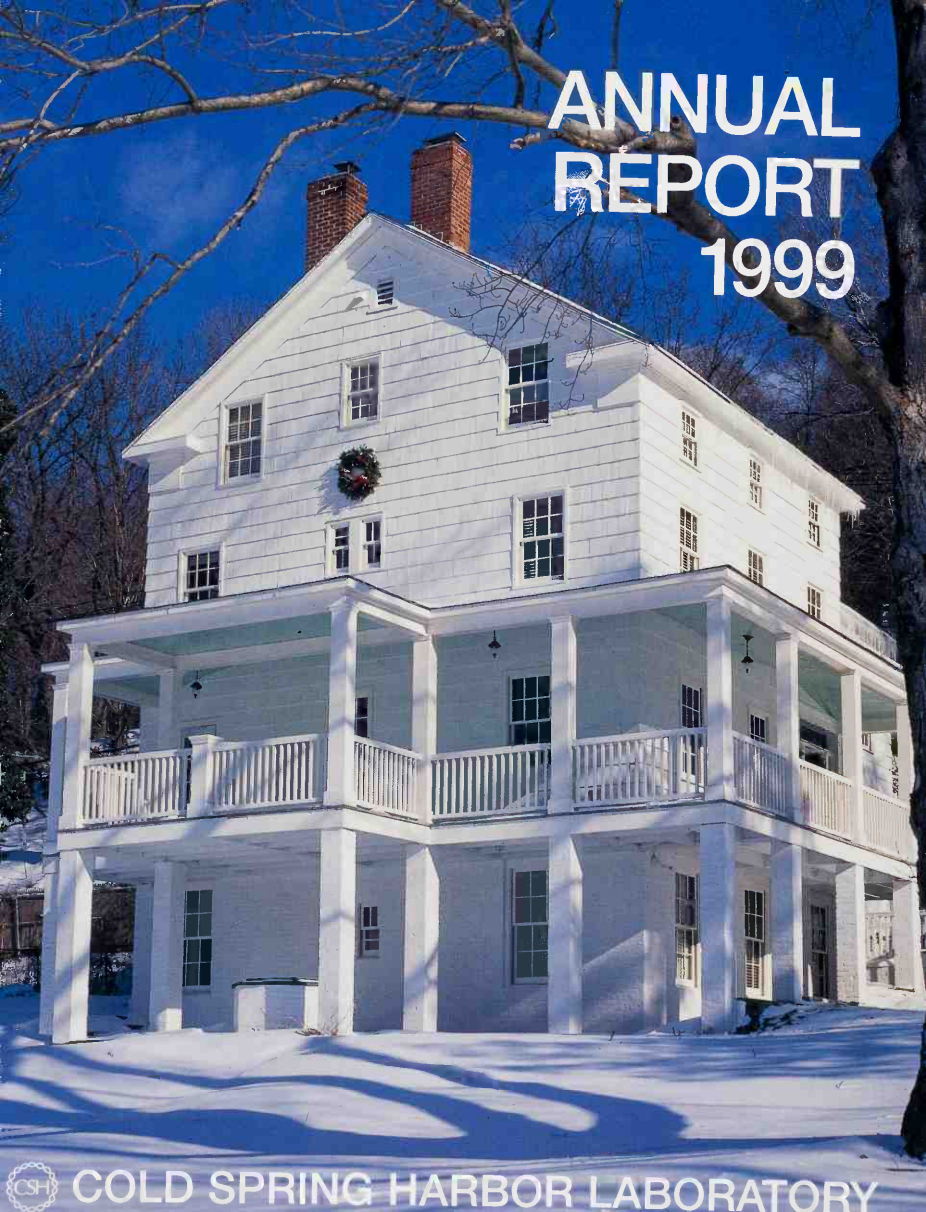

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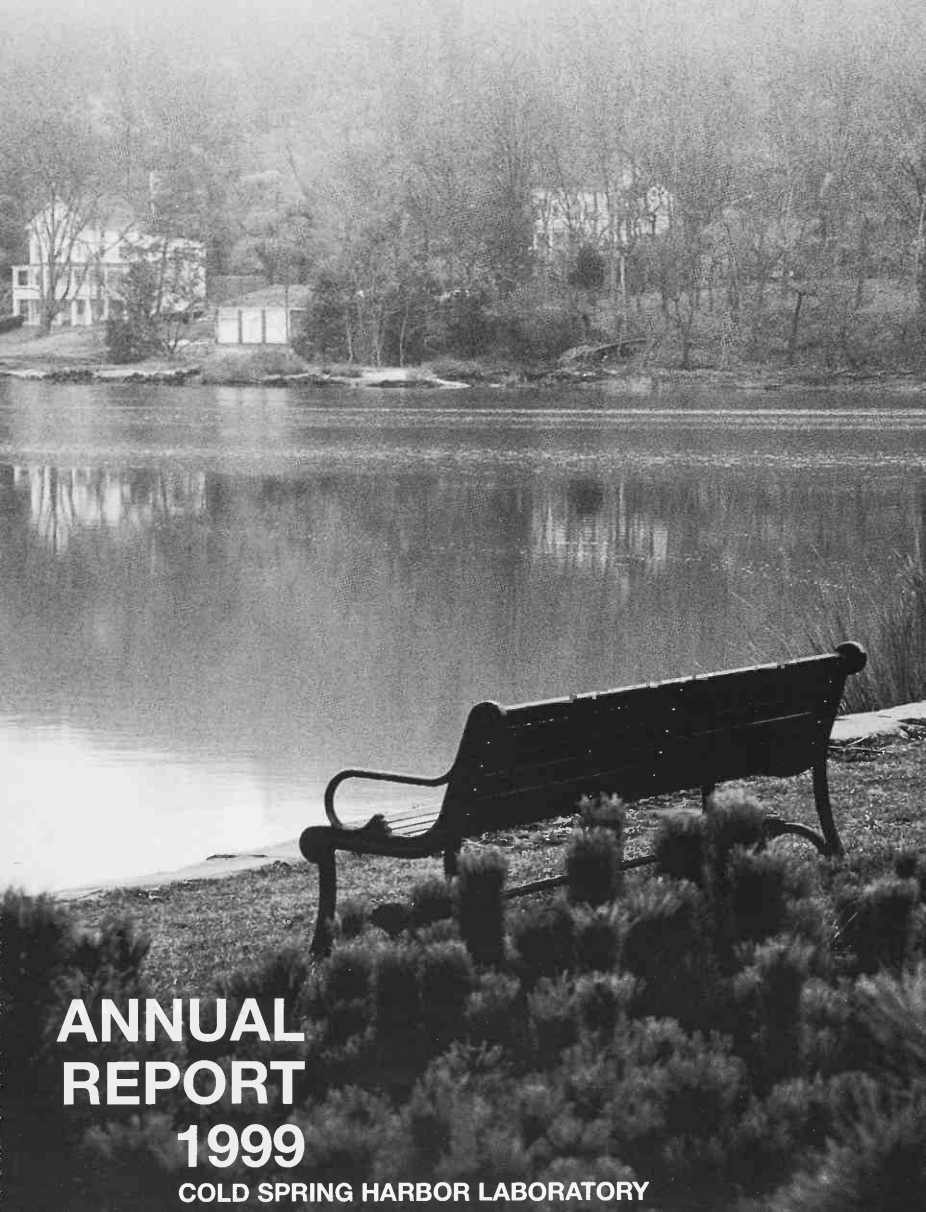
COLD SPRING HARBOR LABORATORY



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Back cover: Nancy and Edwin Marks Laboratory, site of advanced neuroscience imaging at Cold Spring Harbor Laboratory. (Photograph by Miriam Chua.)

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Amyas Ames

Amyas Ames (1906–2000)

Amyas Ames, whose home on gently rolling lands was off Moores Hill Road and who regularly walked over our laboratory's grounds, died January 24, 2000, at the age of 93. He was a member of a distinguished New England family whose name still graces a more than 200-year-old farm-tool-manufacturing company, once called the Ames Shovel Company. It was, however, his family's mid-19th-century investments in railroads, particularly the Union Pacific, that gave his family its great wealth, as exemplified by large country properties at North Easton to the south of Boston and a palatial villa on Lake Como in Italy.

Amyas was an old English version of *Ames* discovered by Amyas's grandmother, whose husband, Oliver Ames, was a governor of Massachusetts in the late 1800s. In turn, that Oliver was the son of Oakes Ames and the nephew of another Oliver Ames, the strong-willed, crafty investor brothers who amassed their family's formidable railroad properties. The name *Oakes* passed on to Amyas's father, who became a distinguished Harvard botanist, directing first its Botanical Garden and later the Botanical Museum and the Arnold Arboretum. Oakes's specialty was orchids, of which he became our country's most renowned expert with his various treatises illustrated by his wife (Amyas's mother), Blanche.

Like his father, Amyas was educated at Harvard. He graduated in 1928 and two years later, married Evelyn Perkins, the daughter of a physics professor at Trinity College in Hartford. Soon after, Amy and Evy moved to New York City, where he rapidly moved up the ranks of the investment banking firm of Kidder Peabody, eventually to be chairman of its Executive Committee as well as twice a governor of the New York Stock Exchange. Before becoming our close neighbors, they first appreciated the beauty of the Cold Spring Harbor community through spending time on the Fort Hill properties of Nan Woods, whose father, William J. Matheson, was our Laboratory's principle patron at the turn of the century. Living there in much more seclusion than Amyas and Evy was Charles Lindbergh, whose enthusiasm for Hitler's pre-War Germany kept him on the sidelines for most of World War II.

The Moores Hill Road property of Amy and Evy was once part of lands along both sides of Stewart Lane belonging to the Laboratory's ill-fated Eugenics Record Station. Milislav Demerec closed down the station as soon as he became our Laboratory's director in 1941. The selling off of its lands—almost 100 acres—began soon thereafter. One of the first buyers was the Cold Spring Harbor School District, which used its purchase as the site for a much-expanded West Side Grammar School. Demerec also bought land, first for use by his sister Vera and later for use by himself, as a site where he could build a house to move into upon his retirement. The monies coming from the sale of the land were later used to help build the greatly needed new research building of the Department of Genetics that was completed in 1953 and now bears Demerec's name. Amy then had just become president of the Long Island Biological Association (LIBA), having become one of its directors two years before. His being also a director of the Carnegie Corporation of New York must have been a plus, since it and the Carnegie Institute of Washington provided the funds needed to build the (now-named) Vannevar Bush Auditorium.

Later, Amy played an important role in the purchase from the irascible Rosalie Jones of 23 acres of her family's land, to the west of Bungtown Road on the way to the sand spit. At that time, this land was not part of the Village of Laurel Hollow and if not purchased by the Laboratory, it could have been the site of dense residential construction. To help finance the purchase, three building plots off Moores Hill were sold—one to Al and Jill Hershey and another to Jim and Jan Eisenman.

Amyas's ever-growing involvement with the New York cultural scene led to his resignation as LIBA president in 1958, and the eight-year-younger Walter Page took up his mantle. By then, Amy was already a director of the New York Philharmonic Orchestra, later becoming its president (1963–1970) and its chairman (1973–1980). He also helped lead Lincoln Center itself as its chairman between 1970 and 1981. Equally important to Amy's life was his identification with environmental causes, helping form in the early 1960s the then Long Island–centered Environmental Defense Fund (EDF) in response to the reputed DDT-induced decline of Long Island's osprey populations. After DDT became banned and the osprey population came back, Amy built a platform-topped pole on his Martha's Vineyard property for a potential osprey nest. It soon found users, and when Liz and I also had a summer home on the Vineyard's Seven Gates Farm, we needed only a five-minute walk to hear the shrill cries coming from the Ames's osprey tenants.

Pesticides were long an anathema to Amy, who led strenuous opposition to our village mayor Bill Smutt's wish to spray the mild pesticide Malathion over our village's trees during an early 1980s' outbreak of the 17-year locusts. The strong feelings then generated by arguments between residents not wanting to see their cherished old oak trees put at risk by a second year of leaf defoliation and those residents not minding the occasional dead tree as long as birds were around to perch on them were not easily forgotten. Many once close friends no longer wanted to speak to each other. Trying to defuse the tension, the Laboratory held an evening meeting so our neighbors could hear scientists on both sides discuss the issues. After it was over, however, neither side saw any reason to move closer to the other's view. Though I was once solidly on the environmentalist side, I was then badly bothered by the EDF seeing recombinant DNA as much its enemy as pesticides. Though I soon warned Amy that his organization's ranting against DNA risked alienating its backing by serious scientists, he saw its good features more important than its possible bad judgment about DNA. In contrast, I saw its well-intentioned trustees, badly misled by staff, creating more of their kind through the pandering of irresponsible scare stories. If what the EDF said about DNA was nonsense, should I trust its views on pesticides?

But even when I so strongly disagreed with Amy, it was impossible not to continue to like and admire him. There was no one in our community more committed to our purposes of education and research or who more enjoyed walking through our woods and telling us how much he valued our existence. To him Cold Spring Harbor, unlike, say, Greenwich across the Sound, would always be much more than a commuter's site for sleeping, where residents were always more involved with where they worked than where they lived. To Amy, it was the mind as exemplified by the achievements of science and the arts that reflected human beings at their best. In working so long and effectively on behalf of Cold Spring Harbor and the cultural scene of New York City, Amy never doubted that benefits would flow out to all the inhabitants of our globe.

Noblesse oblige was never better represented in our times.

James D. Watson

Jack Richards (1925–2000)

Jack Richards, a master contractor whose keen sense of how a good building should be put together underpins the whole fabric of research and education at Cold Spring Harbor, died on March 4, 2000. A Long Islander virtually all his life, he grew up in Stewart Manor to the west of Garden City and attended Sewanhaka High School in Floral Park from 1939 to 1943. With the war then raging, Jack enlisted in the Coast Guard as soon as they would accept him. Upon his return from service, he completed his high-school-equivalency exam. Knowing then what he could do superbly—work with wood, sail, and ski—Jack had no desire for more formal education. After his postwar marriage to his high-school sweetheart, Cory Scala, he worked for 10 years as an employee for the Nassau-Suffolk Lumber Company in Huntington. The first house he built was for the two of them, located on a plot of land in Huntington along Southdown Road just after it turns right to go north toward Lloyd Harbor. This land previously belonged to his employer who, though knowing that Jack and Cory then had no savings, gave them a clear title to the land. He knew that Jack would pay back its several-thousand-dollar cost through deductions from future salary checks.

Soon after he joined Nassau-Suffolk Lumber, Jack first came to the Biological Laboratories along Bungtown Road where he obtained a small order for nails and some lumber. Then, the Biolabs had a reputation for paying their bills very late, with many in the Huntington community believing its eventual bankruptcy would prevent many local merchants from ever being fully paid. In 1959, Jack took the plunge to move out on his own as a self-employed builder of residential houses—sometimes for designated clients but often "on spec," depending on later finding buyers who would let him take home genuine profits from his labors. With his skills as an honest contractor soon becoming known, Jack never was without at least one new home to build.

Jack's first contact with the Laboratory as a builder came through the New York architect Harold Edelman. In the mid-1960s, Harold had several commissions in the Huntington region on which Jack worked. Then Harold was also working for John Cairns, who, as the Laboratory's Director, was in constant need of advice about how to keep the Laboratory's old buildings going for science or human habitation. Initially, none of the renovation projects that Harold asked Jack to bid on were given to him. In particular, he missed being able to rebuild Osterhaut Cottage in 1969 to serve as a residence for Liz and me. So, Jack was more than reluctant when Harold asked him to bid later that year on the addition to James Laboratory that was to provide offices and a seminar room for scientists working on tumor viruses. Jack made the lowest bid and was asked to do the job. Soon after, our then superintendent of Buildings and Grounds, Don Eckels, decided to return to California to take

charge of the City of Davis park systems. We asked Jack whether he might like to build the James Annex, not as an independent contractor but as our Director of Buildings and Grounds. Initially, Jack only perceived trouble in becoming our employee. As self-employed, he could go off on Fridays for weekends of skiing or sailing. In response, we told him that that is how good scientists work and play and he would be a perfect companion to our then two chief scientists, John Cairns and Ray Gesteland. In the end, Jack gave up the independence that all too often gave him clients who never used to pay for late-made changes in construction plans.

From then on, until his official retirement some four years ago, Jack was in effect the contractor for all but the biggest of the Laboratory's construction contracts, using often the same subcontractors whom he had trusted and enjoyed working with as a private contractor. Though official Laboratory policies often forced him to go out for bids, Jack never liked the process, usually knowing what a job should cost and what would be reasonable profits for his subcontractors to take home. At the same time, Jack proved of inestimable value to our young architects, making them aware when they had unnecessarily overdesigned proposed buildings by showing how simpler, less costly architectural solutions would do equally well.

In his early years, when we had far fewer employees, Jack never questioned the need to drive the snowplow or to man a lawn mower when special occasions demanded the Laboratory look its best. Never the person to get his way by loud shots or nasty retorts, Jack's way was to hire individuals who justly took pride in their specific talents and who could be counted on to work through the night—say, in the midst of an ice storm.

For the next two decades, Jack would regularly appear in my office to point out alternative ways of moving ahead. Many of these occasions represented differences in opinion between him and the architects, and he wanted my concurrence before seemingly forcing his opinion on the architect. One such case occurred this past year during the construction of our new Samuel Freeman Building. The decision to give up having a basement then suddenly resulted in a proposed flat furnace addition that looked to us like a square carbuncle. With Jack and me holding firm, our architect, Jim Childress, came up with a new solution that pleases all.

Jack's seemingly instinctive knack for knowing how to behave stayed with him all through his four-year-long fight against prostate cancer. Never one to seek help when he remained capable of taking care of himself, Jack, until the last month of his illness, drove by himself into New York City to receive the radiation or chemotherapy treatments that largely kept him out of pain. But they weakened his body, and finally he could not hide the heavy tiredness that increasingly took over his body at times of intense therapy. Always the best way then to bring back Jack's spirit was to talk about buildings in the course of construction or designs that soon must be made. Though Jack was finally to need a cane to move about, his face until the end was never that of an old or sick person. His brain remained functioning at high capacity, and his advice was as sensible and to the point as ever. Luckily, his end came swiftly and painlessly in Florida, where he went to enjoy the sun and its warmth after still one more round of treatments. For a week, he could enjoy looking out upon the green surrounding him. A bacterial infection then swiftly filled his lungs and his eyes and face sparkled no more.

The respect and affection that he felt for our Laboratory and its purposes will remain an indelible feature of our history. In turn, we shall miss him more than words can ever convey.

James D. Watson

DIRECTOR'S REPORT

In the waning years of the 19th century, the Biological Laboratory at Cold Spring Harbor was expanding its summer program of research and courses that then focused on organism-based biology, encompassing the fields of botany, zoology, bacteriology, and embryology. At the same time, events in Europe would soon have a profound influence on the science at the fledgling laboratory and shape much of biology in the then new 20th century. The hybridist, Hugo de Vries, professor of botany at the University of Amsterdam, completed his extensive, decade-long research on plant hybridization that uncovered quantitative laws of inheritance, research that he first published in 1900. Sparked by the circulation of reprints of the de Vries manuscript, Carl Correns and Erich von Tschermak published accounts of their independent research on plant hybridization in the same year, coming to the same conclusion that traits were inherited as discrete, quantitative units. All three had independently, and to de Vries's disappointment, rediscovered Gregor Mendel's laws of heredity that had remained unappreciated for over three decades. The Darwinian disciple William Bateson, upon receiving de Vries's paper, immediately recognized the importance of the combined discoveries. He soon presented a synthesis of the new field to the Royal Horticultural Society in May of 1900, almost exactly 100 years ago, and, in 1905, coined the term *genetics*.

Charles Davenport, who was by then director of the Biological Laboratory, was aware of these dramatic developments and quickly seized the opportunity to bring Mendelism to Cold Spring Harbor. Davenport was already concerned with understanding inheritance and how it related to evolution. By 1902, he had outlined a plan to the Executive Committee of the Carnegie Institution of Washington for the establishment of a Station for Experimental Evolution, a proposal that was in competition with a rival scheme from the Marine Biological Laboratory at Woods Hole. Fortunately, the Carnegie Institution executive approved Davenport's proposal in 1903, and by 1904, the Station opened with great fanfare. The now celebrated Hugo de Vries was appointed as an honorary associate of the Station for Experimental Evolution and spoke at the opening of the new laboratory. There, he echoed Davenport's belief that understanding the process of evolution necessitates direct experimentation on plants and animals. Remarkably, de Vries chose not to mention Mendel, probably because he considered his 10 or 12 years of studies on plant hybridization to be more extensive and superior to Mendel's work with a limited number of species. Although Mendel was not yet celebrated by those present, Charles Darwin was much in the minds of the assembled, and his revolutionary ideas and studies on evolution of species, by now 45 years old, heavily influenced the goals of the new science. The very name of the station indicated that the principal goal was to understand the laws of inheritance and, thereafter, the secrets of evolution would fall into place.

Evolution and the new science were not only on the minds of the assembled scientists, but clearly were of concern to others present. W.R.T. Jones, brother of the late John D. Jones, one of founders of the Biological Laboratory and governor of the Wawapex Society which owned the Carnegie land, suggested that the new experimental station would revitalize Cold Spring Harbor and its environs, making "our village" well known both at home and abroad. That prediction was more than fulfilled. But Jones also raised concern by stating, "I trust in going back

and investigating, as far as possible, the origin and order in creation, it will find nothing to interfere with the doctrine of the church just around the corner, erected largely by the aid of family relatives, in its efforts for improving morals and explaining to the best of its ability life hereafter." This not so subtle plea reflected the tussle between Darwin's ideas and the doctrines of the Christian church that, unfortunately, has not disappeared after 100 years of enormous insight into the nature of life and the process of evolution. Recent pronouncements by misguided educators in Kansas, who eliminated the teaching of evolution from school curriculum because creation was not taught, make it clear that it is still a challenge for some to separate religious beliefs from scientific reason and progress. What is not appreciated by many is that creation, if it should be taught at all, should be taught within the context of religious education, not in the schools as an alternative to evolution. But the very fact that this debate still exits suggests that science will always be a target for attack both because it often challenges accepted opinion and dogma and because it is sometimes difficult for the public to grasp complex new ideas. Thus, it is easy for a vocal few to twist the scientific progress to confuse others.

One of the first appointments to the Experimental Station at Cold Spring Harbor was Dr. George Shull, who, in 1904, set about preparing the fertile ground for the planting of a variety of species, including species whose seeds were obtained from de Vries. His goal was to confirm the Mendelian laws of inheritance in as many plant species as possible, but within four years, he would clearly state a principle that would change the agricultural economy. By continual self-fertilization of purebred types of Indian corn, the plants reached a state of low vigor that could not be rescued by further selfing of plants of the same type. But superior vigor was obtained routinely by crossing individuals belonging to distinct types, a technique that would dominate agricultural genetics in years to come and cause a revolution in plant breeding.

The new science of genetics progressed rapidly after the rediscovery of Mendel's principles of inheritance, with the period of classic genetics and cytology dominating the 1920s and 1930s. Through the efforts of some of the giants of the classic period, including Thomas Hunt Morgan, Alfred Sturtevant, Calvin Bridges, Herman Muller, and Barbara McClintock (here at Cold Spring Harbor), the chromosomal theory of inheritance was solidified. Units of inheritance were mapped to chromosomes, and their recombination was traced to the physical interchange between chromosomal landmarks. However, it was not until the amazing discovery by Jim Watson and Francis Crick of the double helix that the secret of inheritance was revealed, setting the stage for the dramatic advances in the last half of the 20th century. Two decades after the double helix, recombinant DNA set the stage for remarkable advances in cell and molecular biology, and ushered in the biotechnology era.

It is fitting that just as Mendelism set the stage for the 20th century to become the age of genetics, so determining the complete DNA sequence of the genome of organisms is setting the stage for biology in the 21st century. In just the past five years, the genomes of many microorganisms have been sequenced, as well as those of yeast, the nematode worm *Caenorhabditis elegans*, the first two chromosomes of the plant *Arabidopsis*, and the first human chromosome. Even as I write this, the next milestone—the sequence of the genome of the fruit fly *Drosophila*—will soon be published.

In his 1904 remarks at Cold Spring Harbor, de Vries saw clearly that the new science of genetics would be useful to mankind since "methods would be discovered which might be applied to garden plants and vegetables, and perhaps even to agricultural crops, in order to induce them to yield still more useful novelties." His optimism was fully justified. Manipulation through classical breeding techniques goes back thousands of years to the founding of agriculture itself, but genetics provided a scientific basis for new experimentation leading to such

advances as Shull's hybrid corn. Our new knowledge of all the genes of important organisms will have an even greater benefit for all human beings.

Take, for example, what we have learned from the complete sequences of *Arabidopsis* chromosomes II and IV, determined in part by Dick McCombie and Rob Martienssen at the Cold Spring Harbor Laboratory Genome Sequencing Center. This new information has uncovered much about how genomes evolve to create diversity; large regions of duplication of DNA sequences, exchange between chromosomes, and complicated rearrangement of regions within chromosomes have created new genes for nature to exploit. From a practical point of view, what we learn from the *Arabidopsis* genome will tell us much about genes in other plants. The complete sequence of the *Arabidopsis* genome, expected toward the end of 2000, is eagerly awaited, and insights into the structure of the genome of this plant has propelled efforts, of which we are apart, to determine the complete sequence of the rice genome. Similar comparisons will soon be possible with another international effort to sequence the mouse genome and, eventually, to compare it with the human genome. The foresight of David Luke, immediate past chairman of the Laboratory's Board of Trustees, in helping to establish the Genome Sequencing Center has ensured that Cold Spring Harbor Laboratory remains at the cutting edge of genetics, just as the Carnegie investment did in the early years of the last century.

De Vries could not have imagined the remarkable discoveries of recombinant DNA and the ability to manipulate plant genomes with high precision. But we now have available techniques that enable rational design of desired characteristics that were previously acquired only through prolonged breeding and selection. Genetically modified plants are the future of agriculture and the possibilities they offer are both exciting and unlimited. Creation of disease-resistant crops that do not require the spraying of large amounts of chemicals into the environment makes plain sense. Production of plants that provide much-needed dietary supplements, particularly in the third world, is a moral imperative. Production of varieties that reduce the need for vast amounts of fertilizer also contributes to a better environment and reduces costs to farmers. In the third world, crops that can better survive under adverse conditions will become a necessity as populations expand. The much-touted methods of organic farming are a luxury that only wealthy individuals in economically privileged societies can indulge in, for they cannot serve the needs of the masses. Organic farming on a large scale will be nothing short of an environmental disaster. The developments of 20th-century genetics make all of these possibilities a reality.

Unfortunately, this reality has triggered a reaction from some, particularly in Europe, that is the cause of great concern. The opposition to the use of genetically engineered (GE) food is most often irrational and not targeted at the science, but at peripheral issues that suit the agendas of minority groups. Genetically engineered food has become a sitting duck for groups that are opposed to such diverse issues as the potential dominance of multinational corporations, the demise of the local farmer, scientists playing God, the economic dominance of the United States over technically challenged states, and even international trade. Perhaps more justifiable concerns are whether the technology is safe and what are the long-term consequences of modifying crops. There are even rational arguments for the cultivation of genetically modified, long-lived plants that are designed to be infertile to ensure that they do not mix with native or wild species. But even these concerns are due to not understanding the technology or are based on fear of the unknown. If this type of thinking prevails in society, then we are never going to make any advances, technical or otherwise.

The anti-GE food groups have been quick to jump onto a growing bandwagon that attacks science indirectly. Often, they use the results of scientific investigation to suit their own purposes and in doing so misrepresent the scientific process. Such was the case following the

publication in the journal *Nature* of a study from Cornell University of the forced feeding of pollen from corn that had been genetically engineered to produce an organic, naturally occurring toxin, *Bt*. The study subjected monarch butterflies to a choice of eating milkweed covered with the pollen or nothing at all and reported that about half of the butterflies died in the experiment. The experiment confirmed other studies that insects die when they consume *Bt*. The publication was heralded by a misguided publicity machine from Cornell and was immediately picked up by the anti-GE groups as their *cause célèbre*. The paper has since been criticized by colleagues of the original authors and deemed to be an unlikely event in the field because *Bt* is rapidly inactivated by sunlight not present in the laboratory studies. Moreover, *Bt* has been used for many years, and no adverse effect has been recorded. Despite the fact that further experimentation and studies have not found any support for the demise of monarch butterflies, the anti-GE groups continue to flutter around with multicolored wings, pushing their agendas. Such irrational behavior has induced the further inexplicable decisions of a few major corporations to abandon the use of GE crops, even after they have been using the technology safely for years. Moreover, such protests gloss over the clear documentation of the adverse effects of the use of chemical pesticides.

In the early 1970s, the recombinant DNA debate reached the public forum, and misunderstanding of the science and a fear of the unknown fueled exaggeration of the issues. Now, with the benefit of hindsight, genetic engineering technology has proved to be one of the safest to have been used for the benefit of humankind. Perhaps we should learn a lesson from the recombinant DNA debates about how science, when it receives the attention of the public, should be discussed. First and foremost is that scientists should make every effort to educate the public and keep society abreast of the emerging science. Such efforts eventually won the day in Switzerland, where political events drove the country into referenda about the use of recombinant DNA technology. Here, improved communication between scientists and the public resulted in a slim majority rejecting the excessive claims of the naysayers. Although the issue has not disappeared in Switzerland, the fact that Swiss scientists did not have to leave the country to continue to do their research is due to their efforts in public education.

Genetics and genomics have great promise for the future, both for the insight they will provide into the ways of nature and for the tangible benefits to mankind. For the most part, the scientific community pursues its research and develops technologies in isolation. We are driven by curiosity to shed light on the unknown, but for biology, times have changed, and we must do this as members of a broader society. We also have responsibilities as scientists. There are times when scientists must take a stand against those who seek to manipulate the scientific harvest for purposes that have nothing to do with the science itself. Otherwise, once encouraged by success, science will forever be abused.

HIGHLIGHTS OF THE YEAR

The research and education programs at the Laboratory remain strong and vibrant. The recruitment of the first students in the Watson School of Biological Sciences and the completion of their intensive core courses highlighted progress this year. The DNA Learning Center introduced new, Web-based educational tools, and the meetings and courses programs at the main campus and the Banbury Center continued to provide a valuable service to the scientific community. Significantly, the Laboratory Press saw the continued development of its exciting textbook-publishing program as part of the Laboratory's goal to promote innovative teaching programs for graduate students and senior undergraduate students. These educational programs occur in the surrounding of a strong research environment, with research and education braided together in a common enterprise.

Research

Neuroscience

Roberto Malinow

Roberto Malinow and Karel Svoboda have continued to push the limits of brain imaging by observing the changes that occur in individual neurons as they respond to electrical stimuli. Combining their expertise in electrophysiology and high-resolution imaging of the brain, they study the cellular events that underlie "synaptic plasticity"—the ability of brain neurons to reorganize their connections in response to experience, such as during learning and consolidation of memories. In particular, Roberto and Karel are exploring the mechanisms involved in long-term potentiation (LTP), the strengthening of synaptic connections between nerve cells in response to trained stimuli. Their studies focus on a region of the brain involved in learning and memory called the hippocampus, a region required for a form of memory called declarative memory, or the conscious memory of learned events.

Karel Svoboda

This year, Roberto and Karel studied the distribution of AMPA receptors within dendrites, a nerve cell property they suspect contributes to LTP. AMPA and other receptors mediate the effects of glutamate, a key neurotransmitter that is one chemical which crosses the synapse or gap between neurons. They first engineered hippocampal neurons to express a fluorescent version of the AMPA receptor so they could track its movement within the processes called dendrites that branch from the neuronal cell. They used a high-resolution optical-maging system called two-photon laser-scanning microscopy, both before and after electrical stimulation.

Before stimulation, AMPA receptors were distributed evenly along the branch-like shafts of dendrites, but were largely restricted from the dendritic spines that project from shafts and are where the synapses lie. After stimulation, however, AMPA receptors moved rapidly into the spines. Synapses are typically located at the tips of spines, but without functional AMPA receptors, such synapses are inactive, or "silent." Therefore, the movement of AMPA receptors into dendritic spines may contribute to LTP, at least in part, by converting silent synapses to

active synapses. Moreover, evidence from Roberto's lab suggests that the constant replenishment of AMPA receptors at synapses may be involved in maintaining synapses in an active state over long periods of time. It is an intriguing possibility that this form of synaptic plasticity may underlie the development of the brain's neuronal network and function of neurons in an adult brain.

Roberto and Karel's studies provide some of the first real-time, high-resolution images of the events that are likely to power the restructuring of vast neural networks within an important site for learning and memory in the brain.

Yi Zhong

Yi Zhong and his colleagues are also studying the neurological basis of learning and memory, but in the fruit fly *Drosophila*. They have recently focused their efforts on explaining the puzzling observation that a human disease called neurofibromatosis causes both cancer and learning defects.

Neurofibromatosis is one of the most common inherited genetic disorders affecting humans. The disease is characterized by discoloration and tumors of the skin and is caused by mutations in the *NF1* gene. *NF1* encodes a protein that inhibits the activity of the Ras protooncogene product, another protein that is altered in many human tumors. Thus, when the *NF1* gene is mutated, the resulting uncontrolled activity of Ras can lead to cancer. But how mutations in *NF1* lead to cancer and learning defects was unknown.

From previous work, Yi knew that an enzyme called adenylyl cyclase was required for learning in *Drosophila*. He also knew that adenylyl cyclase activity was stimulated by the *Drosophila* NF1 protein (human and *Drosophila* NF1 are remarkably similar, so much so that human NF1 can substitute for its *Drosophila* counterpart in transgeneic flies). Yi and his colleagues first established that *Drosophila*—like humans and mice—requires NF1 for learning (in this case, learning to avoid a particular odor that was paired with a small electrical shock).

It was first necessary to determine whether the defects in adult flies lacking NF1 were an indirect result of developmental abnormalities due to the loss of NF1 activity as the brain develops. But experiments demonstrated that the learning defect in flies lacking NF1 could be corrected during adulthood by a burst of NF1 from an externally controlled gene. Then, in a series of elegant experiments, Yi showed that the effects of NF1 on learning are mediated by the enzyme protein kinase A, an enzyme that lies downstream from NF1 and adenylyl cyclase in the regulatory pathway controlling learning. Since protein kinase A is a well-characterized enzyme, the studies suggest that by activating it, the learning deficit could be reversed.

Yi's studies reveal the role of NF1 in a new mechanism that contributes to normal learning and memory in *Drosophila*. Because this pathway is conserved from fruit flies to humans, there is a distinct possibility that pharmacological intervention in the adenylyl cyclase pathway might reverse the NF1 defect in humans.

Virus-induced Pathogenicity: The Case of HIV

The genomes of human and simian immunodeficiency viruses, HIV and SIV, encode several auxiliary proteins that modulate the function of infected cells and optimize virus propagation in part by enabling infected cells to evade the host's antiviral immune response. Determining how these auxiliary proteins work is therefore important for understanding the pathogenesis of AIDS and how an effective vaccine might be formulated.

Jacek Skowronski and his colleagues are studying how one of these auxiliary proteins, Nef, promotes HIV infection. The Nef proteins of HIV and SIV are similar, and each fulfills multiple roles in the course of viral infection. The goal of the experiments in Jacek's laboratory is to determine how Nef interacts with protein sorting and signal transduction machinery in T cells and macrophages, two immune system cell types that are infected by HIV.

Jacek and his colleagues have found that Nef is required for the "down-regulation" or removal of particular host-cell proteins from the cell surface (CD4 and class I MHC proteins). Moreover, a collaboration with scientists at Erlangen University (Germany) and Harvard University has led to the identification of certain mutations in Nef that selectively disrupt its ability to down-regulate CD4, thereby greatly decreasing viral replication during the acute phase of viral infection.

One future challenge for the Skowronski group is to determine precisely how down-regulation of CD4 and class I MHC by Nef promotes viral propagation. One possibility is that by removing CD4 and MHC I from the cell surface, Nef enables HIV-infected cells to evade the body's immune response. Alternatively, Nef might be required to optimize host-cell survival so that the virus has a chance to reproduce before it kills the cell.

Cancer Research: Regulation of Cell Death

The genetically programmed death of cells is a normal part of growth and development. In addition, programmed cell death, or "apoptosis," safeguards organisms from cancer by triggering the self-destruction of precancerous cells. A hallmark of many types of cancer is a defect in apoptosis that allows precancerous cells to survive and proliferate, and eventually form a tumor.

Yuri Lazebnik and his colleagues are exploring the possibility that restoring the ability of cancer cells to self-destruct via apoptosis might be an effective therapeutic strategy. They study a family of enzymes called caspases that act in a proteolytic cascade to execute programmed cell death. Among several known caspases, caspases 2, 8, and 9 are thought to be "initiator" caspases. In response to cellular self-destruct signals, these caspases trigger a chain reaction in which a series of "effector" caspases are converted from an inactive to an active form. Once activated, effector caspases cleave key cellular proteins, and cell death follows due to destruction of DNA, disruption of nuclear structure, and disintegration of cells into small vesicles.

Previous work from Yuri's lab suggested that a caspase cascade leading to self-destruction remains functional in cancer cells, but it is uncoupled from the signal that triggers its activation. To study this critical link between a cell death signal and the caspase-mediated cell death machinery, Yuri and his colleagues purified a cellular factor that could trigger caspase-mediated apoptosis. They found that a known protein called APAF-1 (*apo*ptosis *a*ctivation *f*actor-*1*) plus caspase-9 are key components in triggering apoptosis in tumor cells. Another lab had shown that APAF-1 stimulated the biochemical activity of caspase-9, but precisely how APAF-1 worked on caspase-9 remained a puzzle.

Effector caspases are synthesized as inactive precursors that are activated upon cleavage by other "upstream" caspases. For example, caspase-3 (an effector caspase) is cleaved and activated by caspase-9 (an initiator caspase), but how the initiator caspase is cleaved and activated was unclear. The observation that APAF-1 can bind directly to caspase-9 led some scientists to believe that APAF-1 brings two different caspase-9 molecules together, which then cleave and activate each other. To the contrary, Yuri and his colleagues have now

shown that APAF-1 is a regulatory subunit that is in a complex with caspase-9 and causes a 1000-fold increase in caspase-9 activity, probably by triggering the ability of individual caspase-9 molecules to cleave and activate themselves. Now that the process whereby the APAF-1/caspase-9 "holoenzyme" activates the cell death machinery has been investigated, it is possible to investigate ways to recouple this machinery to the self-destruct signals that are generated in cancer cells, but are normally ignored.

Controlling Cell Shape

During development and throughout adulthood, animal cells within multicellular organisms proliferate, migrate various distances, and take up residence as parts of tissues and organs. The regulation of this dynamic process involves the ability of cells to sense and respond to diffusible growth factors and the extracellular matrix (ECM) that surrounds them. Metastatic cancer cells become independent of growth factors for their survival and break free from their extracellular matrix moorings to invade areas of the body outside their normal domain.

David Helfman studies how growth factors and the interaction of cells with the ECM regulate oncogene-mediated signal transduction pathways. In particular, he investigates how growth factors regulate the contractility of the actin/myosin cytoskeleton of cells. All cells have such a system, but it is particularly effective in muscle cells since actin and myosin are responsible for muscle cell contractions. David is interested in how increased contractility stimulates cell-ECM interactions and promotes the growth and survival of cells in part by blocking programmed cell death.

David has discovered that overexpressing a cytoskeletal protein called caldesmon inhibits contractility and interferes with the formation of "focal adhesions," the structures that mediate cell-ECM interactions. His results (obtained in collaboration with Alex Bershadsky of the Weizmann Institute, Rehovot, Israel) identify a new pathway for the regulation of cytoskeletal function, and they suggest that caldesmon can counteract some of the stimulatory effects of the Ras oncogene product on cell growth. Moreover, they provide new information about how the movement of nonmuscle cells is controlled. David's finding that caldesmon affects focal adhesion formation is also important for understanding adhesion-dependent signaling, a process in which the cell's attachment to a substrate triggers changes in cell physiology that promote cell growth and survival.

Epigenetic Inheritance of Gene Expression

Not all clones are created equal. As genetically identical cells (such as those in an embryo) multiply, different sets of genes are switched on and others off, giving rise to cells and tissues with distinctive properties (e.g., liver versus muscle).

Such differential gene expression is often established by alterations in the large-scale architecture, or chromatin structure, of DNA. For example, transcriptionally silent regions of DNA are packaged into forms of chromatin that may be less accessible to certain transcription factors. In contrast, transcriptionally active regions of DNA adopt alternate chromatin structures that may be more accessible to certain transcription factors. A particular DNA sequence can be silent or active, depending on its position within the genome or the type of cell in which it resides. Such states of chromatin are said to be epigenetic because they can

be inherited in a stable manner. Examples occur in inactivation of one of the X chromosomes in female mammals, in certain human cancers, and in parental imprinting of genes.

Shiv Grewal and his colleagues use a powerful model system in the fission yeast *Schizosaccharomyces pombe* to study how the active and silenced states of gene expression are established. Working at the National Cancer Institute, Shiv and former CSHL scientist Amar Klar showed that in a particular region of the fission yeast genome, active and silent states of gene expression can be stably inherited through mitosis (cell division) and, remarkably, through meiosis (the division leading to spores or gametes for the next generation). These findings were striking because scientists had generally believed that different states of chromatin structure were erased during meiosis to enable unbiased gene expression after the fusion of gametes.

In essence, Shiv found that the Mendelian inheritance of traits sometimes depends not only on the faithful replication of DNA sequences, but also on the transmission of higher orders of chromatin structure. The "gene" in these instances is thus not only DNA, but DNA plus associated proteins. Shiv has proposed a chromatin replication model in which both the DNA molecule and higher-order chromatin structure are duplicated.

Since joining the faculty of CSHL in 1998, Shiv has tested several predictions of the chromatin replication model. He has found that among several potential candidates, a particular protein, Swi6, is critical for establishing a silenced state of chromatin structure and that Swi6 is required to maintain silencing through multiple mitotic cell divisions or meiosis.

Histone proteins are major determinants of chromatin structure, and the chemical modification of histones (e.g., acetylation) is known to affect chromatin structure. Shiv has observed that an enzyme that de-acetylates histones, Clr3, is required—in addition to Swi6—for the silenced state of chromatin structure to be maintained. He also showed that Swi6 binding is enriched in a silenced region of DNA and that the histones bound to silenced DNA are less acetylated than are histones bound to the identical region of DNA when it is active.

Because proteins homologous to Swi6 and Clr3 exist in organisms as diverse as fungi, *Drosophila*, and humans, mechanisms for the inheritance of chromatin structure akin to that studied by Shiv are likely to be widespread in nature. In fact, there are proteins in mammalian cells that function like Swi6, and in my own laboratory, we have found that these mammalian cell proteins are bound to a chromatin assembly factor (CAF-1) that is itself tethered to one of the proteins responsible for replicating DNA, called PCNA. Thus, there exists a direct biochemical link between the inheritance of DNA and the inheritance of chromatin. Interestingly, mutations in CAF-1 in the budding yeast *Saccharomyces cerevisiae* cause defects in epigenetic inheritance.

Regulation of Transcription in Cancers

The control of transcription itself is likely a major contributor to progression in human cancers and indeed, many oncogene products are errant transcription factors. Bill Tansey and his colleagues study cell proliferation and how defects in the regulation of transcription can lead to cancer.

The c-*myc* gene encodes a transcription factor that plays a critical role in promoting normal cellular proliferation. Because the Myc transcription factor is a potent cell proliferation stimulator, the level of Myc within cells is normally tightly controlled. An unfortunate consequence of the power of Myc to stimulate cell proliferation is that unregulated Myc activity can lead to cancer.

The amount of Myc protein in cells is determined not only by how much Myc is synthesized in a given time frame, but also by how quickly it is destroyed. Bill is studying how the destruction of the Myc protein is regulated and how defects in this process lead to abnormally high levels of Myc, and potentially to cancer.

First, Bill showed that Myc is normally destroyed by the proteasome, a large complex of enzymes that cuts proteins targeted for destruction into small pieces. This process—called ubiquitin-mediated proteolysis—enables the abundance of many different kinds of proteins, including Myc, to be adjusted in response to particular cellular conditions.

Next, Bill mapped the regions of the Myc protein necessary to target it for destruction by the proteasome. He found that a region encompassing the amino-terminal third of the Myc protein was sufficient to promote the destruction of Myc. Significantly, mutations in this region of Myc are associated with Burkitt's lymphoma, plasmacytomas, and other kinds of cancer. Bill and his colleagues tested five such cancer-associated mutations of Myc and found that four of them blocked Myc degradation. These observations raise the possibility that the mutations contribute to cancer by blocking Myc degradation, thereby increasing the levels of Myc in cells and causing them to promote cell division.

Finally, Bill found that the region required for Myc degradation overlaps with the domain of Myc that mediates its function in activating transcription of certain genes. Thus, he uncovered an intriguing underlying connection between two processes—proteolysis and transcriptional activation—that had not previously been characterized. This connection between proteolysis and transcriptional activation appears to be common in short-lived regulators of gene expression.

Cell Biology in the Nucleus

David Spector and his colleagues are studying the dynamic movements of molecular assemblages that mediate two essential, intimately connected processes in the cell nucleus: transcription and pre-messenger RNA (pre-mRNA) splicing.

Transcription is the process whereby the enzyme RNA polymerase synthesizes RNA molecules from DNA templates. During transcription, RNA polymerase proceeds from one end of a gene to the other, synthesizing a continuously elongating pre-mRNA molecule as it moves along the DNA.

Pre-mRNA molecules contain sequences that encode protein, called exons, as well as noncoding pieces of RNA called introns that are removed by the splicing machinery of the nucleus. Interestingly, splicing often begins before the synthesis of pre-mRNA molecules is completed, i.e., as pre-mRNA molecules emerge from the transcription apparatus. This year, David and his colleagues have discovered important new information about the components of the splicing machinery and how its mode of action is linked to gene transcription.

They showed that truncating the carboxy-terminal domain (CTD) of RNA polymerase prevents the targeting of the splicing machinery to transcription sites and eliminates pre-mRNA splicing. These results indicate that the splicing machinery is recruited to transcription sites by binding to the CTD of RNA polymerase. In essence, the splicing machinery gains easy access to pre-mRNA transcripts by piggybacking on the enzyme that makes the transcripts.

Abundant evidence indicates that splicing factors are assembled and/or stored in large multiprotein complexes called interchromatin granule clusters (IGCs). But in the 40 years since IGCs were described, only a single attempt to purify and characterize them in detail

has been published. David and his colleagues have now succeeded in purifying IGCs, and they have used mass spectroscopy protein sequencing, in collaboration with former CSHL staff member Scott Paterson at Amgen Corporation, to identify over 100 different protein components of these complexes. In addition to several known splicing factors—whose presence in IGCs was known or expected—many were known transcription factors whose presence in IGCs was surprising. In addition, 12 new proteins of unknown function were identified as IGC components and are the subject of ongoing study in David's lab.

Structural Biology

The phenomenon of splicing of pre-mRNA was discovered at CSHL, and Adrian Krainer's laboratory continues to investigate the biochemistry of this process and how different genes are spliced into alternative mRNAs by excising different introns. In collaboration with structural biologist Rui-Ming Xu, Adrian and his colleagues use X-ray crystallography to study—at the atomic level—the precise structural basis of the protein-RNA interactions that mediate pre-mRNA splicing.

Human hnRNP A1 is an abundant, versatile protein that both binds to the single-stranded regions of DNA present at the ends of chromosomes (called telomeres) and, as Adrian's lab discovered, regulates the use of alternative 5′ splice sites, probably by binding to pre-mRNA (although how and where is uncertain).

Rui-Ming and his colleagues, in collaboration with Adrian's lab, have solved the crystal structure of UP1 (a part of human hnRNP A1) which is complexed with telomeric DNA. The structure reveals an interesting "railroad tie" mode of DNA binding in which two antiparallel single-stranded DNA molecules (the rails) bind to the surface of two antiparallel, oblong molecules of UP1 (the ties) oriented at right angles to the DNA. The structure provides a simple and elegant model for how hnRNP A1 binds to telomeres. Moreover, it suggests how hnRNP A1 might bring two strands of RNA into close proximity during the process of pre-mRNA splicing.

Rui-Ming's work with Adrian demonstrates the synergy that can result when structural and molecular biologists collaborate to address a given problem. Two other CSHL scientists, Xiaodong Cheng (now at Emory University) and Winship Herr, benefited in a similar way from their collaborative effort this year to determine the crystal structure of the herpes simplex virus (HSV) transcriptional regulatory protein VP16.

Viruses commandeer the transcriptional machinery of host cells, which they use for the expression of their own genes. During HSV infection, viral gene expression occurs in three classically defined phases: immediate early, delayed early, and late. This cascade of viral gene expression is triggered by VP16, a potent activator of immediate-early gene transcription that is released into cells upon infection. Following its release into cells, VP16 binds to a host cell protein called HCF (*h*ost *c*ell *f*actor). Then the VP16/HCF complex binds—in combination with a second host cell protein called Oct-1—to the DNA target present in the promoters of HSV immediate-early genes. The transcription of these genes is thus activated, and a productive viral infection ensues. VP16 also happens to be one of the most commonly utilized transcription factors for activating genes that have been introduced by experimenters into cells.

To investigate further the role of VP16 in mediating viral gene expression, Xiaodong, Winship, and their colleagues determined the structure of the conserved core of the VP16

molecule using X-ray crystallography. They discovered that VP16 is shaped rather like a chair. The scientists were able to define what parts of VP16 are involved in a particular function by determining where mutations that specifically disrupt one VP16 function but not another map on the surface of the protein. For example, Xiaodong and Winship found that three mutations known to block the assembly of virus particles all cluster on one side of VP16. In contrast, a mutation known to block the interaction of VP16 with Oct-1 maps on the other side of the protein.

Xiaodong and Winship also identified a region of VP16 likely to bind to DNA by examining the charge distribution on the surface of VP16. DNA is negatively charged. Because like charges repel, protein surfaces that bind DNA are usually positively charged. The seat of the VP16 chair-like structure has such a positively charged surface. This region is further implicated in DNA binding by evidence from Winship's lab that mutations in the seat bottom disrupt VP16's DNA-binding activity. In short, DNA sits on the VP16 seat.

Studies of VP16, Oct-1, and HCF by Winship and his colleagues have revealed a great deal of information about how transcription is regulated. The added dimension of structural information provides an even clearer view of how the molecules that mediate this complex and dynamic process interact with each other and with DNA.

X-ray crystallographic data sets for the structures of both hnRNP A1 and VP16 were collected at Brookhaven National Laboratory, where we now maintain a dedicated high-energy beam-line. Our proximity to Brookhaven allows our structural biologists to have immediate access to this natural resource.

Bioinformatics

Lincoln Stein and his colleagues develop computer software programs that enable biologists to organize, work with, and make sense of the vast amounts of information that are becoming available from human and other genome sequencing projects.

Bioinformatics (the application of computer science to the analysis of biological information) is a relatively young field. Ten years ago, the comparison of a newly discovered DNA sequence with known sequences usually required several hours on a personal computer and considerable input by the investigator. Today, the same exercise—which provides clues about the potential function of an uncharacterized sequence of DNA—takes a few seconds and is conducted by scientists all over the world via the Internet. The impact of bioinformatics on the future of basic science, medicine, and agriculture is difficult to overestimate.

Lincoln Stein

One of Lincoln's first projects was a user-friendly database that allows biologists to analyze the nematode worm *Caenorhabditis elegans* genome in several ways, such as to determine what genes are expressed in a particular cell type or what human genes are homologous to a particular *C. elegans* gene. This database currently receives 25,000 "hits" per week and was recognized by a computer chamber of commerce, LISTNET, as the "Best Software of 1999 Developed by a Large Company" on Long Island (and there are some very large computer companies on Long Island).

Lincoln and his colleagues have recently joined a multi-institutional consortium whose goal is to map *s*ingle *n*ucleotide *p*olymorphisms, or SNPs, that are distributed throughout the human genome. SNPs are variations (polymorphisms) among different individuals that occur in sequences of DNA. Although the majority of SNPs

have no physiological consequences, a particular SNP might be located near a mutation that *is* of great physiological consequence (e.g., disease-causing mutation). SNPs are therefore extremely useful as markers for genetic disease, for the development of reagents to diagnose genetic disease, and for the cloning of disease-related genes. In addition, SNPs are invaluable to scientists interested in tracing the flow of genetic information through human populations on global and regional scales.

Lincoln and his colleagues have developed software and hardware systems for storing, analyzing, and disseminating the large amounts of information currently being generated by the SNP consortium. A 1.5-ton uninterruptible power supply, a closet-sized 1-terabyte storage array, and a high-performance cluster of 40 individual computers linked together in a "parallel processing" configuration are now all part of the daily operation in Lincoln's lab.

Symposium LXIV

On June 2–9, our activities centered around the 64th annual CSHL Symposium, entitled Signaling and Gene Expression in the Immune System. On Sunday, June 6, meeting attendee Irving Weissman, of Stanford University School of Medicine, delivered the annual Dorcas Cummings Lecture to a scientific and public audience. His talk "Repairing the Body: The Promise of Blood and Tissue Stem Cells" addressed a topic that is much in the news because of its great potential in repairing human disease tissues. Judging by the enthusiastic reaction of the audience, the lecture was very well received and timely.

Irving Weissman

Watson School of Biological Sciences

The Laboratory's graduate school—the Watson School of Biological Sciences—became a reality this year. Recruitment of the inaugural class was an outstanding success. The six new students arrived late in the summer, beginning classes on September 7. The two core courses—Scientific Reasoning and Logic and Scientific Exposition and Ethics—were supplemented by specialized minicourses and provided an overview of the current state of biology in many fields. The lecturing presented a new challenge to most CSHL scientific faculty and quite happily, everyone involved rose beautifully to the occasion. The huge success of the program is in large measure due to the tireless efforts of Winship Herr and Lilian Gann, both of whom have transformed graduate education at CSHL and we hope beyond.

Winship Herr

In our first official celebration of the Watson School, the Laboratory held a convocation on November 5 at which we bestowed the honorary degree of Doctor of Science *honoris causa* on three esteemed scientists: David Baltimore, president of the California Institute of Technology; Gerald Fink, director of the Whitehead Institute for Biomedical Research and American Cancer Society Professor of Genetics at the Massachusetts Institute of Technology; and Seymour Benzer, the James Griffin Boswell Professor of Neuroscience at the California Institute of Technology. Each of them played a significant part in the education programs at the Laboratory, from under-

graduate research to postgraduate courses. Their citations and acceptance speeches after receiving the degree are printed in the Watson School of Biological Sciences section of this Annual Report.

In November, Jim Watson delivered a series of lectures to commemorate the opening of the graduate school. The lectures were held in Grace Auditorium in anticipation of the sizable audience that might be attracted to hear Jim talk, and we were not disappointed. On each of four evenings, Jim spoke to a full house. The lectures were entitled "Discovering the Double Helix" (Nov. 2); "George Gamow and His Combinatorial Approach to the Genetic Code" (Nov. 9); "Finding the Genes of DNA Tumor Viruses Which Unlock Cellular DNA Synthesis" (Nov. 16); and "Recombinant DNA and the Beginnings of the Human Genome Project" (Nov. 22). These lectures have been captured on videotape and will be available to future students.

Watson School Gala

The Laboratory hosted a gala fund-raiser in Grace Auditorium on October 5th to raise money for the Watson School of Biological Sciences. We were extremely fortunate to have Lola Grace as the gala chairman. Thanks to the efforts of Laboratory Trustee Mark Ptashne, we attracted three of the world's greatest classical musicians—pianist Emanuel Ax, violinist Midori, and cellist Yo-Yo Ma—to perform a joint program of Mendelssohn. Before the exceptional ensemble performance, the audience was treated to a tasting dinner featuring cuisine prepared by chefs from three of New York City's top gourmet restaurants—Charlie Palmer of Aureole (who was present to serve his culinary creations), Daniel Boulud of Daniel, and

(*Top left*) Lola Grace, with daughter Lola and Yo-Yo Ma; (*bottom left*) Chefs of LeBernadin; (*right*) Midori, Emanual Ax, Yo-Yo Ma.

Eric Ripert of Le Bernardin. Exquisite wines, contributed by Iron Horse Vineyard, stunning displays of flowers by J. Barry Ferguson Flowers Ltd., and gifts from Tiffany, Estée Lauder, and Sony Classical helped make the evening a spectacular success for the 350 attendees. The evening was phenomenal, raising more than $750,000 for the Watson School. We must thank Lola once again for organizing such a unique event in our lives.

URP 40th Anniversary Reunion

In August, the Laboratory held a two-day reunion for alumni of the Undergraduate Research Program (URP) in celebration of the program's 40th anniversary. More than 70 people returned to reminisce about living and working at the Laboratory. The event included talks by 14 former URPs and a keynote talk by former URP and Nobel prize winner David Baltimore. Their stories gave current scientists a glimpse of days gone by along Bungtown Road, including parties at the beach, trips to New York City, and, of course, a memorable science experience.

First URP Director Arthur Chovnick and former URP Gerry Rubin

Banbury Center

The first Banbury Center Executives' Conference was held 13 years ago in 1986 and the topic of the meeting was genomics. Although that word had not been invented at the time, the idea of sequencing the human genome had been discussed at the Cold Spring Harbor Symposium earlier the same year, and speakers at that first Executive Conference went on to become leaders of various genome projects. 1999 seemed the right time to review the state of genomics; it was a milestone year in the history of genome research due to the completion of the first sequence of a human chromosome. Among the speakers at the 1999 meeting were Lee Hood of the University of Washington in Seattle and David Botstein of Stanford University, both of whom spoke at the 1986 Banbury meeting. We heard about advances in sequencing, potential applications of data, and the controversies over patenting and commercialization of DNA sequences. Once again, we are indebted to Sandy Warner, chairman of J.P. Morgan and Co., and David Demming for their enthusiastic support of this unique meeting.

Banbury Center has a long history of meetings on human genetic disorders, beginning with a 1982 meeting about the application of recombinant DNA techniques to these disorders. These meetings are of immense immediate value for the discussion of data and ideas, and they also ultimately promote further research. In 1999, we hosted a meeting on ataxia telangectasia (AT), a progressive degenerative disorder involving DNA repair, characterized by degeneration of the brain, lack of muscle control, and immunodeficiency. The meeting was funded by the AT Children's Project. The Children's Project has been a driving force in promoting research on AT, and research sponsored by the Project has led to remarkable advances in our understanding of the basic mechanisms of AT, especially through the identification of the ATM (mutated AT gene) protein. The Banbury AT meeting was designed to encourage critical analysis of the current body of knowledge concerning the protein's structure and function, with particular focus placed on its role in nerve cells. In addition, participants discussed possible applications of current data to the design of new therapies using genes or stem cells.

Banbury's J.P. Morgan Conference

In addition to hosting straightforward scientific meetings, Banbury Center also has a long history of hosting meetings that deal with controversial topics in biology that have important societal implications. In earlier years, these topics dealt largely with environmental carcinogenesis and risk assessment. More recently, we have examined and reviewed possible difficulties arising from new developments in human genetics. In 1999, we focused on xenotransplantation, the transplantation of tissues or organs between species (e.g., from animals to humans). Xenotransplantation raised many of the same issues raised by recombinant DNA technology in the mid 1970s. How, for example, will we deal with what are—for the present at least—unquantifiable risks? In the case of xenotransplantation, one such risk is the transmission of unknown viruses from pig tissues to human transplant recipients. The ambience of a Banbury Center meeting, with 30–40 participants from diverse backgrounds gathered in an intimate setting, is particularly conducive to discussion of such controversial topics.

DNA Learning Center

Student visits to the DNA Learning Center (DNALC) plateaued at 30,000, as all available lab space was used at full capacity. Meanwhile, over 380,000 people visited the DNALC Internet sites, a threefold increase from 1998. The majority visited the animated genetics primer, *DNA from the Beginning*, which went online in January 1999 and was funded by the Josiah Macy, Jr. Foundation. It has proved immensely popular with teachers, students, and casual Web surfers.

Several other Internet projects neared completion during 1999. The *Image Archive of the American Eugenics Movement* is a searchable database of over 1200 photos and docu-

Creators of the *Image Archive of the American Eugenics Movement*. (*Back row, left to right)* Chun-hua Yang, Susan Conova, Shirley Chan, Matthew Christensen; (*front row, left to right)* David Micklos, Susan Lauter, Jan Witkowski.

ments from this dark period in the history of science. It is fitting that Cold Spring Harbor Laboratory be the source of such information, as, indeed, the Eugenics Record Office (ERO) was founded in 1910 by Charles B. Davenport, then director of the Biological Laboratory (which later became CSHL). He set up the headquarters for American eugenics research on land adjacent to the Carnegie Institute's Station for Experimental Evolution (of which he was also director). The ERO was closed in 1940 when the Carnegie Institute withdrew its support.

The new eugenics Web site features a unique interface that displays controversial documents about sterilization, racial stereotyping, and immigration restriction, along with contextual explanations that help users understand how and why the science went so wrong. In a different vein, *Bioservers* offer simple bioinformatics tools that allow students to "mine" information from DNA—including using their own DNA to reconstruct human evolution.

A mobile bioinformatics teaching lab, *VectorNet*, is being constructed under a new $500,000 grant from the Howard Hughes Medical Institute. Ten lap-top computers will connect to a local server running the entire DNALC Web site, bioinformatics software, and gene databases—providing an Internet experience without an Internet connection! During the academic year, *VectorNet* will be deployed in selected New York City schools; during the summer, it will be used to conduct teacher-training workshops at sites around the United States.

CSHL Press

Financially, the year was satisfactory, with a 6% increase in revenues and an operating surplus gained through publication of new books, improved journal advertising revenue, and aggressive marketing efforts. More importantly, the Press continued to serve the scientific community by providing high-quality publications.

The Press published eight new books and a videotape, including the monograph *Prion Biology and Diseases*; the manual *Imaging Neurons*; and the advanced-level textbooks *Essentials of Glycobiology* and *Transcriptional Regulation in Eukaryotes*. Also notable was a collection of the outstanding research papers published at the Laboratory from 1903 to 1968, *Illuminating Life*, which should become a valuable teaching tool. Over 240 titles are in

print, with a strong backlist led by *Using Antibodies* and *At the Bench*. A new, targeted sales program boosted orders through major resellers.

The journal program continued to flourish. *Genes & Development* (*G&D*), *Genome Research* (*GR*), and *Learning & Memory* (*L&M*) increased or maintained subscription levels. Advertising revenues were 30% higher. All three journals were offered more manuscripts than ever before. The impact factors of *G&D* and *GR*, as measured by citation analysis, rose substantially, and *G&D* maintained its rank among the top ten primary research journals. Online editions of all three journals were enhanced by new software tools, and *GR* developed the capacity to publish papers online well ahead of print.

The Protein Society selected CSHL Press from a list of ten candidates as the next publisher of its journal *Protein Science*. The contract is for the period 2001–2005.

A new program to develop and publish textbooks for undergraduates was initiated early in the year. Five promising projects were identified, including a book on evolution, and approaches to potential authors were made. Outlines of prospective books are being developed.

In 1997, the Laboratory purchased the lovely residence of Anne and Wally Meier (daughter and son-in-law of Charles Robertson) adjacent to the Conference Center on our Banbury estate. The house has now been altered to serve as a writing center for authors of the new textbooks. Alex Gann joined our staff as senior editor of textbooks, and his office is located in Meier House as well. Alex earned a Ph.D. in molecular biology after working on restriction enzymes in Noreen Murray's lab at Edinburgh University in Scotland. He did postdoctoral research at Harvard, studying mechanisms of gene regulation with Mark Ptashne, and at University College London, working on newt limb regeneration with Jeremy Brockes. Most recently, he was a lecturer in developmental biology at Lancaster University in England.

Gavin Borden Visiting Fellow

Joseph L. Goldstein, M.D., of University of Texas Southwestern Medical Center and a Nobel laureate for his research on cholesterol metabolism that led to cholesterol-lowering drugs, was the Gavin Borden Visiting Fellow this year. Dr. Goldstein's lecture, held on March 11, was entitled "A Proteolytic Pathway That Controls Cholesterol Content of Membranes, Cells, and Blood."

Joseph L. Goldstein

Titia de Lange, Roel Nusse, Harold Varmus, Steve Hughes, Suzanne Ortiz

Varmus Birthday Celebration

On December 12, 74 scientists gathered at Cold Spring Harbor Laboratory to celebrate the 60th birthday of Harold E. Varmus, M.D., director of the National Institutes of Health. Harold has since become President of Memorial Sloan-Kettering Cancer Center in New York City. He has been coming to meetings at CSHL for over a quarter century and is well known for his research accomplishments—including the discovery of cellular oncogenes with Mike Bishop that garnered them a Noble prize—as well as for his extraordinary leadership. As director of the NIH, Harold stressed the importance of biomedical research for the improvement of the human condition, and oversaw a dramatic increase in public support of biomedical science.

Harold Varmus's birthday celebration at the Laboratory, which took the form of a two-day symposium on cancer cell biology, was organized by Steve Hughes of the National Cancer Institute (NCI); Titia de Lange of Rockefeller University; Roel Nusse, HHMI investigator at Stanford University Medical Center; all former Varmus laboratory members; and Suzanne Oritz of Varmus' lab. at the NCI. Prominent speakers included long-time collaborator Mike Bishop, fellow retrovirologists David Baltimore and Peter Vogt, and former high school mate Gerry Fink.

Undergraduate Research Program (URPs)

The 1999 summer Undergraduate Research Program consisted of 26 students—12 women, 14 men—from 12 countries. They were chosen from among 520 applicants from around the world. The objective of the program is to provide a greater understanding of the principles of biology. It instills in the students an awareness of major topics of investigation, helps develop intellectual tools necessary for modern research, exposes students to the process of research, and allows them to meet top scientists who visit CSHL.

The program received financial support in 1999 from the C. Bliss Memorial Fund, Burroughs Wellcome Fund, Jephson Educational Trust, Dorcas Cummings Memorial

Lecture, Grace Professorship, and the URP Endowment (composed of the Burroughs Welcome Fund, Emmanuel Ax Fund, Garfield Fund, Libby Fund, Olney Fellowship, Shakespeare Fellowship, Von Stade Fellowship, Glass Fund, and the Read Fund).

Partners for the Future

The Partners for the Future program was established in 1990 to give outstanding high school seniors the opportunity to work on original research projects in a laboratory under the supervision of a scientist-mentor. The students spend a minimum of ten hours per week at the Laboratory, beginning in October, and at the conclusion of the program in March, present a scientific summary to an audience of scientists, teachers, and parents. In the process, the students gain valuable research experience and are paid a stipend for their efforts. This program is supported by the DNA Learning Center Corporate Advisory Board from proceeds of their annual fund and golf tournament.

The participants for the 1999/2000 school year were David Rubenstein, Bethpage High School (mentor: Yuri Lazebnik); Michelle Kollmeier, Half Hollow Hills High School West (mentor: Marja Timmermans); Jeffrey Winer, Plainview–Old Bethpage J.F.K. High School (mentor: Masaaki Hamaguchi); Justin Singer, Jericho High School (mentor: Eric Drier); Laura Roche, Cold Spring Harbor High School (mentor: Michael Hengartner); and Michel John Maloof, Garden City High School (mentor: Frances Hannan).

Board of Trustees

C. Thomas Caskey concluded his term on the Board of Trustees in 1999. Since 1996, Tom brought extensive research experience to the Board of Trustees, particularly in the area of human genetics. Tom first visited the Laboratory in the late 1960s to attend a Phage Course. We shall miss his always active participation at Board meetings.

Three new members were elected to the Board in 1999: Robert Lindsay is a managing general partner with Bessemer Partners where he is involved with overseeing the private equity investment activities of Bessemer Securities Corporation. Robert grew up here in Laurel Hollow and his family has had a long-running relationship with the Laboratory—his father, Robert V. Lindsey, served as a Trustee on the Laboratory's Board and treasurer and vice president of LIBA (now CSHLA), and his aunt, Mary D. Lindsay, served as vice chairman on the Laboratory's Board. Ed Scolnick is president of Merck Research Laboratories, and executive vice president of science and technology for Merck and Co., Inc. Before that, he was at the National Cancer Institute where he discovered the viral *RAS* oncogene. Arthur Spiro is president of AMSTEX Enterprises, Inc., a company that deals in corporate and business marketing, planning, acquisitions, mergers, textile patents, investments, licenses and leases. He is also adjunct professor of entrepreneurial studies at the College of Business and Public Affairs at Clemson University in South Carolina.

David L. Luke III

On April 23, former Chairman of the Board of Trustees David L. Luke III was honored at a dinner at the Piping Rock Club in Locust Valley. The dinner was held in appreciation of his contributions to the Laboratory during his 12-year tenure on the Board, and for his role in the establishment of the

Watson School. The keynote speaker was Vartan Gregorian, president of the Carnegie Corporation of New York and former president of Brown University. Ed Harlow of Massachusetts General Hospital Cancer Center and Harvard Medical School offered thoughtful remarks as well. A special treat during the evening was a performance by Kitty Carlyle Hart, whose voice and vocal style from a time past were flawless.

Mr. Luke is now an honorary trustee of the Laboratory and chairs the ongoing $32 million capital campaign to endow the Watson School.

Kitty Carlyle Hart

CSHL Association

The CSHL Association (CSHLA) held its annual meeting on February 2. The membership thanked its retiring directors—W. Dillaway Ayres, David C. Clark, Carol E. Large, Phillip M. Satow, Jordan Saunders, and Lisa Schiff—and elected James Spingarn as president of the CSHLA, and Mary Alice Kolodner, Eileen Pulling, and Larry Remmel as new directors.

The meeting featured a lecture by scientist and author Edward O. Wilson of Harvard University. His lecture was entitled "Consilience: Science Meets the Arts." After the meeting, members of Next Generation Initiative (NGI) were invited to the DNA Learning Center for a special presentation on DNA science by DNALC Director Dave Micklos and then-Assistant Director John Kruper. On March 7, the CSHLA sponsored a lecture by John, introducing the DNA Learning Center's new Web site, *DNA from the Beginning*, the Internet's first authoritative genetics primer.

Fanny Elder, Ann Seifert, E.O. Wilson

On April 17, the Association held a Jazz Benefit. The performance featured Stanley Turrentine and Kenny Blake on saxophone, Harold Betters on trombone, Kevin Moore and Max Leake on piano, Paul Thompson on bass, and John Uskridge and Roger Humphries on drums. The evening included an elegant dinner and raised money in support of young scientists' research at the Laboratory.

The annual major donors cocktail party was held in the Locust Valley home of Carolyn and Ollie Grace. The elegant setting provided an enjoyable opportunity for Laboratory scientists to mingle with those whose generosity helps support their work.

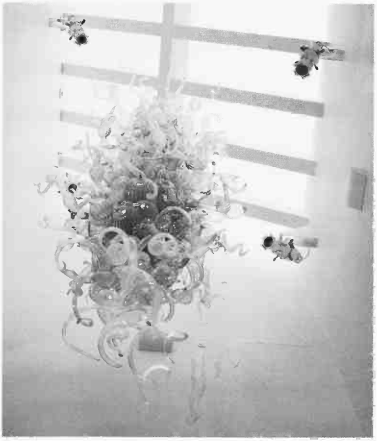
DNALC Corporate Advisory Board (CAB)

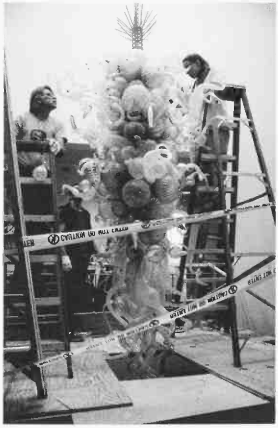
Each year the DNALC Corporate Advisory Board (CAB), now chaired by Jack Leahy, supports the DNALC to the extent of 10% of its operating budget by means of a golf tournament and annual fund.

On October 24, Jim and Liz Watson hosted an evening of cocktails and supper at Ballybung to thank members of the CAB for their support and to introduce potential new candidates for the CAB.

Dedication of the Luke Building

Nancy Marks and Liz Watson at
Marks Laboratory Dedication





Assembly of the Chihuly glass chandelier

Building Projects

Genome Research Center at Woodbury

Renovations are under way at Cold Spring Harbor Laboratory's new Genome Research Center at Woodbury. Located on a 12-acre site, 7 miles south of the Laboratory's main campus, the Genome Research Center will enable the Laboratory to expand its basic research initiatives in genomics and other gene-based technologies. Upon its completion, the Genome Research Center at Woodbury will be a 72,000-square-foot facility, outfitted with state-of-the-art scientific technology.

Research in the center will initially focus on sequencing the *Arabidopsis*, rice, and mouse genomes; identifying genes that are mutated in breast and other cancers; bioinformatics; and establishing a facility funded by the National Science Foundation for identifying mutants of maize. The administrative offices of the CSHL Press, as well as a 125-seat auditorium and a full-service dining commons, will round out this first-class, scientific complex.

Other Building Projects

Two other major building projects were completed in 1999. On June 12, the David and Fanny Luke Building was dedicated. Located on the lower roadway facing the inner harbor, the Luke Building consists of two renovated, and now attached, buildings: the old Carpenter Shop and the Power House. Designed by Jim Childress of Centerbrook Architects, the Luke Building houses the offices of Development, Human Resources, and Public Affairs.

The dedication on October 17 of the new Nancy and Edwin Marks Laboratory for advanced brain imaging was another milestone in the neuroscience program. The state-of-the-art facility was designed by William H. Grover, FAIA, and Walker Burns, AIA, of Centerbrook Architects, and constructed by contractor E.W. Howell, Inc. The Marks Laboratory consists of the Keck Research Laboratories, the Starr Foundation Teaching Laboratory, the Martha Farish Gerry Seminar Room, and shared space for research and support staff. Scientists Karel Svoboda, Zach Mainen, and Tony Zador are studying various aspects of the brain using advanced imaging and electrophysiology techniques in the Marks Laboratory.

The aesthetic centerpiece of the Marks Laboratory is a magnificent glass chandelier created by renowned glass artist Dale Chihuly. Jim and Liz Watson commissioned the piece, which is fashioned after dendrites, and donated it to the Lab.

We are nearing completion of another research facility—the Samuel Freeman Laboratory. There, scientists will use and develop sophisticated computer applications for studying the brain and its function in a relatively new field known as computational neuroscience. The new building, located just north of Beckman Laboratory and across the courtyard from Marks Laboratory, was made possible by a gift from the Samuel Freeman Charitable Trust, headed by CSHL Trustee Bill Murray. We have already recruited one scientist to do research in that building—Dmitri Chklovskii from the Salk Institute—and are looking forward to a dedication of this building in late Spring 2000.

Robertson Research Fund

Since 1973, the Robertson Research Fund has been the primary in-house support for science at the Laboratory. The fund has grown from approximately $8 million in 1973 to now

more than $96 million. Last year, Robertson funds supported cancer research in the labs of David Helfman, Michael Hengarter, Nouria Hernandez, Tatsuya Hirano, Leemor Joshua-Tor, Scott Lowe, Ryuji Kobayashi, Adrian Krainer, Jacek Skowronski, David Spector, and Rui-Ming Xu; neuroscience research in the labs of Grisha Enikolopov, Jerry Yin, and Yi Zhong; and plant research in Rob Martienssen's lab. In addition, Robertson funds supported new investigators Andy Reiner and Shiv Grewal.

The Marie H. Robertson Memorial Fund, devoted to neuroscience, gave support to Grisha Enikolopov's lab and start-up support to scientist Zach Mainen.

Major Gifts

1999 was an exceptional year for fund-raising. We conducted two major campaigns—one to endow the Watson School of Biological Sciences and the other to fund construction of the Nancy and Edwin Marks Laboratory—and the results for both were on target. We are most fortunate to have such generous and supportive friends.

The Watson School of Biological Sciences

Former chairman of our Board of Trustees, David L. Luke III, is spearheading the campaign to endow the Watson School. The progress to date has been very good. The Dean's Chair was established with a pledge of $1,175,000 from the Lita Annenberg Hazen Charitable Trust and $500,000 from the Annenberg Foundation, both made possible by Leon and Cynthia Polsky.

Mr. and Mrs. Leslie C. Quick, Jr., established the Fund for Innovative Graduate Education with a $1 million gift (bringing their gifts to the graduate school to $2.3 million), and Nicholas Forstmann pledged $500,000 in support of a core course, Scientific Exposition and Ethics.

Student fellowships were established by Mr. and Mrs. William A. Miller and The William Stamps Farish Fund, each with a $1 million pledge. Curt Engelhorn established fellowships for European students, known as Engelhorn Scholars, with a $5 million gift through the European Foundation for the Advancement of Medicine.

Faculty lectureships were established by Mr. and Mrs. George W. Cutting, Mr. and Mrs. Norris Darrell, Jr., Mr. and Mrs. Edward H. Gerry, the Esther A. & Joseph Klingenstein Fund, Mary D. Lindsay, and Quick & Reilly Group, with gifts of $300,000 each.

Visiting lectureships were provided by Mr. and Mrs. John P. Cleary, Mr. and Mrs. Edward H. Gerry, and The Seraph Foundation, with gifts of $100,000 each.

Gifts to support the School infrastructure were also appreciated. David H. Deming and Henry U. Harris each gave $50,000 toward the renovation of the students' residence, the Knight House.

Brain Imaging

Gifts to the neuroscience imaging initiative in 1999 were also significant and most welcome. The Starr Foundation gave $1,100,000 to establish a state-of-the-art teaching lab, and the G. Harold and Leila Y. Mathers Charitable Foundation gave $870,419 in start-up funds for research in Karel Svoboda's laboratory.

Although construction and equipment funds are often the hardest to raise, we were most pleased with extraordinary support from the following people and foundations. Toward con-

struction: The Ira W. DeCamp Foundation gave $500,000; The Weezie Foundation gave $100,000; and Mary D. Lindsay gave $50,000. Toward equipment: the Marks Family Foundation gave $1 million (bringing the Marks' gifts to the brain imaging program to $3.5 million through 1999); the Fannie E. Rippel Foundation gave $355,000; the William E. and Maude S. Pritchard Charitable Trust gave $255,000; and the Fairchild Martindale Foundation gave $50,000.

Samuel Freeman Charitable Trust, through William Murray, initiated a $1 million gift toward the construction of the Samuel Freeman building, which will house our new computational neuroscience program.

Research Support

We received the following generous gifts in support of research in 1999: the Davenport Family Foundation gave $325,000 toward cancer research; the American Cancer Society contributed $360,000 for cancer research; and the Breast Cancer Research Foundation gave $200,000 toward breast cancer research. The Ellison Medical Foundation and the Sidney Kimmel Foundation for Cancer Research each gave $200,000 to cancer research.

The Neurofibromatosis Foundation Inc.—through its Illinois, Mass Bay, and Texas chapters—gave $95,000 to neurofibromatosis research at the Laboratory. The Rita Allen Foundation gave $50,000 to cancer research, and the Seraph Foundation gave $50,000.

In postdoctoral support, we received generous gifts from Mr. and Mrs. Alan Seligson, $70,000; the Helen Hay Whitney Foundation, $69,000; and the Goldring Family Foundation, $60,000.

Plant Research

Our plant research program benefitted from the establishment of a Plant Consortium. Westvaco Corporation, Monsanto, Novartis, and Zenica have pledged $135,000 each per year for four years (1998–2002).

Education Support

In April 1999, the Howard Hughes Medical Institute (HHMI) made a grant of $1.32 million in support of the Laboratory's advanced scientific courses in neuroscience, molecular biology, and structural biology, as well as a new program in advanced imaging techniques. HHMI also made a grant of $500,000 to the DNA Learning Center in support of the new *VectorNet*, a mobile bioinformatics teaching lab that will bring bioinformatics education to students at New York City schools during the academic year and training workshops to teachers across the United States during the summer.

HHMI began funding education at the Laboratory back in 1988, with a grant that enabled the Laboratory to extend its program of summer courses to an intense, nearly year-round schedule, and funds earmarked for the construction of the Hughes Teaching Laboratories in the Beckman Neuroscience Laboratory. HHMI's continued support has been the cornerstone of our excellent educational program, for which we are most grateful.

Mr. and Mrs. David L. Luke III gave a generous contribution of $490,234 toward the David and Fanny Luke Building.

President's Council

The President's Council was formed six years ago, bringing together leaders from business, research, and biotechnology who are interested in science and Cold Spring Harbor Laboratory. Members of the President's Council contribute $25,000 or more annually to support the CSHL Fellows program for top young scientists fresh from their Ph.D. or M.D. studies. The program allows promising young researchers to pursue their own high-level, independent research, rather than pursuing a more traditional postdoctoral fellowship in the laboratory of an established scientist.

The 1999 meeting of the President's Council, held May 14–15, focused on the Evolution of Happiness. It began with a luncheon at Ballybung followed by an afternoon lecture by CSHL scientist Jerry Yin on long-term memory formation. The keynote speaker, Dr. Stanley Watson, Professor of Psychiatry and Co-Director of the Mental Health Institute at the University of Michigan, described the role of the molecule POMC in human psychology. The speakers on Saturday were Dr. Huda Akil also from the University of Michigan, Dr. Jonathan Rees from the University of Newcastle (UK) Medical School, and Dr. Stephen O'Rahilly from the Wellcome Trust Clinical Research Facility in Cambridge. The mix of leaders from the business world and the scientific community evoked interesting insights, as well as provocative discussions. The following were members of the 1999 President's Council:

Abraham Appel, Appel Consultants, Inc.
Peter Bloom, General Atlantic Partners, LLC
Michel David-Weill, Lazard Freres & Co., LLC
Jacob Goldfield, Goldman, Sachs & Co.
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.
James H. Simons, Renaissance Technologies Corporation
Ronald P. Stanton, Transammonia, Inc.
Charles L. Stone, Jr., M.D.
Sigi Ziering, Diagnostics Products Corporation

The Harbor Society

The Harbor Society honors those distinguished individuals who have contributed to the CSHL planned giving program by including the Laboratory in their estate planning. The Harbor Society gained six new members (or member couples) in 1999: Barbara and Arthur Crocker, the estate of Lachlan Braden, Lois Learned, Mr. and Mrs. Karl Runkle, Miss Eleanor Greenan, and Mrs. Lawrence Marks. The annual dinner for the Harbor Society with Jim and Liz Watson was held at Ballybung on May 2. We are most grateful for the foresight and generosity of the Harbor Society members.

Breast Cancer Support

In 1999, there was again outstanding support from breast cancer advocacy groups. Through 1 in 9: the Long Island Breast Cancer Action Coalition—our longest-running breast cancer

donor—we received $100,000 including $10,000 from Rick Shalvoy, proceeds from his Row For a Cure. This organization's support since 1994 has totaled $411,000.

The Lillian Goldman Charitable Trust and Mrs. Lillian Goldman (through The Breast Cancer Research Foundation) gave $200,000 to support breast cancer research in Michael Wigler's lab.

We were also pleased to have the support of several groups for the first time in 1999. The Manhasset Breast Cancer Coalition gave $25,000. The Long Island Foundation for the Elimination of Breast Cancer gave $15,750, and the Elizabeth McFarland Foundation (through the Long Island Foundation for the Elimination of Breast Cancer) gave $20,447. The Long Beach Breast Cancer Coalition gave $500.

Special Events and Public Outreach

Cancer Lecture Series

The Laboratory hosted a series of four public lectures about cancer given by four of the country's leading cancer specialists. The first, on March 10, featured Richard D. Klausner, M.D., director of the National Cancer Institute. The title was "The War Against Cancer: Where Has Science Brought Us and Where are We Going?" Former Laboratory staff member Douglas Hanahan, Ph.D., of the University of California at San Francisco, delivered the second lecture on October 12, entitled "The Conspiracy of Cancer Cells."

Richard D. Klausner

The third talk, held on October 19, was given by Neil Caporaso, M.D., of the National Cancer Institute and was entitled "Cracking the Causes of Cancer: What We Know; What We Don't Know." The series concluded on October 26 with Mark Pegram, M.D., Department of Medicine, Division of Hematology/Oncology, University of California at Los Angeles, speaking about "Targeted Therapy for Breast Cancer."

School Lecture Series

We held three Great Moments in DNA Science lectures in 1999. On April 27, John Kruper of the DNA Learning Center spoke about the fruits of the Human Genome Project in a talk titled "Biology's Gold Rush: Mining Genes from the Human Genome Project." On May 4, Laboratory scientist Michael Greenberg spoke about HIV research in a talk titled "Molecular Studies on HIV Nef, an Essential Viral Protein." On May 11, Laboratory scientist Richard McCombie discussed a critical step in genomic research in "Sequence Analysis of Complex Genomes." These lectures are designed for a teen-aged audience and attract students and teachers from many area high schools.

Other Lectures

On September 26, we hosted a public lecture sponsored by the World Wildlife Fund (WWF) in Grace Auditorium. The president of WWF, Kathryn S. Fuller, talked about "A Living Planet for the next Millennium."

On October 3, the organization Teenspeak, together with the Town of Huntington, held a "Festival Within—A Marathon Poetry Reading" in Grace Auditorium. That event included the participation of several Pulitzer-prize-winning poets.

We also continued our Lloyd Harbor Seminars with a lecture by John Coraor of the Heckscher Museum in nearby Huntington on May 20, entitled "The Lives and Works of Arthur Dove and Helen Torr."

Concerts

In addition to the benefit concerts described earlier, we hosted 11 free public concerts during CSHL meetings, when we have a large captive audience. Many meeting participants enjoy the cultural respite from their intense scientific sessions. They were as follows:

April 24	Meng-Chieh Liu, pianist
May 1	Irina Muresanu, violinist, and Tatiana Goncharova, pianist
May 8	Christopher Taylor, pianist
May 15	Judith Ingolfsson, violinist, and Ronald Sat, pianist
May 29	Jennifer Frautschi, violinist, and Benjamin Loeb, pianist
Aug. 21	Ayako Yoshida, violinist, and Andrew Armstrong, pianist
Aug. 28	Dmitri Berlinsky, violinist, and Elena Baksht, pianist
Sept. 4	Sophie Shao, cellist, and Adrienne Kim, pianist
Sept.18	Michael Shih and Patricia Sunwoo, violinists; Ori Kam, violist; and Kristian Reiko Cooper, violoncellist
Oct. 2	Arcadian Trio: Ara Gregorian, violinist; Andrew Russo, pianist; and Raphael Bell, cellist
Oct. 9	Brentano String Quartet: Mark Steinberg and Serena Canin, violinists; Misha Amory, violist; and Nina Maria Lee, cellist

Brentano String Quartet

(*Back row*) Mary Ellen Goldstein, Michael Ockler, Dr. James Watson, Robert Gensel, Dessie Carter, Jim Hope, Bruce Stillman
(*Center rwo*) Daniel Miller, James (Herb) Parsons, Maureen Berejka
(*Front row*) Robert Pace, Carmelita Bautista, Susan Schultz, Katya Davey, Carlos Mendez

Long-term Service

On June 30, employees celebrating milestone anniversaries with the Laboratory were honored at a poolside dinner at Robertson House. Lane Smith, veteran plumber, celebrated 25 years at Cold Spring Harbor Laboratory this past year.

Incredibly to me, I celebrated my 20th anniversary with the Laboratory. Although I had visited the lab in 1978 as a graduate student to speak at the annual CSHL Symposium, I arrived in 1979 to work as postdoctoral fellow in Mike Mathews' lab. The last 20 years have seen great progress in biology at CSHL and I would not have missed a minute of it. All of the following people arrived at the Laboratory that same year, and over the course of two decades, each has had an impact on my life and work at the Laboratory: Maureen Berejka, administrative assistant to the President; Judith Cuddihy, editor for CSHL Press Acquisitions and Development; Katya Davey, hostess for Robertson House; Jim Hope, manager of Food Services; Carlos Mendez, bookkeeper and cash manager; John Meyer, lead painter; Michael Ockler, supervisor of Scientific Art and Photography; James (Herb) Parsons, director of Audiovisual; and Susan Schultz, director of grants contracts.

We also had six people celebrating 15-year anniversaries with the Laboratory: Carmelita Bautista, research associate and facilities manager; Dessie Carter, housekeeper; Robert Gensel, manager of security; Mary Ellen Goldstein, part-time accounts payable supervisor; Daniel Miller, grounds foreman; and Robert Pace, business systems manager.

Changes in Administrative Staff

Maureen Bell joined us as administrative director of the Genomic Research Center. Maureen has a master's degree in biochemistry and an MBA in finance. She was formerly with American International Group (AIG) in New York City as a senior technical services manager. Lilian Gann became assistant dean of the Watson School of Biological Sciences.

Changes in Scientific Staff Titles

Concurrent with the establishment of the Watson School of Biological Sciences, the Laboratory adopted an academic faculty title structure: assistant professor, associate professor, and professor.

Departures

Douglas Conklin went to work at Genetica. Ueli Grossniklaus returned to Switzerland to become a staff scientist at the Friedrich Miescher Institute in Basel. Thomas Misteli (HHMI) became principal investigator at the National Cancer Institute (NIH) at Bethesda, Maryland. Peter Nestler moved back to Germany to accept a position as research scientist at Hoechst Marion Roussel Institute in Frankfurt, Germany. Jing (Jenny) Wang left for a career opportunity at Genica.

New Faculty and Staff

Dmitri Chklovskii was recruited from the Salk Institute as an assistant professor, to initiate studies in computational neuroscience in the newly constructed Samuel Freeman Building. Tony Zador was recruited from the Salk Institute to an assistant professorship, and Zach Mainen, upon completion of his postdoctoral studies with Roberto Malinow and Karel Svoboda at CSHL, was also appointed assistant professor—both as part of the expansion of our neuroscience program. Sang Yong Kim was recruited from the University of Michigan to establish a valuable resource for CSHL scientists: a transgenic mouse and gene targeting facility.

Promotions

Leemor Joshua-Tor and Michael Zhang were both promoted to associate professor. Vivek Mittal completed postdoctoral studies in Nouria Hernandez's lab and was promoted to research investigator in Michael Wigler's lab. Michael Myers was promoted from postdoctoral researcher to senior fellow in Nick Tonks' lab. Zach Mainen (Malinow and Svoboda labs) has been appointed assistant professor here at Cold Spring Harbor Laboratory.

Visiting Scientists

Five visiting scientists joined us this year: Eli Hatchwell came from Wessex Human Genetics Institute at Southampton General Hospital in Southampton, England, to study in Michael Wigler's lab; Boris Kuzin returned from the Russian Academy of Sciences in Moscow to study once again in Grisha Enikolopov's lab; Nathalie Pavy came from Gent University, Belgium in Versailles, France, to study in Lincoln Stein's lab; Hilde Grassmo-Wendler came from the Max Delbrück Centre for Molecular Medicine in Berlin, Germany, to study in Michael Wigler's lab; and Fumio Shiobara came from the National Institute of Agrobiological Resources in Ibaraki, Japan, to study in David Jackson's lab.

Six visiting scientists wrapped up their stays here: Jiaxin An returned from Yi Zhong's lab to his position as professor at the China Academy of Space Technology in Beijing, China; Shern Lin Chew returned from Adrian Krainer's lab to his position as senior lecturer/consultant at St. Bartholomew's Hospital in London; Jyotsna Dhawan returned from David Helfman's lab to India, where she is staff scientist at the Center for Cellular and Molecular Biology in Hyderabod; Imran Siddiqi returned from Ueli Grossniklaus' lab to his position as staff scientist at the Center for Cellular and Molecular Biology in Hyderabod, India; Toshiro Tsukamoto returned from David Spector's lab to Hyogo, Japan where he is a research associate at the Himeji Institute of Technology; and Hannes Buelow, visiting scientist in Linda Van Aelst's lab, left to begin postdoctoral studies with Oliver Hobert at Columbia University in New York City.

Postdoctoral Departures

Christine Berthier (Helfman lab) left to become assistant professor at the University Claude Bernard in Lyon, France.

John Connolly (Tully lab) is continuing his postdoctoral studies at the Foundation Jean Dausset-CEPH in Paris, France.

Francesca Demarchi (Stillman Lab), a visiting postdoc, returned to her postdoctoral position at the ICGEB in Trieste, Italy.

Serge Desnoyers (Hengartner lab) accepted a position as adjunct professor at Laval University in Sainte-Foy, Canada.

Dennis Dong (Wigler lab) is now a staff scientist with ZymoGenetics in Seattle, Washington.

Howard Fearnhead (Lazebnik lab) was appointed principal investigator at the National Cancer Institute Research and Development Center in Frederick, Maryland.

Andrew Fraser (Hengartner lab) is continuing his postdoctoral research in Julie Ahringer's lab at the Wellcome CRC, Cambridge, England.

Anton Gartner (Hengartner lab) accepted an appointment as group leader at the Max Plank Institute in Munich, Germany.

Ilya Ioschikhes (M. Zhang lab) is now an Instructor at the Albert Einstein College of Medicine, Bronx, New York.

Nobuhiro Kashige (Kobayashi lab) accepted an assistant professorship at Fukuoka University, in Japan.

Balazs Lendvai (Svoboda lab) accepted a position as assistant professor at the Institute for Experimental Medicine in Budapest, India.

Hong-Xiang Liu (Krainer lab) went to a research scientist appointment at Phylos, Inc., in Lexington, Massachusetts.

Mireya Marin (Van Aelst lab) is taking some time off to be with her family.

Vivek Mittal (Hernandez lab) moved over to Mike Wigler's lab here at CSHL as a research investigator.

Naoki Nakaya (Enikolopov lab) was appointed assistant professor at Okayama University Medical School in Okayama, Japan. He returned to CSHL shortly thereafter to work as a visiting scientist back in the Enikolopov lab.

Laurence Parnell (McCombie lab) moved to a job at Cereon Genomics, a subsidiary of Monsanto located in Cambridge, Massachusetts.

Jean-Christopher Poncer (Malinow lab) has accepted an assistant professorship at the Pasteur Institute in Paris, France.

Baskar Ramamurthy (Grossniklaus lab) will continue his postdoctoral studies with Ueli at Friedrich Miescher Institute in Basel, Switzerland.

Minoru Saito (Tully lab) became an assistant professor at the Tokyo Metropolitan Institute of Neuroscience in Tokyo, Japan.

Kaetrin Simpson (Stillman lab) moved over to the CSHL Press as a project editor in October and was to be the managing editor of this volume. Much to the deep sorrow of all of us, Kate lost her battle with a malignant brain tumor on January 21, 2000.

Charles Spillane (Grossniklaus lab) will continue his postdoctoral studies with Ueli at Friedrich Miescher Institute in Basel, Switzerland.

Peiqing Sun (Beach lab) accepted an appointment to an assistant professorship at Scripps Research Institute in San Diego, California.

Jack Tabaska (M. Zhang lab) is now a bioinformatics scientist at Monsanto in St. Louis, Missouri.

Daan Van Aalten (Joshua-Tor lab) is now a staff scientist at the University of Dundee in Dundee, Scotland.

Jean-Philippe Vielle-Calzada (Grossniklaus lab) is now a professor at the Center of Investigation and Studies in Avangados, Mexico.

Tomoki Yokochi (Hirano lab) is continuing his postdoctoral research in Alan Wolffe's lab at the National Institute of Child Health and Human Development (NICHD) at the NIH in Bethesda, Maryland.

Shahid Zaman (Malinow lab) is now a staff scientist at the University of Bristol in Bristol, England.

Shao-Hui Zhang (Tonks lab) is now a scientist with Tanabe Research Laboratories in San Diego, California.

Dong-Jing Zou (Cline lab) is continuing postdoctoral research in Stuart Firestein's lab at Columbia University in New York City.

Graduate Students Departures

Robert Babb completed his Ph.D. in Winship Herr's lab and has accepted a postdoctoral position at Novartis Pharmaceuticals Corp. in Livingston, New Jersey.

Grace Chen completed the Ph.D. portion of her M.D./Ph.D. degree in Arne Stenlund's lab and moved to the University of Michigan to complete her medical degree.

Jianzhong Jiang completed his Ph.D. with Rui-Ming Xu and is now a postdoctoral researcher in Rui-Ming's lab at CSHL.

Tracy Kuhlman completed her Ph.D. with Nouria Hernandez and went to work for CSHL Press as a developmental editor based in her Seattle, Washington home.

Qiong Liu completed her Ph.D. with Michael Hengartner and is now considering new positions.

Paul Mintz completed his Ph.D. in David Spector's lab and has gone on to do postdoctoral research in Renata Pasqualini's lab at M.D. Anderson Cancer Center in Houston, Texas.

James Moore went with Ueli Grossniklaus to the Friedrich Miecher Institute in Basel, Switzerland, where he will continue his graduate studies.

Ahmed Samatar left after receiving his Ph.D.

Andrew Mark Settles completed his Ph.D. in Rob Martienssen's lab and went on to a post-doctoral position at theUniversity of Florida, Gainesville.

Tzu-Ling Tseng completed his Ph.D. in Adrian Krainer's lab and has moved on to a post-doctoral position in the lab of Jeff Struewing and Ken Beutow at the Laboratory of Population Genetics at the National Cancer Institute in Bethesda, Maryland.

Concluding Remarks

As we rapidly move into the next century and indeed into the next millennium, it is clear that the opportunities for future discovery in biology and medicine are enormous. The past century of biology will be remembered as a golden era that witnessed a renaissance in science that will have an impact for many years to come. The early years of the new century will be the age when biology will be only limited by our imagination. There is a distinct sense of excitement in the air; a feeling that we must endeavor to transmit to the broader communities that will ultimately benefit from this research.

As the pace of modern science continues to accelerate, the resources of academic institutions will be considerably strained, particularly when compared to biotechnology and pharmaceutical companies which have access to technologies that are far beyond the financial capabilities of academia. This is particularly the case in the genomic era.

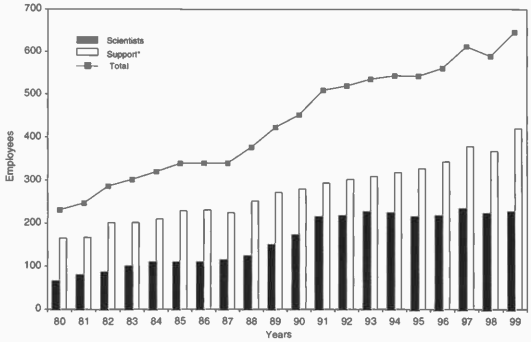
Cold Spring Harbor Laboratory and institutions like us will need considerable financial resources to remain at the forefront of biology, particularly when increases in federal funding of research abate. But leanness might have a positive benefit in the long run, since academic researchers will be forced to think carefully about how these precious resources are utilized, performing the type of basic research that the more remunerative institutions will find increasingly hard to justify. Ideally there will remain a synergism between industry and academia that will be for the benefit of all.

To me, our mission is as clear as it has ever been. We must continue to pursue basic science at the highest possible level, we must make sure that we continue to grow our strong educational programs, making them even more accessible to scientists and students, and we must encourage public support and participation in these exciting ventures at all levels. For together we are entering what will perhaps be the most exciting phase of biology.

March 2000

Bruce Stillman

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



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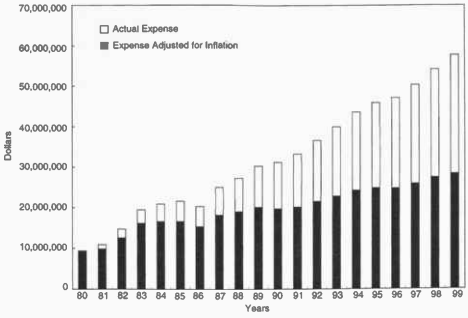
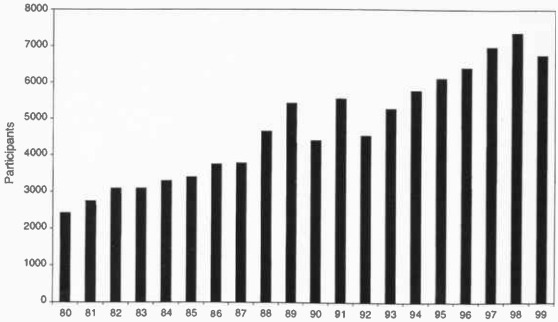


MEETINGS & COURSE PARTICIPANTS



ADMINISTRATION

1999 was a remarkable year for Cold Spring Harbor Laboratory. Our institution was founded in the 19th century, spanned all of the 20th, and now enters the 21st—dynamic, young in spirit, and at the forefront of the biological sciences.

The year began well, with six of the nine Ph.D. candidates offered admission to our new Watson School of Biological Sciences choosing to come to Cold Spring Harbor—a 66% acceptance rate. Two of them held prestigious fellowships from Howard Hughes Medical Institute. All had received other offers of admission from top universities here and abroad. The first class, three men and three women, arrived in late August and in September began the first term of the intense, highly innovative four-year doctoral program. The students' enthusiasm spread to the science faculty and others, many of whom were teaching for the first time and finding enjoyment and satisfaction in the process. During the winter, a very large number of high-quality applications were received for the second class, a good indication that the Laboratory's program has become known as unique and likely to make a major impact on graduate education.

Meanwhile, after several years of planning, the Laboratory embarked on its most-ambitious-ever facilities construction program. Most prominent was the stunning new Nancy and Edwin Marks Laboratory, where neuroscientists now use state-of-the-art excitation laser microscopy to image individual neurons and neural networks deep in the brains of living animals as they experience and react to their environment. The Marks Laboratory was dedicated in November, and its scientists are alreadying working there in two shifts. The Laboratory functions also as a national center where visiting scientists can come to learn new imaging techniques in the new Starr Teaching Laboratory and Gerry Conference Room. A magnificent glass sculpture of a neuron by world-renowned artist Dale Chihuly, a gift from Jim and Liz Watson, graces the main stairwell.

Across a new courtyard opposite the Marks Laboratory, the Samuel Freeman Building for bioinformatics was constructed on the hillside next to the Beckman Neuroscience Center. Here scientists will process the enormous volume of data being generated by the neuroscience and imaging programs. Also located in the building is Lincoln Stein's program to coordinate and process data generated by the SNP Consortium and release it on the Internet. The Consortium, consisting of several pharmaceutical giants—together with five academic partners—aims to scour the human genome to uncover new heritable DNA sequence variations called single nucleotide polymorphisms (SNPs). A large SNP library could help researchers pinpoint genetic origins of diseases and, potentially, the most appropriate drugs to use to treat them. A visual centerpiece of the Freeman Building is the spacious William E. Murray Room, named for our Trustee who heads the Samuel Freeman Charitable Trust, which was instrumental in arranging the funding for this project.

Off campus in Woodbury, construction of the Laboratory's new $25 million Genome Research Center began in December. All but the shell of the 65,000-square-foot building on the 11-acre site, which the Laboratory purchased from the American Institute of Physics in 1998, had already been demolished. To be completed by the end of the year 2000, the new Center will have laboratories for studies of gene sequencing and function and for DNA chip array technology. There will be a plant technology center and an animal facility for the breeding of mouse model systems that are extensively used in cancer research and in neuroscience behavior studies. Cold Spring Harbor Laboratory Press will have new offices in the Center, too.

On the waterfront of our Laurel Hollow campus, the David and Fanny Luke Building was completed and dedicated in May. This represents a complete reconstruction of our old Power House and Carpentry Shop, and it now houses our Human Resources, Development, and Public Affairs Departments. In the Development offices, the new Vernon Merrill Conference Room honors the memory of the greatly revered past president of the Cold Spring Harbor Laboratory Association. In 1997, the Laboratory purchased a seriously deteriorated 18th-century residence that used to serve as Cold Spring Harbor's first general store and post office, directly across the harbor from the Luke Building in the Cold Spring Harbor Historic District. During 1999, the house was completely renovated as a first-year residence for Watson School students. Each student has a private room and bath, individual study area, and use of the centrally located "period" common room. The new residence has been renamed the Townsend Knight House after Townie Knight, an Honorary Trustee of the Laboratory, who grew up and spent much of his early youth living in the original structure.

Finally, all but invisible from Bungtown Road at the site of the Pond Parking Lot, a new heating and air-conditioning facility was constructed to serve many of our lower-campus buildings, such as Freeman, Marks, Sambrook, James, Nichols, Page, and Delbrück, eliminating noisy outdoor chillers and rooftop cooling towers. The simultaneous management of all these projects and many other laboratory and office alterations during a single year is an indication of the capabilities and skill of the Laboratory's Facilities Department, its Director, Art Brings, and the many contractors with whom they work.

In past reports, I have often stressed the importance to the Laboratory of maintaining strong finances. 1999 was no exception to the more than 10-year period during which a balanced budget has been achieved after allowing fully for depreciation. Revenues for the year again reached a new high, advancing approximately 8% to $57,670,000. After expenses of $53,840,000, including depreciation of $3,526,000, there was a modest operating surplus of $304,000. Positive cash flow for the year was $3,830,000. This brings the total funds generated from operations over the past decade to more than $30 million. These funds are used to maintain and modernize our capital plant, purchase increasingly expensive scientific equipment, and pay for the start-up costs of new programs. Financial results were more than $1,000,000 better than projected at the beginning of the year, reflecting our traditional policy of projecting grant revenues conservatively, given the uncertainty associated with federal grant reviews. It was not necessary to use any portion of the $1.9 million of reserves set aside in past years for unanticipated costs of new programs. The good financial results again reflect our scientific staff's success in obtaining grants, another good year of attendance at our meetings and courses, a strong contribution from the Cold Spring Harbor Laboratory Press, better than break-even operations at Banbury Center and the DNA Learning Center, higher than expected investment income, and careful cost control by administrative departments. A modest deficit from the first-year operation of the Watson School was fully absorbed.

Also remarkable during the year was further rapid growth of the Laboratory's permanent endowment, which collectively consists of the Robertson and the Cold Spring Harbor Funds. At year-end, the endowment totaled $220 million, representing an increase of $53 million, or nearly 32%, from $167 million 12 months earlier. We have outstanding $45 million of tax-exempt bonds issued by the Nassau and Suffolk County Industrial Development Agencies. The gain during 1999 was the result of excellent market return and new additions, which included $4,309,432 of new endowment for the Watson School. The market return on the invested funds, of which approximately 40% was allocated to fixed income, was truly exceptional, rivaling even well-managed pure-equity portfolios. Among the three equity-investment managers

employed by the Laboratory, Essex Investment Management returned 111%, exceeding 99% of comparative equity managers; the Vanguard PRIMECAP Fund returned an impressive 42%; and U.S. Trust Company, selected as a new equity manager in October to replace the Miller Anderson & Sherrerd Value Fund, comfortably surpassed the Standard and Poor's 500 Index. Miller Anderson & Sherrerd and U.S. Trust Co. continue to manage the fixed-income investments. As in past years, the Endowment is invested in a balanced mix of equities, fixed income, and short-term instruments. The drawdown policy is 4% per annum based on a three-year moving average of year-end market values. The conservatism of this policy has contributed greatly to the growth of the endowment over the years.

Two specialized funds included in the endowment are worthy of special mention. The Science Fund was established by the Board in 1992 and designated as solely for the future support of science. Since 1992, all equity and royalties received from the Laboratory's technology transfer activities have been accumulated in this Fund. In this way, financial gains from Laboratory research financed by federal and other grants are recycled back into future science. In the early years, the various equities were carried at nominal valuations, and the Fund grew slowly to a total of $490,000 at the end of 1996. Thereafter, many of the equities began to be publicly traded, and under the supervision of a subcommittee led by John Phelan, chairman of our Commercial Relations Committee, some portions of the Laboratory's holdings were sold and converted to more conventional investments. By year-end 1997, 1998, and 1999, the Science Fund was valued at approximately $4.8 million, $6.8 million, and $15.9 million, respectively. By March 31, 2000, it had reached $24.1 million, and $15.2 million of it had been converted to conventional investments managed by the Laboratory's regular managers. The Science Fund was established with the hope that it might one day represent substantial additional support for the science program and replace royalty income the Laboratory has been receiving from a patent expiring June 30th of this year. This goal now seems well within reach. A second fund, the Quest Fund, was established by the Executive Committee in December 1999 and designated for the support of both the science and the education programs at the Laboratory. This Fund consists of the equity ownership in SciQuest Corporation—a "B to B Commerce" Internet company specializing in scientific supplies and equipment—that the Laboratory received from the sale of the Cold Spring Harbor Laboratory Press "Source Book" (catalog) and BioSupplyNet (on-line version) to SciQuest in 1998. In late 1999, SciQuest had a successful IPO, and at March 31, the Quest Fund was valued at approximately $4.7 million, then including $1.6 million in conventional investments.

The success of the Laboratory's technology-transfer activities, which now include some 14 public and private biotech companies based in whole or in part on CSHL research, is a tribute to the skill and entrepreneurial spirit of our scientists. It also reflects the impressive ability of our three-person Technical Transfer Department, headed by John Maroney, assisted by Carol Dempster and Barry Braunstein, in working with the scientific staff, managing patents, and structuring agreements and alliances that have served this institution well. Also, the Department has shouldered responsibility for establishing and managing the Broad Hollow Bioscience Park located on a 20-acre site at SUNY Farmingdale, about a 20-minute drive from the Laboratory. The first building of 50,000 square feet was funded by the State of New York and is scheduled to open in mid-2000 as a home for Laboratory and other young biotech companies.

Another major accomplishment during 1999 was the successful selection, installation, and on-time start-up of new, Laboratory-wide business-computing software. Such projects have taken years and cost several millions of dollars at other institutions. Here, it was accomplished in less than a year and for less than $1 million. The magnitude of this task cannot be overstat-

ed. All administrative departments were involved. The effort was led and coordinated by Dill Ayres, associate administrative director, with the strong support of Bill Keen, comptroller. Lari Russo, assistant comptroller, provided very effective day-to-day direction for the project. Other major participants who worked long hours in their own departments and in the Wawepex "War Room," where training and start-up was centered, include Cheryl Sinclair, Human Resources; Phil Lembo, Purchasing; Susan Schultz, Grants Management; and, very importantly, Jerry Latter and Robert Pace of Information Services.

The Laboratory's payroll, which includes two different pay schedules for more than 700 employees and all the accompanying federal and state tax-reporting requirements, was brought in-house from an outside vendor. On the projected start-up date, January 1, 2000, the whole Laboratory breathed a sigh of relief as newly printed CSHL payroll checks were distributed Laboratory-wide (with only our President going briefly unpaid due to a bank automatic-deposit miscue). The otherwise smooth payroll conversion was attributable primarily to Patty Maroney and to Laura Guterman, who joined the Laboratory in September from Sbarro, Inc., where she was payroll manager. The vendors, Lawson Software and Eisner Consultants, provided the timely guidance and support needed at critical moments. Now, several months into 2000, many administrative personnel continue to put in long hours due to the newness and complexities of the system. It will be some time before all the vastly increased capabilities are fully realized. But those working so hard should have the satisfaction of knowing that their efforts will make it possible to run the Laboratory in this new century on a level consistent with that of the science for which this Institution is known.

The Library, with the irrepressible leadership of Mila Pollack, was another aspect of the Laboratory that was greatly upgraded in 1999. Now staffed weekends, and for longer hours during meetings and courses, the Library has installed the new Web of Science on-line software, which provides scientists with past and present journal articles on their desktop computers. There is a much improved new cataloging system, enhanced color-copying capability, and a staff that is welcoming and anxious to help. The Library can now truly meet the needs of a first-class research institution and is at a level befitting even the small university to which we are being transformed by the new Watson School. Mila is also addressing the task of properly organizing and preserving the extensive archives of the Laboratory and those of Dr. Watson. These embrace many of the highlights of the history of molecular biology and genetics, and it is imperative that they be available for scholars and historians in future years. A recent grant from the Lehrman Institute now makes it possible to hire an experienced archivist and will provide the impetus for an eventual heritage center at Cold Spring Harbor for making available the full story of this fascinating era of science and discovery.

There were a number of key personnel changes in administrative departments during 1999. Deborah Barnes, director of Public Affairs, left the Laboratory at year-end. Among her many contributions, Deborah did much to professionalize the editorial content and look of Laboratory publications. She initiated a widely applauded new series of cancer lectures, the first of which was delivered by Richard Klausner, director of the National Cancer Institute, and another by Doug Hanahan, professor at the University of California in San Francisco and previously on the Cold Spring Harbor Laboratory staff. Deborah was also a most effective lecturer on the art of science writing for the students of the Watson School. She is very talented and a good friend to many of us. We will miss her and hope she visits often in the future.

The year-end marked the departure of Laura Hundt, events coordinator, also in Public Affairs. Laura distinguished herself over the years with too many events to mention. She worked very successfully with the DNA Learning Center Corporate Advisory Board and ran its annual golf

tournament, which year after year set records in participation and fund-raising. Fortunately, we have had the good fortune to find a replacement in Charles Prizzi, who joined us just recently from Long Island University, where he was responsible for all university events on three main—and a number of satellite—campuses.

In Development, we welcomed Peter Wayne as major gifts officer. Peter is concentrating his efforts on raising the $25 million needed for the Woodbury Genome Sequencing Center. Vincent Torti joined the department late in the year as the first development officer specifically for the DNA Learning Center. Vin has much experience in high-school-level education and in fund-raising. He will be responsible for DNALC annual funding, including the golf tournament, and for the capital needed for the planned major building expansion to begin this spring. Cathy Wardell, who for many years has supported so well the activities of the CSHL Association, left to become director of Development at the Portledge School. We will miss Cathy and wish her well in her new position.

All of us, on March 4 of the current year, were greatly saddened by the death of Jack Richards, who for so many years was responsible for Buildings and Grounds at the Laboratory. Dr. Watson wrote extensively about Jack earlier in this report, so suffice it to say that we miss him greatly and extend our deepest sympathy to Cory, Jean, Lynn, and the rest of the Richards family.

No report would be complete without recognition of the invaluable support that Roberta Salant provides for Dill Ayres, John Maroney, and myself and for the members of our Board of Trustees. She is a constant source of needed information, assistance, and good cheer.

In recent years, we have given much thought to planning for the future of administration at the Laboratory. This function at a place such as the Laboratory should be run by someone in their 40s, not in their 60s, as has been my case for several years now. With this in mind, in 1997, I began to look for a successor as administrative director. Fortunately, it was not necessary to look very far. Dill Ayres, a resident of our local Laurel Hollow community, was much interested in science and the Laboratory, and had joined the CSHL Association and become one of its directors. With the considerable help of John Reese, then our treasurer, I was able to persuade Dill to join the Laboratory full-time in 1998 as associate administrative director. In the interim, Dill has had the opportunity to get to know the Laboratory and the staff well. He has taken increasing responsibility for important projects and day-to-day operations. He has vision, good judgment, and a strong business sense. I have much confidence in Dill and, with the strong support of Jim Watson and Bruce Stillman, intend to recommend that the Board of Trustees name him as administrative director at the Annual Board Meeting in November. It is my intention to remain closely associated with the Laboratory, working as needed in areas of future strategic interest. I have much enjoyed my 15 years as administrative director and look forward to a continuation of the challenges and personal relationships that I have found so fulfilling. There can be little doubt that the Laboratory of the 21st century will continue to be a focal point of the biological sciences and an extraordinarily interesting place.

G. Morgan Browne
Administrative Director

April, 2000


RESEARCH

TUMOR VIRUSES

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

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

See previous page for photos of the following scientific staff.
Row 1: Shannon Pendergrast; Setareh Sepehri; Hunter Maats; Antonella Piccini
Row 2: Jason Gregory, Jenny Wang; Joe Fontana, Catherine Kidner, Juana Marie Arroyo, Andrew Groover, Amy Dabrowski; Jose A. Esteban
Row 3: Eli Hatchwell; Katherine Braun, Michael Weinreich; Michael Myers, Helena Palka
Row 4: Roberto Malinow, Julius Zhu; Noriko Saitoh, Susan Janicki; Robert Gasperini, Steve Macnick

TRANSCRIPTIONAL REGULATION

W. Herr E. Julien, D. Aufiero, R. Babb, A. Bubulya, K. Wu, S. Lee, P. Reilly, T. Tubon, J. Wysocka, X. Zhao

We are interested in the mechanisms of transcriptional regulation in human cells. We use the human herpes simplex virus (HSV) as a probe to uncover these mechanisms and to study virus-host cell interactions. Viruses provide simple regulatory networks in which the cellular transcriptional machinery is altered to achieve the goals of virus infection. In a cell infected by HSV, the virus can grow lytically or remain latent for many years. In the lytic cycle, HSV gene expression is initiated by a viral transcription factor called VP16, which is carried in the infecting virion. Before activating transcription, VP16 forms a multiprotein-DNA complex—the VP16-induced complex—on viral immediate-early promoters with two cellular proteins: HCF-1, a protein that regulates cell proliferation, and Oct-1, a POU-homeo domain transcription factor. Once the VP16-induced complex is assembled, VP16 initiates viral gene transcription through a potent transcriptional activation domain.

Our research continues to focus on three principal issues: (1) How do transcriptional regulators activate the basal transcriptional machinery? (2) How do transcription factors, as in the VP16-induced complex, modify their transcriptional activity through selective protein-protein and protein-DNA interactions? (3) What are the natural cellular roles of HCF-1 and Oct-1, and how do they influence HSV infection?

Enhancer Function

T. Tubon, X. Zhao

Our current studies on how transcriptional regulators activate the basal transcriptional machinery focus on two lines of investigation. In the first, we 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. We are using this strategy to dissect the role of the basal transcription factor TFIIB in transcriptional activation by a variety of activators. TFIIB is a particularly attractive subject for study because it is not part of a complex of proteins, making it amenable to genetic and biochemical analyses both in vivo and in vitro.

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 lab here at Cold Spring Harbor Laboratory. The transcriptional activation domains we use to study promoter selectivity of activation domain function discriminate between the mRNA- and snRNA-type promoters. By comparing and contrasting the differing activities of these activators in the variety of promoter contexts, we continue to dissect how activators communicate with the basal transcriptional machinery.

Viral *Trans*-activation

D. Aufiero, R. Babb, A. Bubulya, C. Huang, K. Wu

We study how transcription factors modify their transcriptional activity through selective protein-protein and protein-DNA interaction by studying the structure

and function of the VP16-induced complex. This year, in collaboration with Dr. Xiaodong Cheng (Emory University School of Medicine, formerly of Cold Spring Harbor Laboratory), we reported 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-1 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 was determined at 2.1 Å resolution. The results reveal a novel, seat-like protein structure as illustrated in Figure 1. Over the years, extensive mutagenesis studies by a number of different laboratories have identified residues in VP16 involved in (1) virion formation, (2) DNA interaction, and (3) association with HCF-1 and Oct-1. Mapping of the residues involved in each of these three activities on the VP16 structure indicates that VP16 uses a different surface for each function. Sequences involved in virion assembly and DNA-sequence recognition are structured, but sequences involved in associating with HCF-1 and Oct-1 are disordered (see the dashed line in the center of the figure), suggesting that VP16 undergoes conformational changes during VP16-induced complex assembly. The surface that interacts with DNA is the concave seat (see Fig. 1) and represents a novel DNA-binding domain. Thus, VP16 influences different aspects of the HSV life cycle through use of different surfaces to engineer different types of complexes within the virion and on HSV promoters.

Cellular Functions of HCF-1

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

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

Human HCF-1 is synthesized as a large approximately 2000-amino-acid precursor protein, which is proteolytically cleaved to generate a family of associ-

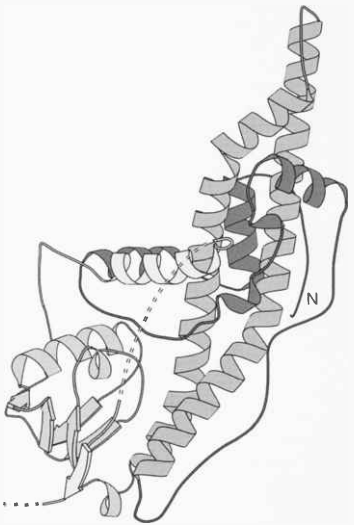


FIGURE 1 A ribbon diagram of the "side-view" of VP16-core protein, illustrating the seat-like structure (adapted from Liu et al. 1999b). The dark-to-light shading is from amino terminus (N) to carboxyl terminus (C).

ated amino- and carboxy-terminal polypeptides. Only the amino-terminal 380 residues of HCF-1, however, are required to associate with VP16 and to stabilize VP16-induced complex formation. This region of HCF-1 is not only involved in interaction with VP16: together with an adjacent basic region, it is also involved in promoting cell proliferation. During the past year, A. Wilson and colleagues described an HCF-1-related protein called HCF-2 (Johnson et al., *J. Virol. 73:* 3930 [1999]). This protein shares the VP16-interaction region with HCF-1 but lacks the basic region of HCF-1, which is also required for cell proliferation.

Last year, in collaboration with the Hengartner lab here at the Laboratory, we reported that HCF proteins have been conserved during metazoan evolution: Extracts from the worm *Caenorhabditis elegans* can stabilize VP16 association with human Oct-1. We showed that *C. elegans* expresses a homolog of human HCF proteins (CeHCF) that can associate with and activate VP16. The pattern of sequence conservation between human and worm HCF, however, is uneven. Like the HCF-2 protein, the amino-terminal sequences involved in interaction with VP16 and cell proliferation are conserved, but the basic region is not conserved in CeHCF. These results suggest that, in its association with HCF-1, VP16 targets the one of two elements of HCF-1 known to be involved in cell proliferation that has been conserved in metazoans. We will continue our studies of CeHCF to uncover the cellular role of this conserved function.

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RNA SPLICING

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

MECHANISMS OF CONSTITUTIVE AND ALTERNATIVE PRE-mRNA SPLICING

RNA splicing is an essential step for the expression of most eukaryotic protein-coding genes. The selection and pairing of authentic splice sites within the spliceosome occur with a very high degree of fidelity, 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 lab has focused on the identification, purification, and molecular characterization of protein factors that are necessary for the catalysis and fidelity of splicing and/or for the regulation of alternative splice site selection.

EXONIC SPLICING ENHANCERS

We have continued to study the nature and function of exonic elements that stimulate the removal of adjacent introns. These elements are thought to be involved in exon definition, splicing fidelity, splicing efficiency, and regulation of alternative exon inclusion. Splicing enhancers are recognized specifically by individual members of the SR protein family. H.-X. Liu developed a powerful method to determine enhancer sequence motifs recognized by individual SR proteins. A natural enhancer in the last exon of the IgM gene was replaced by 20 nucleotides of random sequence, and the resulting library of transcripts was spliced in HeLa S100 extract in the presence of one of four different recombinant SR proteins. Sequences that could promote splicing were selected by an iterative protocol. We found that a very large number of sequences could function as enhancers, suggesting that active enhancers are very common in nature, with perhaps one or more being present in all exons. Splicing in the presence of single SR proteins yielded characteristic short and degenerate consensus motifs, which were defined with the help of M. Zhang here at Cold Spring Harbor Laboratory. These four novel enhancer motifs are more frequent in exons than in introns, and we are currently exploring their predictive value in terms of defining the SR protein requirement for individual transcripts.

Although the motifs we identified may be necessary for enhancer function, they are not sufficient, apparently because of sequence context effects. Exonic silencer elements may have a dominant effect over enhancer elements. For example, A. Mayeda and H.-X. Liu found that a human immunodeficiency virus (HIV) substrate that fails to splice when SC35 is the sole SR protein can do so when a short exonic silencer element is deleted (in collaboration with X.-D. Fu's lab at University of California, San Diego); SC35 enhancer motifs are present in the same exon, but the silencer prevents their recognition or function (Fig. 1). S. Kuusk and J. Zhu showed that a variety of sequences can function as silencers in place of the natural HIV silencer. Although we cannot presently predict what kinds of sequences have silencer activity, A. Zahler's lab (University of California, Santa Cruz) showed, in collaboration with our lab, that a particular silencer in a different HIV exon was recognized by heterogeneous nuclear ribonucleoprotein (hnRNP) A1, and that a high-affinity hnRNP A1-binding site also had silencer function in this context.

S. Chew pursued mechanistic studies of enhancer function. Although we and other investigators had previously determined that enhancers and SR proteins function in the initial stages of spliceosome assembly, the fact that SR proteins remain bound to the spliced exons, and in some cases are exported to the cytoplasm

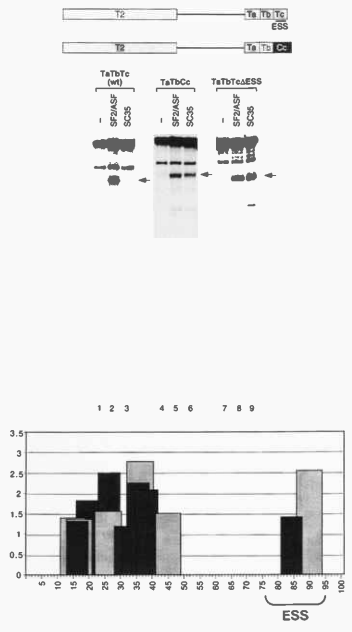

FIGURE 1 An exonic splicing silencer (ESS) in HIV-*tat* pre-mRNA blocks splicing in the presence of a specific SR protein. (*Top*) In vitro splicing of HIV-*tat* pre-mRNA derivatives with replacement or internal deletion of segment Tc in the 3′ exon. The structures of the pre-mRNAs are shown schematically at the top. The deleted ESS element is indicated by a horizontal bar, and the black box shows the Cc segment of IgM C4 exon replacing the Tc segment. The positions of the spliced mRNAs are indicated by arrows. (wt) Wild type. The band between pre-mRNA and mRNA is a cleavage product unrelated to splicing. The ESS blocks splicing in the presence of SC35 but not of SF2/ASF. (*Bottom*) High-score ESE motifs in the HIV-*tat* exon T3. Score matrices derived from functional Selex experiments with SC35 and SF2/ASF were used to search the exon sequence. The resulting scores (*y* axis) were plotted against the nucleotide position along the exon (*x* axis). The vertical bars indicate each SF2/ASF heptanucleotide motif (*dark gray*) or SC35 octanucleotide motif (*light gray*). Because different score matrices were used for each protein, the numerical scores of the two SR proteins cannot be compared. The position of the SC35-specific ESS is indicated.

bound to mRNA, suggested that both the elements and the proteins might have additional roles late in the splicing reaction. Indeed, S. Chew found that both an enhancer and an SR protein are required for the second step of splicing, i.e., intron excision and exon ligation. This requirement was shown 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 this assay, the first step of splicing is carried out in the absence of a 3′ exon, which is later provided in *trans* for the second step. We found that the *trans*-spliced 3′ exon requires an enhancer and a cognate SR protein for efficient bimolecular exon ligation.

STRUCTURE AND FUNCTION OF RRM-CONTAINING SPLICING FACTORS

Several of the splicing factors we have been characterizing contain one or two RNA-recognition motifs (RRMs). The RRM is a particular type of RNA-binding motif, which is very common and is frequently present in two or more copies in a single protein. Among the human proteins we have been investigating, all the SR and hnRNP A/B proteins contain one or two RRMs, p54^{nrb} contains two, and RNPS1 contains one. RNPS1 was identified by A. Mayeda in the course of purifying factors that stimulate alternative 3′ splice-site selection by antagonizing SR proteins. Purified or recombinant RNPS1 has a more general effect on splicing, behaving as an activator that stimulates usage of all splice sites in the presence of SR proteins.

The three-dimensional structures of these proteins are of interest because of the insights they are expected to provide about their mechanisms of action, and also because of their implications for how the conserved RRM fold can be adapted for different functions and ways of binding nucleic acids. Crystallographic studies are being pursued in collaboration with R.-M. Xu's laboratory. The structure of the UP1 fragment of hnRNP A1 was determined at high resolution, providing the first example of how two RRMs are packed within a single protein. This year, the structure of UP1 bound to single-stranded telomeric DNA repeats was determined, revealing an unexpected dimerization of the protein upon nucleic acid binding. The structure suggests the basis of the high-affinity binding of UP1 or hnRNP A1 to DNA or RNA with the telomeric repeat sequence. hnRNP A1 has been linked to alternative splicing regulation (through our previous work) and to telomere length regulation (through B. Chabot's work at the University of Sherbrooke), and therefore it will be of interest to explore the importance of different aspects of the UP1 structure for each of these processes. We are pursuing these functional studies in collaboration with the Xu lab.

The Xu lab also solved the structure of p32, in collaboration with our lab. p32 is a protein that we originally identified several years ago because of its copurification with the SR protein SF2/ASF. p32 was subsequently tied to a wide variety of cellular processes by work in many laboratories. Unexpectedly, we and others have shown that p32 is predominantly, if not exclusively, a mitochondrial protein, although there is some evidence that a change in localization can occur under certain conditions, such as viral infection. The structure revealed that p32 has a novel fold and forms a homotrimer in the shape of a donut with an asymmetric charge distribution.

OTHER STUDIES

M. Murray is continuing his analysis of human PP2Cγ, a type-2C Ser/Thr phosphatase that he showed is required at the onset of spliceosome assembly. He has shown that PP2Cγ is tethered to the spliceosome once this complex assembles and that the activity of the protein in spliceosome assembly requires both a characteristic acidic domain and active site residues responsible for dephosphorylation. He is using a variety of approaches to determine the basis for the physical association of this phosphatase with the spliceosome and to understand the mechanistic consequences of the specific dephosphorylation events it catalyzes in the context of splicing.

M. Hastings is pursuing the characterization of AT-AC intron processing, a second splicing pathway for a small class of introns in higher eukaryotes. She has found that SR proteins are required for this pathway and is currently exploring the factors involved in the interplay between major and minor spliceosomes assembled on adjacent introns of the two types.

H.-X. Liu and L. Cartegni have been studying the relationship between inappropriate exon skipping and the disruption of exonic enhancers by nonsense or other mutations. J. Zhu is exploring the mechanistic link between intron recognition and nonsense-mediated mRNA decay, as well as the functional roles of RS domains in SR proteins.

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REGULATION OF APOPTOSIS IN CANCER CELLS

Y. Lazebnik D. Duelli, L. Faleiro, H. Fearnhead, P. Lassus, X. Opitz-Araya, J. Raychaudhuri, J. Rodriguez

Apoptosis is a fundamental biological process critical for maintaining tissue homeostasis. Consequently, deregulation of apoptosis contributes to cancer. Our laboratory is investigating three questions: What is the apoptotic machinery? How it is regulated? Can it be used to kill cancer cells?

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

We are investigating which caspases are involved in apoptosis and studying 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.

The answer to selective killing of cancer cells may lie in the observation that expression of oncogenes that deregulate the cell cycle can 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.

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

Caspase-9 and APAF-1 Form a Holoenzyme

J. Rodriguez

To understand how E1A can regulate activation of caspase-9, we had to first establish how caspase-9 is activated. Caspase-9 activation requires binding of the precursor to a complex of two proteins, Apaf-1 and cytochrome *c*, and is dependent on hydrolysis of dATP or ATP. However, it was not clear how formation of this complex results in caspase-9 activation and how other initiator caspases are activated. The induced proximity model that was widely accepted at the time argued that the precursor of an initiator caspase has low activity which is sufficient for autocatalytic processing but only when two or more precursor molecules are brought into close proximity by adaptor proteins, such as Apaf-1. The processed caspase becomes fully active and is free to cleave its substrates.

We previously postulated an alternative model in which proteins like Apaf-1 facilitate autocatalysis of

the caspase precursors by increasing their activity through a conformational change. During the last year, we indeed found that the proteolytic activity of caspase-9 in a complex with Apaf-1 is several orders of magnitude higher than that of the free enzyme. Thus, this complex functions as a holoenzyme where caspase-9 is the catalytic subunit and Apaf-1 its regulator. We argue that this regulation is allosteric and suggest that this mechanism is common for other initiator caspases. The proposed model has several implications.

A practical implication is that in vitro screens for caspase-9 substrates and inhibitors, which are actively sought by pharmaceutical companies as potential therapeutics, should use the caspase-9 holoenzyme, rather than caspase-9 alone. Another implication is that caspase-9 activity may be regulated even after caspase-9 processing, for example, by sequestering caspase-9 from the holoenzyme or by the amount of available Apaf-1 oligomer. This would explain the otherwise difficult to explain high efficiency of the caspase-9 dominant-negative mutant and the naturally occurring caspase-9 "decoys" as inhibitors of apoptosis. This would also mean that a mutant caspase-9 can inhibit apoptosis even if expressed by only one allele in a tumor cell.

We are investigating how expression of E1A affects formation of the caspase-9 holoenzyme, as well as whether this holoenzyme contains cofactors other than Apaf-1. We also initiated a collaboration with Dr. Joshua-Tor's lab here at Cold Spring Harbor to determine the three-dimensional structure of the holoenzyme.

E1A Regulates Cytochrome *c* Release from Mitochondria

D. Duelli, J. Raychaudhuri

To investigate how expression of E1A affects cytochrome *c* release, we initiated two approaches. One was to set up a cell-free system in which extracts from cells expressing E1A induce cytochrome *c* release from mitochondria. The results obtained with this system indicated that extracts from cells expressing E1A contain an activity that is able to release cytochrome *c*. This activity is being purified. Another approach was to establish whether expression of E1A regulates inhibitors or activators of cytochrome *c* release. This was achieved by a complementation assay in which cells that express E1A are fused with untransformed cells. We found that expression of E1A triggers at least two pathways, one of which represses and one that inhibits cytochrome *c* release. This inhibitor is being identified.

Does Caspase-9 Mediate Oncogene and Drug-induced Apoptosis in All Cancer Cells?

P. Lassus

Some observations obtained in our and other laboratories suggest that drug-induced apoptosis of cancer cells can be mediated by caspases other than caspase-9. During the last year, we identified an experimental system where apoptosis does not appear to involve processing of known caspases yet is prevented by a caspase inhibitor, indicating that a caspase activity is involved. We have established approaches to identify this putative caspase and are pursuing them.

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REGULATION OF APOPTOSIS AND SENESCENCE BY CANCER GENES

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G. Ferbeyre A. Samuelson
A. Lin C. Schmitt
M. McCurrach M. Soengas

Apoptosis is a genetically controlled form of cell death that is important for normal development and tissue homeostasis. Senescence produces "genetic death" in that senescent cells permanently arrest and are incapable of further propagation. Both processes are frequently disrupted in cancer cells, implying that each can limit tumor development. Moreover, radiation and many chemotherapeutic agents can induce apoptosis or senescence, raising the possibility that the integrity of these programmed responses influences the outcome of cancer therapy in patients. The goal of our research is to understand how cancer genes control apoptosis and senescence in normal cells, and how mutations that disrupt these processes impact tumor development and therapy. Our approach emphasizes genetics, and we typically exploit simple cellular systems to study cancer gene function. More recently, we have developed animal models to confirm the relevance of our simple systems for multistep carcinogenesis and cancer therapy in vivo.

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

Oncogene Signaling to p53: The ARF-p53 Tumor Suppressor Pathway

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

Last year, we and others identified the ARF tumor suppressor as an important intermediate in oncogene signaling to p53 (de Stanchina et al., *Genes Dev. 12:* 2434 [1998]). These observations raised the possibility that disruption of ARF, like p53 loss, would accelerate tumorigenesis initiated by mitogenic oncogenes. To test this possibility, we examined the impact of *INK4a/ARF* and *p53* loci on tumor development and treatment response in *Eμ-myc* transgenic mice (Schmitt et al. 1999). These mice constitutively express the *myc* oncogene in B cells and, hence, develop B-cell lymphoma. *Eμ-myc* transgenic mice were bred with mice harboring deletions of several tumor suppressor loci to generate genetically defined tumors with mutations in the *INK4a/ARF, Rb*, or *p53* genes. Like *p53* null lymphomas, *INK4a/ARF* null lymphomas formed rapidly and were highly invasive. Furthermore, *INK4a/ARF*$^{-/-}$ lymphomas displayed reduced p53 activity despite the presence of wild-type *p53* genes. These results establish that ARF is impor-

tant for oncogene-induced apoptosis in developing tumors and demonstrate that *INK4a/ARF* and *p53* mutations lead to aggressive tumors by disrupting overlapping tumor suppressor functions. We continue to study ARF action and ARF-independent pathways involved in oncogene signaling to p53.

p53 Effector Mechanisms

M. Soengas [in collaboration with T. Mak, Amgen Institute and Ontario Cancer Center]

How p53 promotes apoptosis is controversial (see Lowe 1999; Wallace-Brodeur and Lowe 1999). Although many investigators have focused on the transcriptional targets of p53, our recent efforts have examined how p53 signaling integrates with the caspases. Caspases are a family of cysteine proteases that are expressed as inactive pro-enzymes that become activated during apoptosis. "Signaling" caspases link pro-death signals to the effector caspases, whereas "effector" caspases disassemble the cell. Importantly, signaling pro-caspases must associate with specific adaptor molecules to initiate the caspase cascade. These complexes include FADD/Casp-8, RAIDD/Casp-2, and Apaf-1/Casp-9.

Using embryonic fibroblasts derived from various knockout mice, we examined the requirements for several caspases or their adapters on apoptosis in response to the *E1A* and c-*myc* oncogenes. Previously, we showed that Casp-3 and the FADD/Casp-8 and RAIDD/Casp-2 complexes were dispensable for p53-mediated cell death (Woo et al., *Genes Dev. 12:* 806 [1998]; Yeh et al., *Science 279:* 1954 [1998]; J. Polyakova, J. Yuan, and S. Lowe, unpubl.). This year, we examined the contribution of Casp-9 and its cofactor Apaf-1 to p53 action during Myc-induced apoptosis (Soengas et al. 1999). Like p53 null cells, Apaf-1- and Casp-9-deficient cells expressing c-Myc were resistant to apoptotic stimuli that mimic conditions in developing tumors. In contrast, ectopic p53 expression efficiently induced cell death in p53-deficient cells but had no pro-apoptotic effect in cells lacking Apaf-1 or Casp-9. Remarkably, inactivation of Apaf-1 or Casp-9 substituted for p53 loss in promoting the oncogenic transformation of Myc-expressing cells.

These studies demonstrate that Apaf-1 and Casp-9 can act as essential downstream components of p53 in Myc-induced apoptosis and identify Apaf-1 and Casp-9 as candidate tumor suppressors. Although we do not believe that p53 directly interacts with either Apaf-1 or Casp-9, it seems likely that the immediate effectors of p53 converge on the Apaf-1/Casp-9 death effector complex—or at least require it for their activity. To extend these observations, we are testing the oncogenic capacity of Apaf-1/Casp-9 disruption in transgenic mice, and screening human tumors for *Apaf-1* and *Casp-9* mutations.

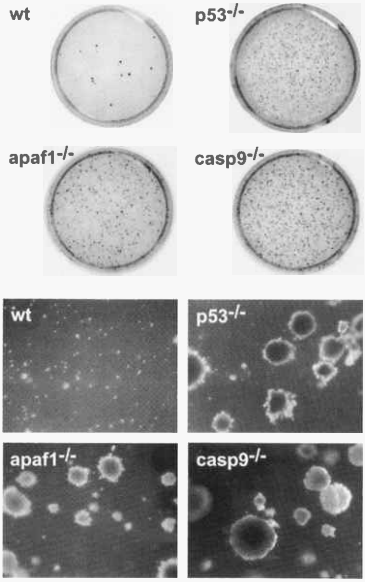

FIGURE 1 Effect of Apaf-1 and Casp-9 on colony formation in soft agar. The ability of Myc/Ras-expressing MEFs to form colonies in soft agar is illustrated by Giemsa staining (*top*) and phase-contrast microscopy (*bottom*); 2 x 10^4 cells of the indicated genotypes were resuspended in 0.3% Noble agar (in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum) and plated on 6-cm plates containing a solidified bottom layer (0.5% Noble agar in growth medium). Shown is a representative experiment two weeks after plating. Note that the size and number of colonies derived from *Apaf-1*$^{-/-}$ and *Casp-9*$^{-/-}$ cells were comparable to those of *p53*$^{-/-}$ cells.

E1A and Chemosensitivity

A. Samuelson, J. Polyakova [in collaboration with Y. Lazebnik, Cold Spring Harbor Laboratory]

E1A has the remarkable property of enhancing cellular sensitivity to anticancer-agent-induced death. We have previously shown that this activity involves at least two separable functions: The first involves inactivation of Rb and the second requires an amino-terminal E1A region. Remarkably, E1A mutants unable to inactivate Rb but retaining the amino-terminal function selectively promote chemosensitivity in cells lacking an intact Rb pathway. As a result, elucidating the amino-terminal function(s) involved in chemosensitivity may suggest new drug targets. In collaboration with Y. Lazebnik, we have shown that E1A induces several caspases through a posttranscriptional mechanism that acts independently of p53 and involves inactivation of the retinoblastoma gene product (J. Polyakova, unpubl.). Although pro-caspase induction by E1A cannot solely explain its pro-apoptotic activity, it seems likely that it contributes to the p53-independent apoptosis induced by oncogenes. Although our previous studies associated the amino-terminal E1A function that contributes to apoptosis with the E1A-p300/CBP interaction, we now have used more refined mutants to separate p300/CBP binding from E1A-induced apoptosis (A. Samuelson, unpubl.). We hope that these results will facilitate a more complete understanding of this E1A action in chemosensitivity.

Control of Cellular Senescence

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

Our previous work demonstrates that expression of oncogenic *ras* in primary human or rodent cells results in a permanent arrest accompanied by accumulation of p53, p16, and $p19^{ARF}$ and has the hallmarks of cellular senescence. Negation of *ras*-induced senescence appears to account for the phenomenon of oncogene cooperation and may be important for tumor development. Currently, we are addressing several questions: How does *ras* signaling activate a permanent cell cycle arrest? How do tumor suppressors such as p53, p16, and $p19^{ARF}$ initiate and maintain this arrest? What is the relationship between *ras*-induced arrest and replicative senescence? How do epithelial cells respond to *ras*? What is the impact of *ras*-induced arrest for multistep carcinogenesis in vivo?

This year, we have established an epithelial system to study *ras*-induced arrest. We chose to study murine keratinocytes because (1) protocols have been established to culture mouse keratinocytes and (2) the genetics of chemically induced skin carcinogenesis involves cooperation between *ras* and *p53* mutations. Like fibroblasts, keratinocytes respond to oncogenic *ras* by inducing $p19^{ARF}$, p53, and cell cycle arrest (A. Lin, unpubl.). Hence, the phenomenon of *ras*-induced "senescence" extends to epithelial cells, which give rise to the majority of human tumors (i.e., carcinomas). In addition, with W. Funk (Geron Corporation), we used a 1000-gene cDNA microarray to compare gene expression profiles in normal diploid human fibroblasts that were (1) exponentially growing, (2) quiescent, (3) *ras*-arrested, or (4) senescent. Although *ras*-arrested and senescent cells showed differences, they were much more similar to each other than to quiescent cells (D. Shelton et al., submitted). The common changes observed in *ras*-arrested and senescent cells were reminiscent of an inflammatory response and raise the possibility that senescent cells are ultimately cleared by signaling to the immune system. In any case, these data demonstrate that *ras* arrest and replicative senescence are highly related processes. We are now expanding our analysis to survey more than 18,000 genes and expressed sequence tags, and hope that this effort will provide additional insights into the nature of *ras*-induced arrest.

In Vivo Models of Drug Sensitivity and Resistance

C. Schmitt, M. McCurrach, E. de Stanchina

Our laboratory has a long-standing interest in the molecular genetics of drug sensitivity and resistance. Although we continue to use in vitro models, we have concluded that some aspects of therapy response must be studied in vivo. We hypothesized that the *Eμ-myc* transgenic mouse (described above) 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) large numbers of pure tumor cells can be isolated from mice undergoing therapy, (3) therapy is performed in immunocompetent mice, and (4) lymphoma cells readily adapt to culture and can be transplanted into syngeneic mice.

Eμ-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, *Eμ-myc* lymphomas lacking functional p53 (generated by crossing to the p53 knockout mouse) display reduced apoptosis and are typically resistant to these same therapies. Interestingly, tumors lacking the *INK4a/ARF* locus were nearly as resistant as p53-deficient lymphomas. Moreover, these tumors displayed attenuated p53 activity and apoptosis compared to those arising in a control background. Thus, disruption of the p53 pathway—through a *p53* mutation or by an extragenic mutation—dramatically reduces the responsiveness of *Eμ-myc* lymphomas to therapy (Schmitt et al. 1999).

These data have important implications for understanding p53 and the clinical behavior of human tumors. For example, our data demonstrate that tumors with extragenic mutations in the p53 pathway can display properties of *p53* mutant tumors. This fundamental point is crucial for interpreting studies relating *p53* gene status to clinical parameters in human patients and may explain why some studies fail to correlate *p53* mutations with adverse clinical features. In addition, we provide the first evidence that *INK4a/ARF* mutations can have a negative impact on the outcome of cancer therapy. Although the extent to which this impacts human cancer is as yet unknown, it is noteworthy that *p53* mutations are associated with highly aggressive tumors and drug resistance in hematological malignancies (Schmitt and Lowe 1999), indicating that *Eμ-myc* lymphomas can recapitulate the behavior of human tumors. We are continuing to use this tractable system to identify additional determinants of chemosensitivity in vivo.

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CELL SIGNALING IN HIV PATHOGENESIS

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Our interest is in understanding the molecular mechanisms underlying the pathogenesis of AIDS and, in particular, 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) that is an important determinant of viral pathogenicity. Important questions concerning the normal function of this gene have been raised on the basis of observations that *nef*- viruses replicate poorly in the host where their presence correlates with attenuated development of AIDS. In fact, deleting the *nef* gene is a promising strategy to generate attenuated immunodeficiency viruses for use as live vaccines.

Natural Nef protein isolates 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 the cell surface 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. Nef also down-regulates expression of class I MHC complexes at the cell surface, and this effect may enable infected cells to evade the immune response in the infected host. We showed previously that HIV-1 Nef uses two different mechanisms to down-regulate expression of CD4 and class I MHC molecules. In previous years, we probed and elucidated the molecular mechanism(s) that mediate these effects by the HIV-1-encoded Nef protein, but this year, we also initiated a similar analysis of Nef encoded by SIV. These experiments identified mutant Nef proteins that were selectively defective for individual functions of Nef, which in turn opened the way to initiate experiments addressing the importance of individual functions and molecular interactions of Nef for viral replication in cell culture and in SIV-infected rhesus macaques.

HIV-1 NEF INDUCES CD4 ENDOCYTOSIS VIA AN AP-2-CLATHRIN ADAPTOR-DEPENDENT PATHWAY

Evidence from experiments performed in our laboratory suggest a model where Nef accelerates CD4 internalization from the cell surface by promoting the recruitment of CD4 molecules expressed at the cell surface to AP-2 clathrin-adaptor complexes (AP-2) at the plasma membrane. This is supported by observations that Nef is concentrated at plasma membrane sites containing AP-2 and clathrin heavy chains, two core elements of clathrin-coated pits that mediate endocytosis of a number of cell surface receptors (Le Borgne and Hoflack 1998), that CD4 is redistributed to these sites in cells expressing Nef or a green fluorescent protein (GFP)-tagged Nef, and that mutations in Nef that disrupt its ability to colocalize with AP-2 also disrupt its ability to accelerate CD4 internalization.

During constitutive endocytosis, the CD4 molecule contacts the endocytic machinery via a dileucine sorting signal in its cytoplasmic domain, a signaling motif which, in vitro, has been shown to interact directly with the β subunits of golgi and plasma-membrane-associated (AP-1 and AP-2) adaptor complexes. Since we found that this dileucine sequence is part of a larger hydrophobic element in the CD4 cytoplasmic domain required for the ability of Nef to down-regulate CD4 molecules, we believe that in the presence of Nef, CD4 makes contacts with elements of the endocytic machinery similar to the contacts that take place in the absence of Nef during CD4 endocytosis and that Nef likely stabilizes the normal interaction(s) of the CD4 cytoplasmic domain with the endocytic machinery, rather than linking CD4 to AP-2.

SIV NEF USES SURFACES AND MOLECULAR INTERACTIONS DIFFERENT FROM THOSE OF HIV-1 NEF TO DOWN-REGULATE CD4 EXPRESSION AND TO INTERACT WITH AP-2

We previously found that HIV-1 Nef regions mediating the interaction of Nef with AP-2 in vivo and in vitro

map to the disordered loop in the carboxy-terminal region of the Nef molecule. SIV Nef also uses a similar mechanism to down-regulate expression of the CD4 molecule. However, there is little similarity in the amino acid sequences in the disordered regions of SIV and HIV-1 Nef proteins. Therefore, to understand how SIV Nef interacts with AP-2 and down-regulates CD4, we set out to determine sequences in SIV Nef that are required for these functions. We found that the amino-terminal disordered region in SIV Nef is necessary and sufficient for the ability of Nef to associate with partially purified AP-2 adaptor complex in vitro, and for its ability to target heterologous proteins to AP-2-containing-clathrin coats in vivo. Thus, different regions in the SIV and HIV-1 Nef proteins mediate interactions with AP-2 clathrin, which is essential for the ability of Nef to induce CD4 endocyosis via the clathrin pathway. Interestingly, we also know of additional differences in the functional organization of HIV-1 and SIV Nef in the core domains of the two molecules. For example, the PPII helix in HIV-1 Nef is essential for its ability to modulate CD3-initiated signaling in T cells and to induce endocytosis of class I MHC molecules, but not for the ability of SIV Nef to perform these functions. The observation that HIV-1 and SIV Nef proteins have evolved different surfaces to carry out similar functions is striking. That genetic selection would maintain similar molecular interactions via different surfaces in SIV and HIV-1 Nef proteins is evidence that these interactions have critical roles for the viral life cycle in vivo.

TWO ELEMENTS TARGET SIV NEF TO AP-2, BUT ONLY ONE IS REQUIRED FOR THE INDUCTION OF CD4 ENDOCYTOSIS

The observations that SIV Nef and HIV Nef proteins use different surfaces to contact AP-2 raised the possibility that the two proteins may use different strategies to down-regulate CD4 expression. To address this possibility, we studied in detail the elements in SIV Nef that are required for its interaction with AP-2 and for down-regulation of CD4 from the cell surface. We found that SIV Nef interacts with AP-2 via two elements located in the amino-terminal region of the Nef molecule. Interestingly, only the N-distal element is required to induce CD4 endocytosis. This N-distal AP-2 targeting element contains no canonical endocytic signals and therefore probably contacts the AP-2 complex via a novel interaction surface. The data support a model where SIV Nef induces CD4 endocytosis by promoting the normal interactions between the dileucine sorting signal in the CD4 cytoplasmic domain and AP-2 but does not substitute for the CD4-AP-2 adaptor interaction. Neither element is important for the induction of class I MHC endocytosis, thus confirming our previous conclusion from studies of HIV-1 Nef that different mechanisms underlie the induction of class I MHC and CD4 endocytosis by Nef.

Interestingly, our data suggest different functional roles for the N-proximal and N-distal AP-2 interacting elements in 239-Nef. Why the interaction of 239-Nef with AP-2 via the N-proximal element is not sufficient to induce CD4 endocytosis is not known. One possible explanation of these observations is that the interaction involving the N-distal element, but not the N-proximal element, reorients the 239-Nef molecule with respect to the AP-2 complex in a very specific way that promotes the recruitment of CD4 to AP-2 and the induction of CD4 endocytosis. Alternatively, the N-distal element in the amino-terminal loop of SIV Nef may have an additional function besides linking Nef to the AP-2 complex, such as a direct or indirect interaction with CD4 or perhaps with other molecules that control the recruitment of CD4 and/or morphogenesis of the coated pit. Finally, it is possible that the N-proximal element is important for other sorting events that involve CD4, perhaps in the context of other adaptor complexes such as, for example, AP-3 or that it is involved in another function such as the sorting of membrane proteins other than CD4. Results from these experiments will now permit us to design mutant 239-Nef proteins defective in each of the two AP-2 interacting elements to assess the importance of these interactions with AP-2 for SIV replication in SIV-infected rhesus monkeys (in collaboration with Dr. Frank Kirchhoff, University of Erlangen, Germany).

THE HIV-1 *NEF* GENE CAN TO A LARGE EXTENT REPLACE SIV NEF IN VIVO

We collaborated with Dr. Frank Kirchhoff's group (University of Erlangen, Germany) to assess whether HIV-1 Nef can functionally replace SIV Nef in vivo. If so, it would provide a means by which to study the function of HIV-1 Nef in vivo. Initially, two rhesus macaques were infected with the chimeric viruses (Nef-SHIVs) carrying a large population of HIV-1 *nef* genes isolated directly from HIV-1-infected patients. It

was expected that this would permit selection of HIV-1 *nef* genes that are most robust in rhesus macaques. As a result of these initial experiments, a single molecular clone, named SHIV-40K6, was selected. The SHIV-40K6 *nef* allele was active in CD4 and class I MHC down-regulation and enhanced viral infectivity and replication. Notably, all of the macaques inoculated with SHIV-40K6 showed high levels of viral replication early in infection, and approximately half of the animals maintained high viral loads and developed immunodeficiency within 1 year from the infection. In contrast, infection of rhesus macaques with control SIVs where the *nef*-coding sequence was inactivated by deletion does not result in development of AIDS in a significant fraction of the infected animals. These data demonstrate that HIV-1 *nef* can to a large extent functionally replace SIVmac *nef* in vivo.

EFFECT OF THE ATTENUATING DELETION AND OF SEQUENCE ALTERATIONS EVOLVING IN VIVO ON SIV C8-NEF FUNCTION

The SIVmacC8 variant has been used in a European Community Concerted Action project to study the efficacy and safety of live attenuated SIV vaccines in a large number of macaques. The attenuating deletion in the SIVmacC8 *nef* long-terminal-repeat region encompasses only 12 bp and is "repaired" in a subset of infected animals. It is unknown whether C8-Nef retains some activity. Since it is important to use only well-characterized deletion mutants in live attenuated vaccine studies, we collaborated with Dr. F. Kirchhoff to analyze the relevance of the deletion and the duplications and point mutations selected in infected macaques for Nef function in vitro. The deletion, affecting amino acids 143–146 (DMYL), resulted in a dramatic decrease in Nef stability and function. The initial 12-bp duplication resulted in efficient Nef expression and an intermediate phenotype in infectivity assays, but it did not significantly restore the ability of Nef to stimulate viral replication and to down-modulate CD4 and class I MHC cell surface expression. The additional substitutions, however, which subsequently evolved in vivo, gradually restored these Nef functions. It was noteworthy that coinfection experiments in the T-lymphoid 221 cell line revealed that even SIVmac *nef* variants carrying the original 12-bp deletion readily outgrew an otherwise isogenic virus containing a 182-bp deletion in the *nef* gene. Thus, although C8-Nef is unstable and severely impaired in in vitro assays, it maintains enough residual activity to stimulate viral replication. Thus, the *nef* mutation in SIVmacC8 does not have the characteristics that would be suitable for use in live attenuated vaccine studies.

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MOLECULAR BIOLOGY OF PAPILLOMAVIRUSES

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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 more than 80% of all cervical carcinomas.

A key impediment to the study of papillomaviruses has been the inability to define simple in vitro cell culture systems for human papillomaviruses, largely due to the fact that these viruses normally require specialized differentiating cells that only with 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 that 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 addition, the viral DNA replication machinery represents the one of the most promising targets for antiviral therapy.

In previous years, we have reported the characterization of the papillomavirus replicon and the identification of the viral components that are required for viral DNA replication. More recently, we have directed our attention toward detailed biochemical analysis of initiation of DNA replication. 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. On the basis of these studies, we now have a relatively clear picture of the roles of the E1 and E2 proteins in replication. E1 has all of 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 and loading 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 duplex, 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. This sequential assembly generates different complexes that in turn recognize the ori, destabilize the double helix at the ori, and function as a replication helicase.

Binding Sites for the E1 Initiator Protein in the ori

G. Chen

The initiator protein E1 recognizes the papillomavirus origin of DNA replication and thus determines where DNA replication initiates. To understand how the E1 protein can form oligomeric structures on the ori, we have analyzed the stoichiometry of binding as well as defined the sequences that constitute specific recognition sites for E1 using a minimal E1 DNA-binding

domain. Because of the variety of forms of E1 that can bind to ori, it has been difficult to determine exactly what constitutes a recognition sequence for E1. However, we have recently demonstrated that E1, in the presence of E2, can bind to the ori in monomeric form. We have analyzed binding of this specific complex, in combination with mutational analysis of the ori sequence, to determine what specific sequences are required for binding of E1. These studies have demonstrated that a monomer of E1 recognizes the hexanucleotide sequence AACAAT and that binding of a dimer of E1 occurs through the recognition of two of these hexameric sequences, arranged as an inverted repeat and positioned 3 bp apart. Inspection of the ori sequence demonstrated that in addition to this pair of E1 recognition sequences, four additional putative E1 recognition sequences are present. Two of these are arranged in a similar fashion, but overlapping the first pair by 3 bp. The final two putative sites are present flanking the four sites in the overlapping array. Importantly, a similar arrangement of this recognition sequence is present in the majority of other papillomavirus ori sequences, indicating that both the recognition sequence and the arrangement of binding sites are conserved features. Through the use of high-resolution footprinting and a combination of interference and mutational analyses, we have been able to demonstrate simultaneous binding of four molecules of E1 to the four overlapping E1-binding sites. This mode of binding has interesting implications both for how E1 recognizes its specific site and for how E1 induces structural changes in the ori DNA.

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

E. Gillitzer

Cooperative binding of the E1 and E2 proteins to the ori is essential for viral DNA replication in vivo. We have previously demonstrated that the interaction between the E1 and E2 proteins has two separate components. The DNA-binding domains (DBDs) of the two proteins interact with each other, and the activation domain of E2 interacts with the helicase domain of the E1 protein. The interaction between the activation domain of E2 and E1 is the productive interaction, whereas the interaction between the two DBDs serves to facilitate this interaction. To determine how the physical interaction between the two DBDs triggers the productive interaction, we have analyzed the structure of the ori in response to binding of the two DBDs. Both the E1 and E2 DBDs individually generate modest bends in the DNA upon binding. However, when the two proteins are bound cooperatively, a much sharper bend centered between the two binding sites results. This sharp bend (which approaches 90°) could result either from the combined intrinsic bends contributed by the individual proteins or from the interaction between the two proteins. To distinguish between these possibilities, we utilized mutants in the E2 DBD that fail to interact with the E1 DBD. Interestingly, although the intrinsic bend generated by the mutant E2 DBDs was of similar magnitude to that generated by wild-type E2 DBD, the mutant E2 DBDs failed to generate a sharp bend in combination with the E1 DBD. These results suggested that the physical interaction between E1 and E2 DBDs gave rise to the sharp bend in the sequences between the two sites. To determine whether bending of the DNA was also a requirement for cooperative interaction, we wanted to change the bendability of the DNA sequence between the E1- and E2-binding sites. This sequence includes the sequence AAT, which is not involved in binding of either E1 or E2. It is well established that homopolymeric A and T stretches are intrinsically stiff. We therefore generated a double substitution, changing the two A-T base pairs to T-A base pairs. This creates a T4 sequence that is predicted to be intrinsically stiff. As a control, we also generated the two individual mutations. To determine if the mutations increased the stiffness compared to the wild-type sequence, we digested the wild-type and mutant DNAs with with DNase I. Binding of DNase I to the minor groove requires bending of the DNA. As a result, stiff DNA is cleaved less efficiently than flexible DNA. When tested by DNase I cleavage, the double mutant was cleaved less efficiently than the wild-type sequence or the individual point mutations, consistent with increased stiffness of the double mutant. As expected, none of these mutations had a significant effect on binding of E1 or E2 alone. Likewise, the individual mutations had little or no effect on cooperative binding of E1 and E2. Interestingly, however, the double mutation which increased stiffness resulted in a severe reduction of both cooperative binding and bending. This is consistent with the idea that bendability of the DNA sequence is important for the interaction between the two DBDs and that bending of the DNA

is a prerequisite for cooperative DNA binding. Furthermore, these results demonstrate that the interaction between the E1 and E2 DBDs serves a predominantly architectural role, analogous to the function of cellular factors such as HMG proteins, which can modify the architecture of a protein-DNA complex by introducing bends in DNA.

Initiator Complex Assembly

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 also serves 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. In agreement with such a model, we have previously demonstrated that a dimeric form of E1 recognizes the ori together with E2 and that the form of E1 which has helicase activity is a hexamer. Our current understanding of the assembly pathway of these complexes is that a sequence-specific complex of a dimer of E1 and a dimer of E2 together bind to the ori and initially recognize the ori. In an ATP-dependent manner, the E2 dimer is displaced and additional E1 molecules are added to the complex. These are bound sequence-specifically, forming a tetramer on the four paired E1-binding sites in the core. Additional E1 molecules are recruited to this complex, binding to the DNA sequences on both sides of the core, and resulting in a protection of approximately 60 bp of ori sequence. The binding of E1 to the flanking sequences shows little dependence on DNA sequence but is dependent on protein-protein interactions with the E1 molecules bound to the core. This large complex, which appears to be a precursor of the DNA helicase, is capable of distortion of the ori giving rise to $KMnO_4$ sensitivity on both sides of the core. Interestingly, the two halves of this large complex appear to be independent of each other, since distortion can be detected with only one half of the ori present. This indicates that the distortion complex is composed of two functional units, each one of which initiates distortion on one side of the core, presumably allowing for subsequent bidirectional unwinding and initiation of DNA synthesis. Thus, as predicted, the assembly of initiator complexes represents a gradual progression from smaller to larger E1 complexes. Interestingly, in conjunction with the assembly of progressively more complex structures, a transition from a high to low degree of sequence specificity occurs.

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DNA SYNTHESIS

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Studies on the mechanism and control of the initiation of replication of DNA in eukaryotes and the mechanism of chromatin inheritance continued this year with good progress. During the last 3 years or so, many aspects of the mechanism of initiation of DNA replication at each origin have become apparent. The process begins with the origin recognition complex (ORC) acting as a landing pad for a series of cell-cycle-regulated protein-protein interactions that ultimately lead to the initiation of DNA synthesis at each origin. Cdc6p and the six minichromosome maintenance proteins (Mcm2–7p) form a prereplication complex at each origin before S phase, followed by the formation of a preinitiation complex (pre-IC) containing additional proteins as each origin fires. We have further characterized both complexes and their regulation by cell-cycle-regulated protein kinases.

Other studies have followed on our observation that the chromatin assembly factor-1 (CAF-1) protein interacts with an essential DNA replication protein called the proliferating cell nuclear antigen (PCNA) and facilitates the inheritance of epigenetically determined states of gene expression. This mechanism may account for how heterochromatin, the bulk of the transcriptionally silent chromatin in higher eukaryotes, is copied from one generation to the next.

INITIATION OF CHROMOSOMAL DNA REPLICATION

Cdc6p: The mechanism for selection of origins of DNA replication within eukaryotic chromosomes has long been of interest. When we found that ORC bound in a DNA-sequence-specific and ATP-dependent manner to origins of DNA replication, it was logical to think that ORC was the only protein that defined origin selection. We had demonstrated previously that Cdc6p could bind to ORC and that overexpression of Cdc6p could rescue a defective ORC mutant. In the last year, we have found that Cdc6p can influence the DNA sequences to which ORC binds and that Cdc6p may alter ORC function on the DNA.

Coprecipitation experiments have shown that ORC and Cdc6p form a tertiary complex on the DNA and that Cdc6p ensures that ORC binds only to origin DNA. When ORC was bound to nonspecific DNAs, addition of Cdc6p destabilized ORC binding. This reaction required the functional nucleotide-binding site within Cdc6p and occurred in the presence of ATP, but not in the presence of the ATP analog ATP-γ-S that is not easily hydrolyzed. Thus, we have been able to reconstitute in vitro the first step in origin-dependent assembly of proteins onto origin-bound ORC. The reaction occurs very efficiently and with stoichiometric amounts of ORC, Cdc6p, and origin DNA. We also have evidence that this complex can attract the MCM proteins onto the origin in a reaction that requires an extract from G_1-phase cells, but not with an extract from G_2-phase cells. Thus, it will be possible to begin to reconstitute origin-dependent assembly of the prereplication complex (pre-RC) in vitro.

Recent studies on Cdc6p show that the nucleotide-binding motif in this protein is required for assembly of the MCM proteins onto the chromatin during the G_1 phase of the cell cycle. A mutation of the Walker A domain of the Cdc6p nucleotide-binding motif prevents initiation of DNA replication, and the cells arrest with a G_1 content of unreplicated DNA. Interestingly, when the nonessential amino terminus of Cdc6p was deleted in the inactive Cdc6p, the cells failed to arrest in late G_1 phase and progressed through M phase. Thus, the amino terminus of Cdc6p contains a regulatory domain that prevents cells from progressing through the cell cycle until cells initiate DNA replication. We are mapping the sites within the Cdc6p amino terminus that perform this cell-cycle-checkpoint function.

Assembly of the Preinitiation Complex: The pre-RC is believed to be a precursor for assembly of other proteins onto the origin after activation of the S-phase

cyclin-dependent protein kinase (CDK) and another protein kinase called Cdc7p/Dbf4p. We demonstrated in past years that Cdc45p, an MCM-binding protein that is essential for the initiation of DNA replication, binds to the MCM proteins in an S-phase CDK-dependent manner. We have now shown that Cdc45p origin association also required activation of the S-phase CDK. In the yeast *Saccharomyces cerevisiae*, these kinases are Clb5p and Clb6p cyclins that associate with the Cdc28p kinase. Interestingly, efficient association of Cdc45p with the origin also requires another protein kinase complex, the Cdc7p/Dbf4p kinase. Thus, the loading of Cdc45p onto the origin defines a critical step in the process leading to initiation of DNA replication.

Consistent with the role of Cdc45p in initiation of DNA replication, we have shown that the cellular single-stranded DNA-binding protein, replication protein A (RPA), also loads on to the origin at the same time as Cdc45p. Both Cdc45p and RPA coimmunoprecipitate with each other and with the DNA polymerase ε, a DNA polymerase that functions at the replication fork. It is possible that other proteins also load onto the origin at this time, and we are searching for these proteins by direct protein sequencing. The assembly of Cdc45p and RPA onto the DNA is similar to the events that involve SV40 T-antigen recruiting RPA to the SV40 origin of DNA replication.

Cdc7p/Dbf4p Kinase: The requirement for the Cdc7p/Dbf4p kinase for initiation of DNA replication has been known for some time, but how it regulates initiation of DNA replication has only recently begun to emerge. Other investigators and we demonstrated that Cdc7p is present in the cells throughout the cell division cycle and a large proportion is bound to chromatin. On the other hand, the Dbf4p subunit is highly cell-cycle-regulated. The protein is synthesized at the G_1- to S-phase transition and associates with the Cdc7p subunit to form an active protein kinase. In vitro, Cdc7p can phosphorylate five of the six MCM subunits, as well as the catalytic subunit of DNA polymerase α, another polymerase required for initiation of DNA replication. The absence of Dbf4p in the G_1 phase of the cell cycle is due in part to the fact that it is a substrate for the anaphase-promoting complex (APC), a mediator of ubiquitin-dependent degradation of proteins in late mitosis and in G_1 phase of the cell cycle.

The activity of Cdc7p kinase is cell-cycle-regulated by the levels of the Dbf4p subunit. But in the presence of a block to DNA replication during S phase, we found that Cdc7p/Dbf4p kinase activity was reduced. This would have the consequence of preventing initiation of DNA replication at those origins that have not yet assembled the pre-IC. The reduction in Cdc7p kinase activity correlated with hyperphosphorylation of the Dbf4p subunit, a process that we demonstrated requires the Rad53p kinase. Rad53p is a cell cycle checkpoint protein that is required to block cells from entering mitosis before DNA replication has finished, or before DNA damage has been repaired. Thus, the Cdc7p/Dbf4p kinase appears to orchestrate progression through S phase and monitor events that might lead to inheritance of DNA damage.

MCM DNA Helicase: There are six sequence-related MCM proteins in all eukaryotes tested to date, and they all have some similarity to DNA helicases. Recent studies from Ishimi's group in Japan have shown a weak DNA helicase activity using three of the six MCM proteins from either mouse or human cells. To date, however, no helicase activity has been found with the six-subunit complex.

With the sequencing of the genomes of a number of archaea, in collaboration with Rui-Ming Xu's group here at Cold Spring Harbor Laboratory, we noticed that many of these species contained only a single MCM protein. Thus, the gene encoding a number of archaea MCM proteins was cloned, and the proteins were expressed using baculovirus vectors in insect cells. The *Methanobacterium autothermotrophicum* MCM (MtMCM) was purified and shown to contain an intrinsic DNA-activated ATPase and DNA helicase. The helicase migrated in the 3′ to 5′ direction on the template stand, which is the same direction that the SV40 T-antigen helicase migrates on DNA. We also demonstrated that the MtMCM helicase functions as a double hexamer, again similar to that of the SV40 T-antigen helicase. Thus, it is likely that the MCM proteins function at each origin to help unwind DNA. Whether they function at the DNA replication fork as the principal DNA helicase remains to be determined.

EPIGENETIC INHERITANCE OF CHROMOSOME STATES

PCNA and CAF-1: In addition to investigating the mechanism and control of initiation of DNA replication, we are interested in the inheritance of chromatin and the transcriptional states of gene expression that are passed on during each cell division. We reported last year that CAF-1 interacted with the DNA replication protein PCNA and that PCNA marked replicating DNA for assembly of chromatin. CAF-1 is required for

the efficient inheritance of silenced states of chromatin in yeast and for replication-dependent assembly of nucleosomes during SV40 DNA replication. Thus, the interaction between CAF-1 and PCNA may be the mechanism for linking inheritance of chromatin with inheritance of the DNA itself.

The role of PCNA in epigenetic inheritance was investigated by first determining the sites in PCNA that bind CAF-1, with the hypothesis that these interaction sites might define a new functional domain in PCNA. Using existing and new mutations in the yeast PCNA, we found a number that failed to interact with CAF-1, yet were able to support cell proliferation. The mutants were then tested for their ability to inherit the silent mating-type locus (HMR) or the silenced state of a gene placed near the telomeres of chromosome VII in yeast. The PCNA mutants fell into two groups. One group displayed reduced silencing at both the telomere and the HMR locus, indicating that PCNA is required for efficient maintenance of the silenced state. The other class displayed a variegated phenotype, switching from the on state to the off state as cells divided. We conclude that PCNA is required for the establishment and stable inheritance of a silenced transcriptional state of chromatin. These experiments reinforce the role of this interesting DNA replication protein in inheritance of gene expression states of chromatin, providing a possible link between DNA replication and the determination of states of gene expression as an organism develops.

CAF-1 and Heterochromatin Proteins: In collaboration with Drs. Natalia Murzina and Ernest Laue at Cambridge University, we found that human CAF-1 interacted with the mammalian heterochromatin HP1-like proteins Mod1 and Mod2 and HP1-α. These proteins are related to the *Drosophila* HP1 protein that is found associated with heterochromatin. HP1 mutants suppress position effect variegation (PEV), a variable state of expression of genes that happen to be located near heterochromatin. We found that CAF-1 could mediate the loading of Mod1 onto chromatin that was replicated in vitro. Furthermore, both CAF-1 and Mod1 remained associated with heterochomatin in cells after S phase. Interestingly, the Mod1 protein was removed from chromatin as the chromatin condensed during passage through mitosis but was reloaded in early G_1 phase cells. Thus, we have uncovered a molecular link between a DNA replication protein (PCNA) and a protein required for inheritance of transcriptionally repressed chromatin (Mod1) that is mediated by the chromatin assembly factor CAF-1.

KAETRIN SIMPSON (1972–2000)

Kaetrin Simpson came to this laboratory as a Postdoctoral Fellow in 1997 following a rather brilliant career as a student at Cambridge University and at the Imperial College in London. We were deeply saddened by her all to soon death in January of the New Year.

Kate attended secondary school at the Reading School and entered Cambridge University in 1990 with great promise. In her three years there, she emerged as one of the top students in biology, graduating with many honors. During two summer breaks from her studies at Cambridge, spent near her parents home in Glasgow, Kate worked in the laboratories of Professor A. Curtis and Dr. M. Frame where she excelled in research, publishing two papers as a result of these summer studies. After graduating with honors from Cambridge, Kate moved to Imperial College where she studied for her Ph.D., the replication of Epstein-Barr virus DNA, with Clare Huxley. There, Kate achieved a great deal, being able to utilize the EBV origin of DNA replication to make stable artificial chromosomes in mouse cells. These studies were at the cutting edge of that field. Kate first came to Cold Spring Harbor Laboratory as a teaching assistant in the Yeast Artificial Chromosome course in 1995. At the relatively young age of 25, Kate returned to Cold Spring Harbor to take up postdoctoral studies, working to understand how the sites of initiation of DNA replication are determined in human chromosomes. This was an ambitious project on which she made excellent progress before succumbing to a brain tumor.

Kate was a very bright, vibrant, and a fun-loving person who was as charming as she was smart. With a determination to succeed in life and in academia, the sky was the limit as she began her career. We will all miss her always up-beat personality and only wonder about the contributions that Kate would have made to science.

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ONCOPROTEIN DESTRUCTION

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S.Y. Kim K. Tworkowski
M. Muratani

The maintenance of normal cellular growth and differentiation ultimately depends on mechanisms that regulate cell cycle progression. Two of the most important of these regulatory mechanisms are transcriptional activation and proteolytic destruction, which together control the appropriate appearance and disappearance of key cell cycle players. Work in our laboratory explores the intersection between these two processes to understand how 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. Myc is also a highly unstable protein that is destroyed within minutes of its synthesis. The rapid destruction of Myc plays an important part in keeping intracellular levels of the Myc protein low and responsive to environmental stimuli. Our laboratory studies how cells control Myc activity by controlling Myc destruction, and how this process goes wrong in cancer.

We have previously shown that Myc is an unstable protein because it is destroyed by a process known as ubiquitin-mediated proteolysis. In this process, the covalent attachment of Myc to the protein ubiquitin (Ub) signals its destruction by 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, Ub is then transferred to a lysine residue within the target protein. This process is then repeated many times to produce a highly ubiquitylated substrate that is rapidly destroyed by the proteasome. Because 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 Ub-mediated proteolysis.

We have mapped the elements in Myc that control its destruction and have found that the Myc degron overlaps closely with the transcriptional activation domain of Myc. We have also shown that cancer-associated mutations within the Myc degron—which are observed in a very high percentage of lymphomas—subvert the normal destruction of Myc, stabilize the protein, and allow Myc to accumulate. Importantly, some of these mutations have also been shown to enhance the transforming ability of Myc in vitro, suggesting that enhanced protein stability is a mechanism of Myc activation in cancer.

We continue to study mechanisms of Myc proteolysis. We are exploiting an extraordinary conservation of Myc destruction in yeast to identify molecules that participate in this process. Using this approach, we have identified several components of the Ub-proteasome pathway that are required for the Ub-mediated destruction of Myc in yeast; we are currently characterizing their mechanisms of action and asking whether the human cell homologs of these genes participate in Myc destruction in human cells. We have also established a novel tagging system that allows rapid and sensitive measurement of Myc protein stability. Previous studies of Myc protein turnover have been hampered by difficulties in detection of the Myc protein. Using our tagging system and retroviral gene transfer, however, we are able to express Myc at physiological levels in untransformed cells and accurately determine its stability. We are currently using this system to complete our characterization of the elements in Myc that control its stability and to identify molecules that affect Myc protein stability.

In addition to our studies of Myc, we are also interested in more general aspects of how transcriptional activators are targeted for Ub-mediated destruction. Like Myc, many of the transcription factors that control cell growth are unstable proteins that are destroyed by Ub-mediated proteolysis. We wish to apply what we have learned about Myc protein destruction to other unstable transcriptional regulators. Recent studies in our laboratory have suggested that a common mechanism underlies transcription factor destruction, because they have revealed that a transcriptional activation

domain per se can signal Ub-mediated proteolysis. As mentioned above, we had previously shown that the Myc degron overlaps closely with the transcriptional activation domain of Myc. We have found that these two elements overlap in almost all unstable transcription factors. We have also found that acidic activation domains, but not those classified as glutamine-rich or proline rich, can signal protein instability. We used a family of synthetic activation domains to show that there is an intimate relationship between the transcriptional activation domain and degron function (see Fig. 1), with more potent activation domains always being more effective at signaling protein instability than their less active counterparts. We have also shown in collaboration with Herman Wijnen and Bruce Futcher that degrons from yeast cyclins, when tethered to a DNA-binding domain, can activate transcription. The extensive overlap of activation domains and degrons, and the close functional relationship between the two activities, reveals an unexpected convergence of two very different processes and suggests that many transcription factors are destroyed because of their ability to activate transcription. We are currently performing genetic analyses in yeast to understand the true nature of the split personality of these destruction and activation domains (DADs).

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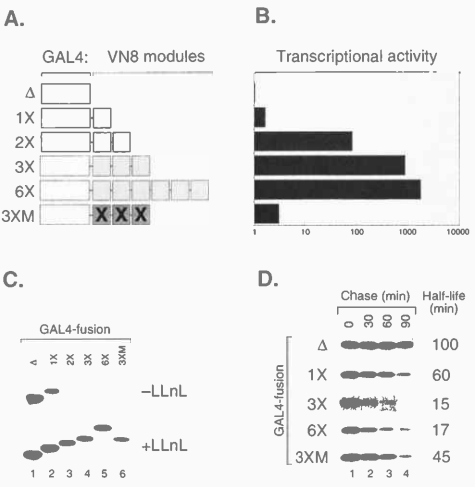

FIGURE 1 Intimate functional overlap between activation domain and degron function in a family of synthetic activators. To explore the relationship between transcriptional activation domains and degrons, we asked whether a family of synthetic activation domains, derived solely on the basis of their ability to activate transcription, could function as degrons. (*A*) Structure of synthetic activators. Activators were constructed by fusing 1x, 2x, 3x, or 6x copies of an 8-amino-acid sequence from the VP16 activation domain (VN8: DFDLDMLG) or 3x copies of a mutant sequence (3XM: DADADMLG) to the DNA-binding domain (DBD) of the yeast transcription factor GAL4. (*B*) Relative transcriptional potency of each activator. GAL4 fusion proteins were assayed for transcriptional activation in human HeLa cells. Relative transcriptional activity is shown on a logarithmic scale, setting the transcriptional activity of the GAL4 DBD alone (Δ) at 1. (*C*) Steady-state levels of the GAL4-VN8 activators. Human HeLa cells were transiently transfected with expression constructs encoding the indicated GAL4 fusion proteins. Following transfection, cells were either untreated (–LLnL) or treated with the proteasome inhibitor LLnL (+LLnL). Total proteins were prepared and HA-tagged GAL4 fusion proteins revealed by SDS-PAGE and immunoblotting analysis. As can be seen, potent activation domains prevent accumulation of the GAL4 DBD, unless the proteasome is inhibited (+LLnL). (*D*) Stability of GAL4-VN8 activators. GAL4-VN8 proteins were transiently expressed in HeLa cells, and their stabilities were determined by pulse-chase analysis. Potent activation domains destabilize the GAL4 DBD more than weak activators, demonstrating that the ability of VN8 modules to activate transcription correlates with their ability to signal protein instability.

MOLECULAR GENETICS

The section on molecular genetics studies problems relating to growth, development, and programmed cell death in humans and model organisms. The groups in this section share a genetic approach to these problems.

- Dr. Futcher's lab studies the control of cell division in yeast with special emphasis on cyclin-dependent protein kinases and their substrates and also on the regulation of telomeres. Recent studies have identified critical localization features of the cyclins, identified mutants of telomere maintenance, and identified a cluster of genes under the regulation of "Forkhead" transcription factors.
- Dr. Grossniklaus's group studies sexual function in plants (*Arabidopsis* and maize), in particular the development of the female gametophyte. They also work on developing methods for the asexual reproduction of plants.
- Dr. Jackson's group also studies *Arabidopsis* and maize, in particular the development of meristems. They continue to study cell-to-cell communication in plants involving the intercellular trafficking of regulatory proteins and have developed genetic screens for mutations in this pathway.
- Dr. Martienssen's lab focuses on the genomic architecture of *Arabidopsis* and the development of whole genome approaches, especially enhancer traps, to define genes important in development.
- Dr. Hamaguchi's group studies human cancer genes. They have identified two candidate tumor suppressors from the region of chromosome 8p22-23. They are now screening tumors for point mutations in these genes in order to validate these candidates.
- Dr. Wigler's group also studies tumor suppressors and is developing new genetic tools for the study of human disease. These tools are increasingly based on microarrays for identifying deletions and amplifications in cancer, and microarray methods for the functional analysis of candidate cancer genes. These methods are also being applied to the analysis of spontaneous mutations linked to sporadic hereditary diseases.
- Dr. Hannon's group has developed new technology for the genetic manipulation of mammalian cells, using highly modified retroviral tools. This technology has been used to identify new candidate oncogenes that may function in the pathways of programmed cell death and cellular senescence.
- Dr. Hengartner's group uses the nematode *C. elegans*, a simple metazoan, to study highly conserved but complex biological processes, with an emphasis on apoptosis (programmed cell death) and developmental neurobiology. They have recently identified a gene, *CED-6*, required for engulfment of the apoptotic cell and have found that germ line cells have a DNA-damage-induced cell death pathway. Recently, they have identified mutations that disrupt axonal guidance.

CELL CYCLE CONTROL IN *SACCHAROMYCES CEREVISIAE*

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

Our main interest is the regulation of Start and mitosis in *Saccharomyces cerevisiae*. We are most interested in the tethering of division to cell growth and in the generation of the basic cell cycle oscillation. Many key cell cycle events are regulated by protein kinase complexes formed between Cdc28p and one of nine cyclins. These fall into two broad groups: the G_1 cyclins, which include Cln1, Cln2, and Cln3, that regulate Start and the mitotic B-type cyclins, which include Clb1, Clb2, Clb3, and Clb4. Two other cyclins, Clb5 and Clb6, are very important for DNA replication, but they also have roles at Start and perhaps also in early mitosis. A second interest is yeast and human telomerase, and how telomere length relates to cell senescence.

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. A previously known mutant allele of Cln3p, which lacked a few basic amino acids at its carboxyl terminus, is reverted to wild type by the nuclear tag, suggesting that these missing amino acids are at least part of a nuclear localization signal (NLS). Nuclear forms of Cln3p can complement all *CLN3* functions, whereas cytoplasmic forms of Cln3p cannot complement any *CLN3* function, suggesting that Cln3p is solely nuclear.

Cln2p, on the other hand, is both cytoplasmic and nuclear. Subcellular fractionation and fluorescence microscopy with Cln2p-GFP (green fluorescent protein) show mainly cytoplasmic localization, with some nuclear localization. The cytoplasmic Cln2p is mostly at the sites of bud growth or polarized growth, suggesting that the known involvement of Cln2p in these processes is direct. Neither cytoplasmic nor nuclear Cln2p can fully complement all functions of *CLN2*, showing that Cln2p has important functions in both locations. In particular, the function of *CLN2* that overlaps with the *CLB5, CLB6* function is a nuclear one, whereas the *CLN2* functions that overlap with the functions of *PCL1, PCL2*, or *BUD2* are cytoplasmic ones.

Two Yeast Forkhead Transcription Factors Regulate a Cluster of Genes at Mitosis

T. Volpe and B. Futcher [in collaboration with Gefeng Zhu, University of Washington, Seattle; Tricia Davis, University of Washington, Seattle; Paul Spellman, Stanford; and David Botstein, Stanford]

Previously, we used microarrays to identify 800 cell-cycle-regulated genes in yeast. Among these were a cluster of 35 genes called the "*CLB2*" cluster, whose transcription peaked in mitosis. This cluster included many genes important for mitosis including the mitotic cyclins *CLB1* and *CLB2*, and the transcription factors, *SWI5* and *ACE2*. Computer analysis of the promoters of the genes in this cluster revealed that they contained a binding site for the transcription factor Mcm1p immediately adjacent to the motif GTAAAYAA. A variant of the later motif, AACAA, had previously been identified as important for the expression of *CLB1, CLB2*, and *SWI5*. It was thought

to be the binding site for a hypothetical transcription factor called "Swi Five Factor" (SFF).

We wished to identify SFF. Because most other transcription factors for cell-cycle-regulated genes are themselves cell-cycle-regulated, we searched among the 800 cell-cycle-regulated genes for putative transcription factors of unknown function. Furthermore, since previous genetic screens for SFF had been unsuccessful, we guessed that SFF might be encoded by redundant genes. Thus, we were especially interested in pairs of homologs of cell-cycle-regulated putative transcription factors and found only three such pairs. One of the pairs was Fkh1p and Fkh2p, which encode transcription factors of the Forkhead family. At about this time, Gefeng Zhu and Tricia Davis determined that the binding site of a GST-Fkh1p fusion was GTAAACAAA, which is essentially identical to the SFF site. Therefore, we examined the regulation of the *CLB2* cluster of genes in an *fkh1 fkh2* mutant.

When an *fkh1 fkh2* mutant was released from an α-factor block, the *SWI5* transcript (a member of the *CLB2* cluster) failed to oscillate, although control cell cycle transcripts from other clusters did. mRNAs from this experiment were then used for microarray analysis. None of the mRNAs encoded in the *CLB2* cluster oscillated, whereas most other cell-cycle-regulated transcripts oscillated normally. However, there was one additional cluster, the *SIC1* cluster, with aberrant regulation in the *fkh1 fkh2* mutant. The genes of this cluster are regulated by the transcription factors Swi5p and Ace2p, which are encoded in the *CLB2* cluster.

We formed the hypothesis that Fkh1p and Fkh2p regulate the *CLB2* cluster directly, by binding to the SFF sites of the promoters of these genes. On the other hand, we hypothesized that they regulate the *SIC1* cluster indirectly, by altering the transcription of *SWI5* and *ACE2*. To test these hypotheses, we did cross-linking chromatin immunoprecipitations using an Fkh2p tagged with three tandem copies of the HA epitope. Immunoprecipitation of this tagged Fkh2p gave specific coprecipitation of four out of four promoters of the *CLB2* cluster (*CLB2, SWI5, HST3, YJL051w*). However, there was no coprecipitation of two out of two promoters from the *SIC1* cluster (*EGT2, SIC1*). This suggests that the hypotheses are correct.

The identification of Fkh1p and Fkh2p as critical components of SFF is significant in two ways. First, it will allow a detailed understanding of the regulation of a large cluster of important mitotic genes. Second, it appears that the *FKH1* and *FKH2* genes are indirectly under the control of the G_1 cyclin and the G_1 phase transcription factors SBF and MBF. Thus, we will be able to establish a chain of events linking early G_1 to late mitosis, bringing us almost all the way around the cycle.

Mechanisms of Transcriptional Activation by Cyclin-Cdc28p Complexes

H. Wijnen

One model for activation 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) inhibit RB, an inhibitor of E2F (analogous to SBF). To look at this model, we have selected for mutants in which SBF is active even in the absence of *CLN3*. Several such mutants have been found. One is in the *CHD1* gene, a chromatin remodeling protein containing chromodomains. The *CHD1* gene is highly conserved throughout evolution, with homologs in humans, and it seems to be involved in repression. Interestingly, RB recruits a similar chromatin remodeling protein to E2F-regulated promoters. Other mutations allowing SBF activity in the absence of *CLN3* were in *YMR131c* (slightly homologous to an RB-binding protein), *YDR295c* (a putative transcription factor), and *YGL004c* (a WD40 repeat protein). We are still characterizing these mutants.

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 is *mtr10*, a karyopherin β involved in nuclear import. The *mtr10* mutant has short telomeres, and although it is viable, it has a partial senescence phenotype. The short telomere and senescence phenotype was suppressed by overexpression of the telomerase RNA Tlc1p, but not by other telomerase components. Furthermore, Northern analysis showed that Tlc1p levels were unusually low in the *mtr10* mutant, and kinetic experiments showed that the Tlc1p RNA has an unusually short half-life in the mutant. The RNA may also have an aberrant length. Tlc1p is known to be processed posttranscriptionally, and it may be that transport via Mtr10p is important for this processing. In the absence of Mtr10p, transport and processing may be abnormal, preventing accumulation of the stable, mature form of Tlc1p.

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DEVELOPMENTAL GENETICS OF PLANT REPRODUCTION

U. Grossniklaus R. Baskar, C. Spillane, A. Coluccio, J. Moore, J. Thomas, J.-P. Vielle-Calzada

We use genetic and molecular approaches to identify genes controlling plant reproduction and focus on the complex processes that lead to the formation of the female gametes. The key events in female gametogenesis occur within ovules where a single cell gets committed to undergo meiosis. Only one of the meiotic products survives and gives rise to the haploid female gametophyte, a multicellular structure unique to the plant life cycle. Two cells in the female gametophyte differentiate into gametes: the egg and central cell. Double fertilization of these cells by two sperms delivered by the pollen tube (male gametophyte) gives rise to the embryo and endosperm, respectively. We are particularly interested in unraveling the molecular mechanisms controlling cell fate decisions that are at the center of these developmental processes.

In addition to yielding insights into fundamental concepts of plant development, our research also provides new tools to manipulate the reproductive system. We focus on engineering components of apomixis, a naturally occurring asexual form of reproduction through seeds. In apomixis, the developmental events we study in sexual systems are short-circuited or modified to prevent meiotic reduction and fertilization. As a result, the progeny of an apomictic plant is genetically identical to the mother plant. The introduction of apomixis into sexual crops promises economic and social benefits exceeding those of the green revolution. However, the synthesis of apomixis requires knowledge of the genes controlling all relevant developmental processes. International collaboration is crucial to harness this complex trait and to provide free and equal access to apomixis technology (see: http://billie.harvard.edu/apomixis).

1999 brought major changes for our group. After spending 7 months in our laboratory, visiting scientist I. Siddiqi returned to the Center for Cellular and Molecular Biology in Hyderabad, India. But we welcomed new visitors, N. Huck (Friedrich Miescher Institute, Switzerland) and J.G. Ramike Pimentel (CINVESTAV, Plant Biology Unit, Mexico), who visited the lab over the summer. In March, J. Moore, R. Baskar, and C. Spillane relocated to the Friedrich Miescher Institute in Basel, Switzerland, where I took a position as Staff Scientist. J.-P. Vielle-Calzada, A. Coluccio, and J. Thomas stayed at the Laboratory until the end of the year. We would like to thank everyone who made the last 5 years at Cold Spring Harbor a stimulating and enjoyable time for us and our families.

Isolation of Genes Involved in Megasporogenesis

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

Sexual and apomictic reproduction are closely related. The determination of apomictic initial cells usually occurs during megasporogenesis. Some of the genes regulating sexual development may also be responsible for the induction of apomixis. Therefore, we screened our transposant collection for (1) genes expressed during megasporogenesis about which we have reported previously and (2) mutants resulting in female sterility. We identified 46 families that segregate sterile plants among 3700 transposant families tested. The large majority of these are male sterile mutants or show obvious floral defects that affect reproduction (33/41 tested). A small class of mutants (3/41) are male and female-sterile and may affect meiosis. A few mutants (6/41) are female-specific and most affect ovule morphogenesis. In one mutant, *coatlicue* (*coa*), female sterility is not caused by abnormal ovule morphology but by a defect in megasporogenesis. In *coa* ovules, the functional megaspores arrest at the onset of the gametophytic phase, suggesting that *COA* may be required for the initiation of the mitotic division cycles during megagametogenesis.

Cell Specification in the Megagametophyte: The Egg Cell

R. Baskar, J. Moore, U. Grossniklaus

Although the seven cells of the megagametophyte are clonally derived, they develop along four alternative developmental pathways. The small number of cell

types in the female gametophyte make it an ideal system to study cell specification, although the inaccessibility of this tissue has been a major obstacle for molecular investigations. We are particularly interested in the specification and function of the egg cell. We identified two transposants with expression in the egg. Reporter gene constructs containing up to 4.5 kb of 5′ sequences of one of these genes have been introduced into plants by *Agrobacterium*-mediated transformation. The transformants show reporter gene expression in the female gameotphyte, but expression is not restricted to the egg cell. This may be due to the presence of a second transcription unit on this fragment which we identified recently. We are analyzing deletion derivatives that do not contain this second gene with the aim of identifying egg-cell-specific regulatory elements. These will be used for genetic cell ablation and misexpression of putative regulatory genes to investigate cellular interactions between the cells in the female gametophyte and to probe the potential of the egg cell for autonomous activation, an important component of apomictic reproduction.

Developmental Genetics of Megagametogenesis

J. Moore, U. Grossniklaus [in collaboration with N. Huck, D. Page, and S. Lienhard, Friedrich Miescher Institute, Switzerland]

Despite the importance of megagametogenesis for plant breeding, only a small number of mutants affecting this process have been described. In our transposant collection, we identified about 50 mutants affecting the gametophyte. We characterized 14 of these at the genetic and morphological level. All mutants have been mapped and were found to be distributed across the five *Arabidopsis* chromosomes. Some mutants affect distinct steps along the developmental pathway, such as the mitotic divisions, nuclear migration, cellularization, and nuclear fusion. In contrast, others form phenotypically normal female gametophytes but are infertile, suggesting that fertilization is affected. To gain a better understanding of the phenotypes, we made use of enhancer detector lines with *GUS* expression in specific cell types of the megagametophyte. We crossed these molecular markers into mutant backgrounds in order to observe how cell-type-specific gene expression was affected. In a pilot study, we found expression of an egg-cell-specific marker in gametophytes that arrested development prior to the mitotic divisions. Does the megagametophyte have a basic egg cell nature even when prevented from forming an egg cell? Is cell type-specific gene expression directed from the cells surrounding the female gametophyte? Does a unicellular gametophyte express characters of all its cell types? We hope to address these questions by extending this study in the coming year.

Genetic Control of Genomic Imprinting in Plants

C. Spillane, J. Thomas, J.-P. Vielle-Calzada, U. Grossniklaus [in collaboration with S. Michele Nunes and D. Page, Friedrich Miescher Institute, Switzerland]

Imprinted genes are differentially expressed depending on their parental origin. Gene-specific genomic imprinting was first described for the maize *red color* locus (Kermicle, *Genetics 66:* 69 [1970]) and was later found to play a crucial part in mouse embryogenesis (Surani et al., *Nature 308:* 548 [1984]). Despite the importance of genomic imprinting in development, human disease, and plant breeding, the underlying genetic mechanisms remain largely unknown. We have shown that the *MEDEA* (*MEA*) gene is regulated by genomic imprinting. *MEA* is the first plant gene that displays characteristics of imprinted genes in mammals: *MEA* is the only known imprinted plant gene that affects seed morphogenesis and where imprinting is not allele-specific.

Our current efforts focus on the elucidation of the genetic control of imprinting. The *mea* mutant provides a unique entry point for identifying genes involved in the establishment and maintenance of genomic imprinting. We are using *mea* to gain insight into the imprinting process by isolating second-site modifiers. The genetic and molecular characterization of such modifiers will yield fundamental insights into the poorly understood genetic regulation of imprinting. We are currently pursuing three independent approaches to identify modifiers of *mea*. (1) We have screened approximately 2000 chemically mutagenized plants for second site mutations that alter the seed abortion characteristics of *mea/MEA* heterozygous plants. For instance, a mutation in a gene that is required for the activation of the maternal *MEA* allele will show a higher degree of seed abortion (75% aborted seeds instead of 50%, if the mutation is fully penetrant and unlinked). To date, we have identified 15 putative modifiers that are being analyzed in more detail. (2) We have screened for natural variants in

over 25 *Arabidopsis* ecotypes and identified two ecotypes where silencing of the paternal *MEA* copy appears to be less stable. (3) We are screening through collections of mutants known to affect methylation or transgene silencing to identify genes that also affect genomic imprinting. This approach has led to the identification of the first *trans*-acting factor involved in maintaining a genomic imprint, the *DECREASE IN DNA METHYLATION* (*DDM1*) gene.

Genetic Screens for Mutants Displaying Apomictic Traits in Maize

U. Grossniklaus, A. Coluccio

Three main steps are relevant to the engineering of gametophytic apomixis. (1) Avoidance of meiosis, (2) parthenogenesis, and (3) normal endosperm development. During the past few years, we have developed maize stocks for genetic screens that should allow the isolation of mutants which are relevant to these aspects. For instance, no systematic screens for mutants that produce unreduced gametophytes have been performed, but some were found fortuitously, e.g., the *elongate1* (*el1*) mutant that produces 30–50% unreduced megagametophytes (Rhoades and Dempsey, *Genetics 54:* 505 [1966]). In maize, normal kernel development strictly requires a 2m:1p ratio of maternal to paternal genomes in the endosperm. If a unreduced central cell is fertilized by a normal sperm, the kernel aborts due to an imbalance of imprinted genes in the endosperm. We developed a tetraploid stock tetraplex for *R-Navajo* (*R-nj*) (allows the identification of contaminants). Fertilization of an unreduced megagametophyte with pollen from this stock results in a 4m:2p genomic ratio ensuring normal endosperm development. We screened more than 450 families that were derived from individuals with high *Mutator* activity for plants producing plumb kernels presumably derived from unreduced megagametophytes. Several putative mutants were identified and are currently being analyzed.

Delayed Activation of the Paternal Genome during Seed Development

J.-P. Vielle-Calzada, R. Baskar, A. Coluccio, J. Thomas, U. Grossniklaus

While we were studying imprinting at the *mea* locus, we discovered a related phenomenon that apparently affects most, if not all, of the paternal genome. Because plant embryos can develop from somatic cells or microspores, it is generally considered that the maternal contribution is not crucial for embryogenesis. Early acting embryo lethal mutants in *Arabidopsis*, including *emb30/gnom* that affects the first zygotic division, have fueled the perception that both the maternal and paternal genomes are active immediately after fertilization. We identified 19 transposants that show reporter gene expression in the developing embryo and/or endosperm after fertilization. To determine if *GUS* expression was the result of transcription from one or both alleles, we performed reciprocal crosses between the wild type and these transposants. For all 19 lines, *GUS* expression was strictly dependent on maternal transmission (Fig. 1), suggesting that the paternally inherited allele is not expressed during early embryo and endosperm development. To determine whether the absence of paternally derived *GUS* expression was due to inactivation of the *GUS* transgene or if it reflected silencing of endogenous genes, we analyzed gene expression using allele-specific reverse transcriptase–polymerase chain reaction (RT-PCR). For *PROLIFERA* (*PRL*) (Springer et al., *Science 268:* 877 [1995]) in which we recovered a new insertion, only transcripts derived from the maternal allele could be detected during the first 3–4 days after pollination, after which the paternal allele was activated.

We isolated and sequenced genomic regions flanking 12 of the *Ds* elements. Putative functions based on homology could be assigned to eight of the detected genes. They encode proteins with a wide variety of functions and are located throughout the *Arabidopsis* genome, suggesting that other genes known to be expressed at early stages may also be affected by this regulation. To test this prediction, we examined *EMB30/GNOM* expression using allele-specific RT-PCR. The early phenotype of *emb30* (nearly symmetrical division of the zygote) has been interpreted as being caused by a recessive mutation that affects a gene active during the earliest diploid phase of embryogenesis. At 24 hours after pollination (HAP), *EMB30* mRNA derived from the paternally inherited allele cannot be detected. To determine if allele-specific expression is correlated with genetic activity, we crossed heterozygous *emb30/EMB30* plants to the wild type and analyzed developing seeds 24–48 HAP. If the paternally inherited allele is active following fertilization, heterozygous *emb30/EMB30* embryos that inherit a wild-type allele from their father should undergo normal development. We found no difference in the frequency of early defects between F_1 seeds

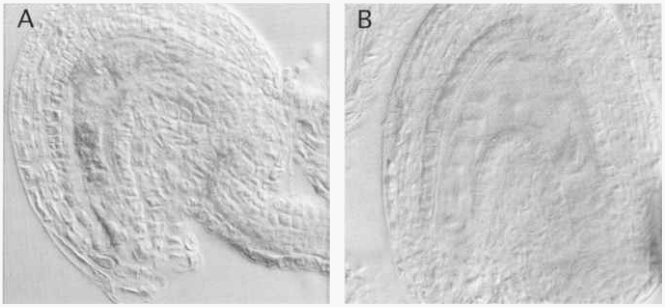

FIGURE 1 Silencing of paternally inherited gene activity during seed development in *Arabidopsis*. (*A*) When an enhancer detector is used as a female parent in crosses to wild type, reporter gene expression can be detected in the developing embryo and endosperm of F_1 seeds 48 hr after pollination (HAP). (*B*) When the same enhancer detector is used as the male parent in crosses to wild-type plants, *GUS* expression is absent from the embryo and endosperm 48 HAP. The same observation is true for all 19 enhancer detector lines tested.

derived from self-fertilization and an out-cross to the wild type. However, all F_1 seeds derived from the out-cross were normal and viable at maturity, suggesting that late expression of the paternally derived *EMB30* allele is sufficient to rescue the *emb30* defects. Thus, early defects are caused by a maternal effect that can be rescued by a paternally inherited wild-type allele. Our results strongly suggest that the first few days of embryogenesis and endosperm development are largely, if not exclusively, under maternal control, a finding that should lead to a reinterpretation of the genetic control of early seed development in flowering plants.

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TUMOR SUPPRESSOR GENES AND BREAST CANCER

M. Hamaguchi R. Jones J. Troge
J. Meth J. Winer
T. Odawara

Breast cancer is by far the most common cancer and the second leading cause of cancer death in women. Expanding knowledge of the genetic basis of breast cancer will be helpful in overcoming this disease in several ways. Molecular probes can be used to predict which individuals are at high risk for breast cancer. Molecular diagnoses may furnish us with useful information to determine if treatment should be aggressive or conservative. A better understanding of the causes of breast cancer will bring new insights into cancer development in general and therefore provide new ideas for prevention and treatment of cancer.

Tumor cells have been studied for this purpose and have been found to have multiple genetic mutations, some of which are commonly observed among tumors. Mutations of certain genes are now believed to play a critical part in cancer development. A class of these genes called tumor suppressor genes prevent cells from growing out of control. Loss of their function drives the normal cells to act in a malignant manner. As for breast cancer, some tumor suppressor genes have been cloned in familial cases, but genes for nonfamilial cancer have not yet been discovered. Certain chromosomal regions are suspected of containing tumor suppressors because they are frequently deleted in breast cancer cells. In fact, we have identified two homozygous deletions at 8p22 and 8p23. Furthermore, loss of heterozygosity (LOH) of the short arm of chromosome 8 has reportedly occurred in more than half of breast cancers. LOH represents the status that one allele is eliminated and therefore only one intact gene is left. These facts imply the presence of tumor suppressor genes in these loci. Our efforts have been directed at cloning these suppressor genes.

Positional Cloning (Gene Hunting)

M. Hamaguchi, R. Jones, J. Meth, J. Troge [in collaboration with D. Esposito and M. Wigler, Cold Spring Harbor Laboratory]

The deletion probes in breast cancer have been mapped by radiation hybrid (RH) mapping. Radiation hybrids are rodent cells containing human genomic DNA fragments generated by irradiation. If two DNA markers are close to each other, the retention patterns in these RHs should be similar. Thousands of known sequence tagged sites (STSs) have been screened as a framework. Relative distances between a known STS and the probe in question are obtained by comparison of their retention pattern. One deletion probe was mapped to 8p22 and another to 8p23. Thus, RH panels are useful for obtaining approximate map locations at a resolution of approximately 500 kbp.

Detail maps of the loci are critical for further investigation, and thus we have constructed physical maps of these loci. Library screening and database searches generated a number of genomic mega clones (20 YACs, 11 BACs, and 2 P1s). STSs around the original deletion probes have been isolated by database searches, subcloning of genomic clones, and sequencing of P1s. These STSs have been further mapped by RH and analyzed by polymerase chain reaction (PCR) against genomic clones to determine their physical order. We have utilized 83 STSs to refine the physical map (see Fig. 1).

Deletion mapping has been carried out by quantitative PCR (Q-PCR) on 200 breast tumors using 18 STSs. The nuclei of tumor and normal cells have been separated by FACS (fluorescence-activated cell sorter). A method to immortalize DNA is required because only a limited amount of nuclei can be obtained by FACS. We have applied "high complexity representation" (HCR) developed by Dr. Wigler as a solution for this problem. Two deletion epicenters have been demarcated by these analyses, and the size of each deletion is estimated at 500 kb (see Fig. 1).

A transcript map has subsequently been constructed by database searches, rapid isolation of cDNA by hybridization (RICH), exon trapping, and sequencing. RICH is a new genetic tool to identify transcribed sequences developed in our lab. To improve efficiency, we have also designed a new vector for exon trapping. Since discovery of genes from a given genomic region is the rate-limiting step in positional cloning, we have been devoted to develop-

ing and employing new technologies for this purpose. This procedure has provided us with 35 candidate sequences, including 19 expressed sequence tags (ESTs), 7 RICH products, 2 trapped exons, and 7 exons predicted by computational analysis. By RT-PCR analysis, 7 out of 35 fragments have been found to be expressed in the normal mammary glands and utilized to isolate full-length cDNAs.

Mutational Analysis of Candidate Genes

M. Hamaguchi, J. Meth, T. Odawara, J. Winer [in collaboration with M.C. King, University of Washington, and S. Scherneck, Max-Delbruck-Centrum for Molecular Medicine]

We have cloned the full-length cDNA of six genes, all of which are deleted in breast tumors. Our first step was mutational analyses of these genes in cell lines; 60 cell lines have been screened by the protein truncation test (PTT) as follows: Open reading frames of the genes are amplified by PCR with specially designed primers so that the PCR fragments have the T7 RNA polymerase promoter at the 5′ ends and the polyadenylation signal at the 3′ ends. The amplified fragments are transcribed in vitro using T7 RNA polymerase and then translated into protein with labeling, and the produced protein is analyzed by electrophoresis. If a gene has a deletion/insertion that causes frame shift or a mutation that generates a stop codon, the gene product will be shorter than normal, thus, the term truncation.

We have first examined two genes, *Bravo* and *Charlie*, because they are most frequently deleted (6/200). They are located in the center of deletion with their 3′ ends overlapping. We have detected a truncated protein of *Bravo* in a breast tumor. The causative mutation was confirmed by sequencing. These facts support that *Bravo* as being a strong candidate for the tumor suppressor gene, although its biological function is unclear at this moment. *Charlie* has turned out to be a TRAIL receptor. TRAIL receptors are related to programmed cell death, which qualifies them as a tumor suppressor candidate. We have not detected its truncating mutation in cell lines. We are screening for mutations of *Bravo* and *Charlie* on surgical specimens and familial cases. No truncating mutations of the other four genes have been demonstrated.

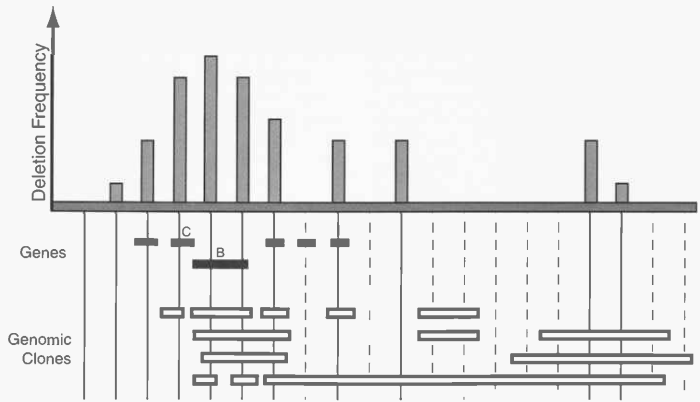

FIGURE 1 Map of chromosome 8p22. (*Vertical lines*) STSs; (*solid lines*) SSTs used for both deletion and physical mapping; (*dotted lines*) SSTs used only for physical mapping. (*Top*) Deletion map; the height of each column represents deletion frequency. (*Middle*) Transcript map; each solid box represents a candidate gene. B and C represent *Bravo* and *Charlie*, respectively. (*Bottom*) Physical map; the open boxes represent genomic clones.

Expression Study of a Tumor Suppressor Candidate

M. Hamaguchi, J. Meth, T. Odawara [in collaboration with M. Wigler, Cold Spring Harbor Laboratory, and K. Kitamura, National Institute of Health (Japan)]

We have carried out expression studies of *Bravo* and *Charlie* on cell lines, resulting in identification of several cell lines that do not express these genes. Two breast cancer cell lines have been demonstrated not to express either *Bravo* or *Charlie*. To clarify their biological roles, we are performing a forced expression study on these two cell lines. *Bravo* and *Charlie* have been cloned into a vector with a promoter so that they can be expressed in the target cells. We will observe phenotypic changes of the cells caused by their expression. We will also compare the expression patterns between the original and transformed cells.

Two inducible gene expression systems have been developed by Dr. Wigler and Dr. Kitamura. Dr. Wigler utilizes the retrovirus-mediated ecdysone system, and Dr. Kitamura uses the vaccinia-virus-mediated RNA polymerase system. Both systems along with the DNA microarray will be used to scrutinize the biological function of the candidate genes. The changes in the expression pattern by induction of the genes examined are detected by DNA microarray technology to identify the genes residing downstream from their signal transduction pathway.

GROWTH CONTROL IN MAMMALIAN CELLS

G. Hannon E. Bernstein S. Hammond
S. Boettcher Y. Seger
J. Du

The interests of my laboratory remain the basic mechanisms underlying neoplastic transformation. This has been reflected in the past year by continued efforts to dissect the transformation process through genetic approaches in cultured mammalian cells. In addition, we have also persisted in the development of tools and technologies that might aid our efforts by improving our ability to manipulate mammalian cells in vitro. Finally, our laboratory has delved into an area that is new for us: homology-dependent gene silencing.

This year, two graduate students, Yvette Seger and Emily Bernstein, and one postdoc, Jian Du joined our laboratory. Scott Hammond came as a visiting scientist from Genetica Inc. Sabrina Boettcher left her position as media maker in the McClintock lab to become a technician in our laboratory.

Cancer Genetics

J. Du, Y. Seger

The transformation of a normal cell into a cancer cell occurs via multiple genetic alterations that release a cell from the constraints which normally regulate proliferation. These include decreased sensitivity to extracellular signals that control growth and a disruption of internal controls that ensure that each cell cycle is completed without error. Ultimately, most normal cells also have a preprogrammed limitation on the number of cell divisions that can be executed. During the past year, we have continued our investigations into tumor suppression mechanisms, devoting most of our efforts to seeding new long-term projects that are designed to reveal basic aspects of the transformation process. Specifically, we wish to understand the mechanisms by which the *myc* oncogene contributes to tumor formation (Y. Seger) and to probe genetic alterations that can provoke genomic instability, a hallmark of cancer cells (J. Du). We have also continued work on oncogene-induced apoptosis.

Protection from Oncogene-dependent Apoptosis

G. Hannon, R. Maestro

Normal cells often respond to pro-oncogenic alterations by activating homeostatic responses that are likely to act as tumor suppression mechanisms. These can manifest themselves as growth inhibitory (e.g., senescence) or as predisposing to programmed cell death (apotosis). Since oncogenes such as *myc* predispose normal cells to apoptosis, additional genetic alterations must occur during tumorigenesis that counteract these proapoptotic effects. Last year, I described the results of a screen to identify genes that could protect normal cells from oncogene-dependent cell death. One of the genes isolated through this approach is a developmentally regulated transcription factor, Twist. This effort has been carried out as a collaboration with Roberta Maestro, a former visiting scientist here at Cold Spring Harbor Laboratory and present faculty member at the CRO in Aviano, Italy. This year has seen the publication of this work and the emergence of some hints with respect to the pathways by which Twist might contribute to tumor formation.

Oncogene-dependent apoptosis proceeds largely through a mechanism that requires an intact p53 tumor suppressor protein. Last year, we reported that 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 (CSHL). Twist could also interfere with p53-dependent growth arrest. The p53 protein is a transcriptional activator that invokes biological responses through the activation of downstream target genes. Twist expression suppressed induction of p53 targets that are known to contribute to growth arrest (p21) and cell death (bax).

The p53 tumor suppressor protein is regulated in a variety of ways. These include control over its abun-

dance and control over its specific activity. In addition, p53 is regulated through association with proteins that control activity, stability, and protein trafficking. As a step toward determining the mechanism by which Twist interferes with p53, we examined the abundance of known p53 regulators. We found that Twist expression reduced the abundance of the ARF (an *a*lternative *r*eading *f*rame of the *INK4a* locus) tumor suppressor mRNA by approximately 5–10-fold.

ARF regulates p53 through its interaction with MDM2 (*m*ouse *d*ouble *m*inute, a regulator of p53). ARF, in turn, is regulated by proapoptotic oncogenes such as *myc* and *E1A*. ARF-null cells are impaired in the ability to undergo programmed cell death in response to these proapoptotic oncogenes, thus mimicking the phenotype of Twist-expressing cells. Although Twist could impact the p53 pathway in other ways as well, suppression of ARF synthesis is sufficient to account for the effects on p53 activity that we observe. Work during the next year will focus on the mechanisms that underlie the effect of Twist on ARF expression.

Homology-dependent Gene Silencing

E. Bernstein, S. Hammond

In most organisms, the presence of double-stranded RNA (dsRNA) signals trouble. For example, dsRNAs could indicate invasion by pathogens or could reflect the activity of mutagenic and potentially deleterious mobile genetic elements. Therefore, cells have evolved mechanisms for detecting and responding to these threats. In mammals, responses are rather general and include suppression of translation and nonselective RNA degradation. However, in a variety of other systems including *Caenorhabditis elegans*, *Drosophila*, trypanosomes, and plants, the response is specific, interfering only with the expression of sequences that are homologous to the dsRNA. The subject of dsRNA-induced gene silencing (also known as RNA interference or RNAi) has recently achieved a more prominent place in the general consciousness because of its application as a reverse genetic tool. This has highlighted the almost complete mystery surrounding the mechanisms by which dsRNAs can suppress the expression of specific target genes.

We sought to establish a biochemically tractable model in which such mechanisms could be investigated. In part because of our familiarity with cell culture systems, we asked whether dsRNA-induced gene silencing could be provoked in a *Drosophila* cell line. To test this possibility, we introduced into cultured *Drosophila* S2 cells a dsRNA corresponding to *Drosophila* cyclin E, a gene that is essential for progression into S phase of the cell cycle. S2 cells normally reside mostly in G_2. However, those transfected with cyclin E dsRNA arrested in the G_1 phase of the cell cycle (Fig. 1). One hallmark of RNAi is a reduction in the level of mRNAs that are homologous to the dsRNA. Cells transfected with the cyclin E dsRNA showed diminished endogenous cyclin E mRNA as compared with control cells (Fig. 1). Similarly, transfection of cells with dsRNAs homologous to *fizzy*, a component of the *a*naphase *p*romoting *c*omplex (APC)

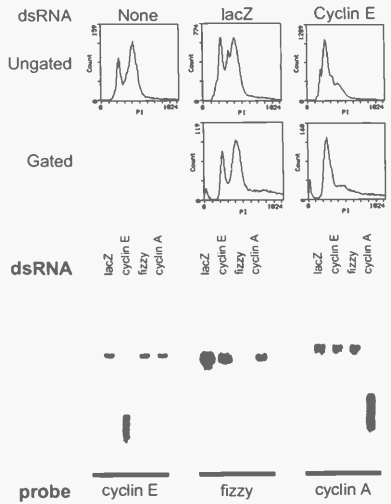

FIGURE 1 (*Top*) Cells were transfected with dsRNAs corresponding to cyclin E or with a control dsRNA (*lacZ*), as indicated. Cells that successfully incorporated cotransfected DNA (*lower panels*, Gated) were marked using a plasmid that directs expression of a membrane-inked green fluorescent protein (GFP). (*Bottom*) *Drosophila* S2 cells were transfected with the indicated dsRNAs. After 3 days, cells were lysed and total RNA was prepared. This was analyzed by Northern blotting with the indicated probes. Equal loading was ensured by overprobing the blot with the RP49 cDNA (encoding a ribosomal protein).

or *cyclin A*, a cyclin that acts in S, G_2, and M, also caused reduction of their cognate mRNAs (Fig. 1). These results suggest that RNAi may be a generally applicable method for probing gene function in cultured *Drosophila* cells.

Mechanistic studies of RNA interference would be greatly aided by the availability of a cell-free system in which the process could be attacked biochemically. We therefore tested cellular extracts for activities that might contribute to dsRNA-induced gene silencing. Lysates prepared from cells transfected with cyclin E dsRNA efficiently degraded a synthetic cyclin E transcript; however, a heterologous *lacZ* transcript was stable in these lysates. Conversely, lysates from cells transfected with the *lacZ* dsRNA degraded the *lacZ* transcript but left the cyclin E mRNA intact. These results suggest that RNA interference ablates target mRNAs through the generation of a sequence-specific nuclease activity and provide a context in which the properties of that enzyme can be studied.

Gene silencing provoked by dsRNA is sequence-specific. A plausible mechanism for determining specificity would be incorporation of nucleic acid guide sequences into the complexes that accomplish silencing. In accord with this notion, pretreatment of extracts with a Ca^{++}-dependent nuclease (micrococcal nuclease) abolished the ability of these extracts to degrade cognate mRNA. Therefore, our results support the possibility that the RNAi nuclease is a ribonucleoprotein (RNP), requiring both RNA and protein components.

In plants, the phenomenon of cosuppression has been associated with the existence of small (~25 nucleotides) RNAs that correspond to the gene that is being silenced (Hamilton and Baulcombe, *Science 286:* 950 [1999]). To address the possibility that a similar RNA might exist in *Drosophila* and guide the sequence-specific nuclease in the choice of substrate, we partially purified our activity through several fractionation steps. Active nuclease fractions also contained an RNA species of 25 nucleotides that is homologous to the cyclin E target. This band is likely to represent a family of discrete RNAs since it could be detected with probes specific for both the sense and antisense cyclin E sequences and with probes derived from distinct segments of the dsRNA.

RNA interference allows an adaptive defense against both exogenous and endogenous dsRNAs, providing something akin to a dsRNA immune response. Our results have begun to provide a framework within which models of dsRNA-induced gene silencing can be created. According to our data, introduction of dsRNA into *Drosophila* cells provokes the assembly of a sequence-specific nuclease. This occurs through conversion of the dsRNA, either via processing or replication, into small RNAs that are homologous to the substrate. These are incorporated into a nuclease that is apparently an RNP particle, containing both essential protein and RNA subunits. Fractionation of the nuclease and Northern analysis of the 25-nucleotide RNA indicates that a family of nucleases is created, with each member having a 25-mer that targets a specific portion of the substrate.

Our data draw a striking parallel between RNA interference in *Drosophila* and posttranscriptional gene silencing (PTGS) in plants. As stated above, plants in which PTGS is occurring contain 25-nucleotide RNA species that are homologous to the gene that is being suppressed. The identical size of putative specificity determinants in plants and animals predicts a conservation of both the mechanisms and the components of dsRNA-induced, posttranscriptional gene silencing in diverse organisms. During the next year, we hope not only to deepen our understanding of this intriguing gene regulatory mechanism, but also to extend through that understanding the reach of RNAi as a tool to study gene function.

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CAENORHABDITIS ELEGANS DEVELOPMENTAL GENETICS

M. Hengartner J. Bikoff, H. Cosel-Pieper (URP), S. Desnoyers, L. Englmeier, A. Fraser, A. Gartner, T. Gumienny, D. Hoeppner, R. Hofmann, Q. Liu, S. Milstein, B. Schumacher, M. Spector, S. Tharin, J. Ye

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

Despite its humble dimensions and simple anatomy, one should not loose 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.

PROGRAMMED CELL DEATH

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

The proximal cause of apoptosis in *C. elegans* is the activation of the caspase homolog CED-3 from the inactive zymogen (proCED-3) into the mature protease. This activation is mediated by the Apaf-1 homolog CED-4. In cells that should survive, CED-3 and CED-4 are antagonized by the Bcl-2 family member CED-9. We previously suggested that CED-9 prevents death by sequestering CED-4, and possibly proCED-3, in an inactive, mitochondria-associated complex, the apoptosome. In cells fated to die, CED-9 is in turn inactivated by the proapoptotic BH3 domain-containing protein EGL-1. All four proteins are structurally and functionally conserved between nematodes and mammals, strongly suggesting that the apoptotic program is ancient in origin and that all metazoans share a common mechanism of apoptotic cell killing.

The* C. elegans *IAP Homolog BIR-1 Plays a Conserved Part in Cell Division, Not Apoptosis: IAPs (inhibitor of apoptosis proteins) have been shown to suppress apoptotic cell death in a variety of model systems, including mammals and *Drosophila*, and have been suggested to inhibit caspases directly. All IAPs contain at least one BIR (baculovirus IAP repeat), a conserved 50–70-amino-acid domain with putative Zn^{++}-binding properties. To determine whether this gene family also controls apoptosis in *C. elegans*, we cloned and characterized two worm BIRPs (BIR-containing proteins), BIR-1 and BIR-2; these are probably the only BIRPs in *C. elegans*. Expression of either BIR-1 or BIR-2 is unable to inhibit programmed cell death in *C. elegans*. Rather, ablation of BIR-1 expression causes a complete defect in cytokinesis with resultant embryonic lethality. Interestingly, this defect can be partially rescued by transgenic expression of survivin, the mammalian IAP most similar to BIR-1. Our results suggest that BIRPs not only function in the regulation of apoptosis, but rather can also participate in diverse biological processes, including cell division.

Physiological Cell Death in the* C. elegans *Germ Line: 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 is very common in the adult hermaphrodite germ line: More than half of the germ cells that differentiate along the oogenic pathway undergo PCD. 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.

To understand 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 mutations in over half a dozen genes that result in excessive germ cell death. We are now in the process of genetically characterizing these genes and are cloning two representative members of this group.

***DNA Damage-induced Apoptosis in* C. elegans:** During our studies of the regulation of germ cell death, we noticed that genotoxic injury such as exposure to ionizing radiation or chemical mutagens can induce germ cell death, as well as germ cell proliferation arrest. These observations suggested that *C. elegans*, like mammals, possesses DNA damage checkpoints that promote the elimination of damaged cells via apoptosis. To better understand how DNA damage activates the apoptotic machinery, we set out to characterize this pathway in more detail.

We found that DNA-damage-induced apoptosis requires the apoptotic core machinery but is genetically distinct from somatic cell death and physiological germ cell death. Interestingly, most mutations that affect physiological cell death did not significantly affect checkpoint-induced cell death, demonstrating that multiple death-inducing pathways exist within the *C. elegans* germ cell. We have identified mutations in three checkpoint genes that are required for DNA-damage-dependent cell proliferation arrest and apoptosis. One of these genes, *mrt-2*, encodes a homolog of the conserved *Schizosaccharomyces pombe rad1*/*S. cerevisiae RAD17* checkpoint gene. This result for the first time implicates a *rad1* homolog in DNA-damage-induced apoptosis in animals and suggests that induction of apoptosis in response to DNA damage might be an ancient, conserved biological response (Fig. 1). We hope that further genetic analysis of radiation-induced cell death in the worm will help us understand the molecular basis of DNA-damage-induced apoptosis in general and in particular why and how tumor cells eventually become resistant to chemotherapy/radiotherapy-induced apoptosis.

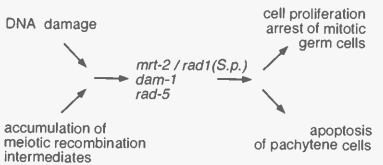

FIGURE 1 *C. elegans* DNA damage checkpoint pathway. The *C. elegans* checkpoint proteins MRT-2, RAD-5, and DAM-1 are required for both cell proliferation arrest and apoptosis following genotoxic events. Arrows indicate activation events.

Genes Involved in Engulfment of Dying Cells: 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*. Last year, we 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, *ced-6* possesses a highly conserved homolog in mammals. We cloned this homolog, which we named *hced-6*, and found that it is widely expressed in humans. Overexpression of *hced-6* in worms can partially rescue the engulfment defect of *ced-6* mutants, suggesting that the two genes are functionally conserved and that the signaling pathway(s) in which CED-6 participates is conserved from worms to humans.

NERVOUS SYSTEM FUNCTION AND DEVELOPMENT

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

Cell Death in the Developing Nervous System: Programmed cell death is very common in the developing *C. elegans* nervous system. To better understand how developing neurons decide between differentiation and death, we have undertaken a detailed study of cell fate determination in the hermaphrodite-specific neuron (HSN), a sexually dimorphic motor neuron, which drives egg laying in hermaphrodites but dies in males. We have isolated more than a dozen mutations in at least three genes that specifically rescue the HSN neuron from death. We expect that a genetic and molecular analysis of these genes will lead to a better understanding of how the HSN neuron controls activation of the apoptotic machinery.

Axonal Guidance and Outgrowth: *C. elegans* has proven to be a useful system to identify conserved genes that function in nervous system development. We are currently concentrating our attention on *unc-69*, which we found to be required for proper guidance of many axons in the developing worm. The *unc-69* gene 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. *unc-69* is highly conserved through evolution, and expression of the human *unc-69* homolog (tentatively named *hunc-69*) completely rescues the locomotion defect of *unc-69* mutant worms. Our current data are consistent with a model where *unc-69* participates in a conserved signal transduction pathway that relays external guidance cues to the growth cone cytoskeleton.

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PLANT DEVELOPMENT

D. Jackson G. Birkenmeier, F. Taguchi Shiobara, Z. Yuan, M. Krishnaswami, G. Sabino, J. Thomas, D. Bikoff

The research in our lab aims at elucidating the molecular mechanisms of development, using *Arabidopsis* and maize as model systems. Plants are an interesting system in which to study development because their mode of development is unique and fundamentally different from that of animal development. In particular, plants show high developmental plasticity since they continue to initiate new organs throughout their life cycle from groups of stem cells called meristems. We are therefore trying to determine how patterns of developmental gene expression are established and to determine the role of cell-to-cell communication in the shoot meristem. It is clear that the domestication of crop plants for agricultural use involved the selection of specific morphological variants, in some cases affecting the meristems either directly or indirectly, e.g., in selection for increases in fruit size or of the number of rows of kernels on an ear of corn. Therefore, a better understanding of the mechanisms of plant development will have significant contributions to agriculture.

Analysis of Mosaic Sectors of KNOTTED1 in *Arabidopsis*

G. Birkenmeier

Cell-to-cell communication is an essential step in the development of any multicellular organism; cells need to talk to each other in order to coordinate their proper development in space and time. We previously described a novel pathway of cell-to-cell communication involving the intercellular trafficking of the plant homeodomain protein KNOTTED1. KN1 is normally expressed in the shoot apical meristem, and dominant mutations show nonautonomous behavior which we correlated with the movement of KN1 protein between leaf cells through specialized channels called plasmodesmata. KN1 is also required for the development of the shoot meristem; loss of function mutations of the *Arabidopsis* KN1 ortholog fail to make or to maintain the meristem.

As a first step to characterize the range and biological significance of cell-to-cell transport of KN1, we designed a system to make marked sectors of KN1 expressing cells in *Arabidopsis*. This system uses an organellar targeted green fluorescent protein (GFP) as a cell autonomous sector marker. This constitutively expressed marker is flanked by *lox* sites and is directly followed by a promoterless GFP-KN1 fusion. Stochastic excision of the *lox*-organellar GFP gene in a single cell early in development, achieved by induction of a heat shock CRE transgene, leads to the production of clonal sectors where the organellar GFP is no longer present and the GFP-KN1 fusion protein is now expressed. By careful observation of sector boundaries, we can determine the range of movement of GFP-KN1 at different stages of development. Since overexpression of GFP-KN1 induces a morphological phenotype of leaf lobing and aberrant growth, we may also be able to use this system to determine the biological consequence of cell-to-cell trafficking, i.e., whether movement of KN1 protein into cells where *kn1* is not being transcribed leads to a discernible phenotype.

Using this system, we have produced sectors of KN1 expression that are easily scored phenotypically; for example, we observe half-leaf sectors where one half of the leaf is normal and the other half has the characteristic overexpression phenotype with severe lobing. Sector boundaries are visualized by a distinct boundary of organellar GFP. However, the fluorescence from the GFP-KN1 fusion has been difficult to detect, presumably because the fusion protein level is regulated and is much lower than that of the organellar GFP-expressing cells. To solve this problem, we are using immunohistochemistry to localize the organellar GFP and the KN1-GFP fusion to assess the range of

cell-to-cell movement of KN1. This will provide important information on the range of signaling that we can expect for this novel mode of cell-to-cell communication.

Development of a New GFP Enhancer Trap in *Arabidopsis*

Z. Yuan, D. Bikoff

GFP has revolutionized cell biology in its ability to reveal patterns of gene expression in living cells and organisms. We are using the GFP as a tool to visualize the cell-to-cell trafficking of a number of plant proteins. An interesting question is to what extent the ability of proteins like KN1 to traffic through plasmodesmata is regulated in different cell and tissue types. To address this question, we are using a novel enhancer trap system developed by Dr. Jim Haseloff (Cambridge University, U.K.). This system relies on genomic enhancers that activate a GAL4-VP16 fusion protein, which in turn activates GFP under the control of the GAL4 UAS. In this system, the GAL4-VP16 and the UAS-GFP are linked on the same transgene, which is a problem because this can interfere with visualization of other GFP fusion proteins driven by the UAS. To overcome this problem, we separated the two components into separate transgenes with different antibiotic selectable markers and are starting to generate enhancer trap lines with this new system. Using these lines, we will *trans*-activate GFP fusions to different plant proteins in order to assess directly their ability to traffic through plasmodesmata connecting different cell and tissue types. We therefore hope to shed light on the regulation of plasmodesmal trafficking, which is proposed to be an important step in developmental regulation as well as in systemic signaling in viral defense and posttranscriptional gene silencing.

Isolation of the *fae2* Gene of Maize

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

We have isolated a series of mutations in maize that cause aberrant proliferation and growth (fasciation) of the ear inflorescence meristem, leading to an increase in the number of flowers initiated by the ear. The normal function of these genes is therefore important either to control stem cell proliferation or to promote lateral organ formation. One of the mutations, *fasciated ear2* (*fae2*) arose in a nontargeted *Mutator* transposon tagging screen, and we previously identified and cloned a *Mu8* element and flanking genomic DNA that cosegregated with the *fae2* mutation. In the past year, we isolated a putative new allele of *fae2*. The plant carrying this new mutation also showed a novel DNA polymorphism when probed with the *Mu8* flanking

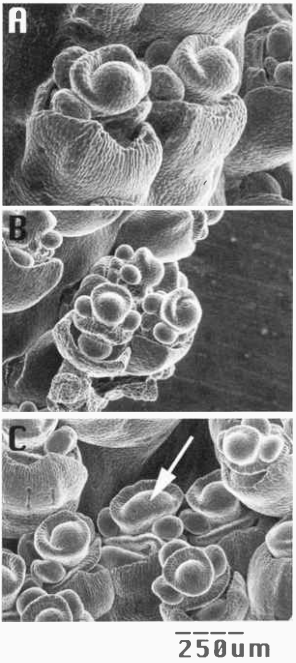

FIGURE 1 Flower development in normal and *fae2* ears. Scanning electron micrographs of maize flowers which on normal ears are borne in pairs (*A*). In *fae2* mutants, however, this pattern is disrupted and flowers arise in irregular groups of three or more (*B*). Within the flower, the development of the female organ, the gynoecium, is often abnormal (*C*).

probe, indicating that it is highly likely that we have cloned the *fae2* gene.

We identified an transcript of approximately 2.7 kb, and its sequence reveals significant similarity to a class of leucine-rich repeat (LRR) proteins that have been implicated in various aspects of plant signaling, including resistance to pathogens and response to plant growth regulators. Expression analysis by northerns and in situ hybridization indicates that *FAE2* is expressed in ear primordia, in both inflorescence and floral meristems. This is consistent with our phenotypic analysis which indicated that the mutant ears not only have an enlarged inflorescence meristem, but also show increased numbers of floral primordia and increased gynoecium size (see Fig. 1). By analogy to other known LRR genes, it is likely that *FAE2* encodes a receptor that interacts with an unknown extracellular ligand to regulate stem cell division or lateral organ specification.

In the last year, we have also received two further fasciated ear mutants from maize collaborators and are testing allelism to the two other mutants in this class. It is apparent that several loci in maize can mutate to give a fasciated phenotype, although our analysis of genetic interactions suggests that at least some may act in the same pathway to control meristem development.

Progress toward Transposon Tagging of the *abphyl1* Gene

M. Krishnaswami, Z. Yuan

The *abphyl1* mutation of maize is the only mutation described in plants that changes patterns of leaf initiation from one regular pattern to another. *abph1* mutant plants initiate leaves in opposite pairs rather than individually, which is the norm for all grass species. The *abph1* mutation arose spontaneously, and we have therefore carried out directed transposon screens to isolate additional alleles that will be amenable to cloning of the *abph1* locus by transposon tagging. Specifically, we are using the *Mutator* transposon system, which shows a high forward mutation rate and no strong target site specificity. We isolated and confirmed genetically three new *abph1* alleles and have several more that await testing. For one of the alleles, *abph1*-43*, we tested for cosegregation of *Mu* transposons with the new allele using several different *Mu* probes specific for different classes of *Mu* autonomous and nonautonomous transposons. In one case, we have found a *Mu7* hybridizing fragment of approximately 4 kb that cosegregates with the *abph1*-43* allele, and we are testing this cosegregation on a larger number of individuals. If the cosegregation holds up, we will then clone the transposon band and isolate the flanking sequence to test whether it corresponds to the *abph1* gene. The question of what controls the arrangement of leaves, or phyllotaxis, is one of the oldest problems in plant biology, and the isolation of the *abph1* gene should provide a unique opportunity to study this problem from a molecular perspective.

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PLANT DEVELOPMENTAL GENETICS AND FUNCTIONAL GENOMICS

R. Martienssen J. Arroyo, M. Byrne, A. Dubrowski, J. Fontana, A. Groover, C. Kidner, B. May, P. Rabinowicz, R. Shen, J. Simorowski, T. Singer, E. Vollbrecht, C. Yordan

The DNA sequence of the first higher plant genome, that of the model plant *Arabidopsis thaliana*, is scheduled to be finished by the end of this year (see report from the McCombie laboratory). Taking advantage of this landmark in plant genetics, we are systematically determining the function of genes in *Arabidopsis* and maize by using transposable elements to monitor gene expression and disrupt gene function. We are also continuing to study genes involved in organogenesis and the elaboration of the plant body plan: We have characterized the roles of three genes (*ramosa1, asymmetric*, and *argonaute*), in stem cell determination, differentiation and maintenance, and we have begun to explore the epigenetic mechanisms that may underlie these processes. This year, we reported the structural and functional characterization of the first completely sequenced heterochromatic region in a higher organism, a cytogenetically visible structure in *Arabidopsis* that resembles the chromosomal "knobs" of maize described by Barbara McClintock. The genetic consequences of plant genome architecture, which is largely controlled by transposons, remains a focus of the research in our laboratory.

Functional Genomics: Maize Targeted Mutagenesis

B. May, E. Vollbrecht, P. Rabinowicz, R. Martienssen
[in collaboration with M. Freeling, University of California, Berkeley; D. Alexander, Novartis Agricultural Biotechnology Research Institute; and L. Stein, Cold Spring Harbor Laboratory]

We have established a transposon library in maize for the purposes of targeted selection of gene knockouts. Searches are performed by amplifying DNA from these populations using specific primers from the transposon and from the gene. We are using Robertson's *Mutator* transposons, and in 1998 and 1999, 40,000 plants were grown at the Novartis nursery in Kauai, individually bar-coded, and sampled. All plants were self-pollinated, harvested ears were shelled, and the kernels were bagged and bar-coded. Individual leaf samples were sorted into row-column grids of 48 x 48 (2304 plants) in pools.

In the fall of 1998, 23,000 ears from Hawaii were screened for lethal mutations before they were shelled. Background mutations in the parental lines were identified by pedigree analysis and subtracted from the totals.

New defective kernel mutations (dek):	3589/14188 (25.3%)
New viviparous mutations:	171/22724 (0.8%)
New reduced fertility mutations:	138/15088 (0.9%)

There are 15 known loci that contribute to kernel vivipary, and we have recovered 171 new mutations. This is an allele frequency greater than ten alleles per locus. DNA was extracted from nine grids (864 leaf pools), and polymerase chain reaction (PCR) amplification of the pools was used in a double-blind assay to identify correctly a new allele of *vp1* found in the screen. We are now ready to begin screening for insertions in new genes.

Differential Methylation of Genes and Retrotransposons Facilitates Shotgun Sequencing of the Maize Genome

P. Rabinowicz, C. Yordan, R. Martienssen
[in collaboration with W.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, but the evidence for this is anecdotal and controversial. We have shown that repeat sequences in

maize, because of their sensitivity to bacterial restriction-modification systems, can be largely excluded from genomic shotgun libraries by the selection of an appropriate host strain. In contrast, unmethylated genic regions are preserved in these genetically filtered libraries if the insert size is less than the average size of genes. This demonstrates that repeats are the primary targets of methylation in maize. The use of filtered libraries in genome shotgun sequencing in plant genomes allows significant enrichment of genes, greatly reducing the number of reactions required for genome shotgun sequencing analysis.

Chromosome Structure and Organization in *Arabidopsis*

C. Yordan, J. Simorowski, B. May, R. Martienssen
[in collaboration with D. Preuss, University of Chicago;
P. Fransz, Gatersleben Institute of Plant Genetics;
R. Wilson, Washington University; E. Chen, PE Biosystems;
and W.R. McCombie, Cold Spring Harbor Laboratory]

Heterochromatin, constitutively condensed chromosomal material, is widespread among eukaryotes, but the complete DNA sequence of such a region had not been previously determined. The Cold Spring Harbor Laboratory/Washington University Genome Sequencing Center/PE Biosystems *Arabidopsis* sequencing consortium has sequenced and analyzed 3.0 Mb from *A. thaliana* chromosome IV, which includes 0.5–0.7 Mb of isolated heterochromatin resembling the chromosomal knobs of maize first described by Barbara McClintock. The knob on chromosome IV is characterized by a low density of expressed genes, low levels of recombination, and a low incidence of gene trap insertion. Tandem arrays of long repeats were found, as well as many individual transposons. Methylation of repeats and transposons was dependent on chromatin remodeling, as methylation was lost in *ddm1* (*decrease in DNA methylation*) mutant strains. Clustered repeats, comprising genes as well as transposons, were associated with condensed chromosomal domains even when their heterochromatic nature was not immediately apparent. The complete sequence of a heterochromatic island provides a unique opportunity to study sequence determinants of chromosome condensation in a higher eukaryote.

Robertson's *Mutator* Transposons Are Regulated by DNA Methylation and Chromatin Remodeling in *Arabidopsis*

C. Yordan, T. Singer, R. Martienssen

Transposable elements of the Robertson's *Mutator* family in maize are very aggressive and widely used for mutagenesis. Active elements are undermethylated in maize. We have identified at least 20 intact and many more defective *Mutator* transposons in the *Arabidopsis* genome. Many of them are located in heterochromatic regions and are heavily methylated. In strains of Columbia carrying the *ddm1* mutation, these elements become demethylated and transpose at high frequencies. *DDM1* is required for methylation of heterochromatic repeats in *Arabidopsis* (Vongs et al., *Science 260:* 1926 [1993]) and was recently shown to encode an SNF2 chromatin remodeling enzyme (Jeddeloh et al., *Nat. Genet. 22:* 94 [1999]). Thus, *Mutator* transposons are indeed regulated by DNA methylation and chromatin remodeling. Furthermore, these results indicate a link between heterochromatin, transposon regulation, and other epigenetic phenomena regulated by *ddm1*, such as imprinting and paramutation.

Systematic Functional Genomics in *Arabidopsis* Using Gene Traps

B. May, C. Yordan, J. Simorowski, J. Arroyo, R. Shen,
A. Dubrowski, J. Fontana, A. Groover, R. Martienssen
[in collaboration with W.R. McCombie, J. Healy, and
A. Reiner, Cold Spring Harbor Laboratory]

We are developing a database of *Arabidopsis* gene function by generating several thousand strains, each with a unique gene trap or enhancer trap transposon located somewhere in the genome. DNA is prepared from each line, and the insertion site is amplified and sequenced. During the last year, we generated several thousand new lines and sequenced more than half of them. Each line is now being routinely screened for early lethals and then stained for reporter gene expression. So far, we have mapped more than 2300 insertions and disrupted close to 1000 genes. The sequences and staining patterns are entered into a relational database that can be queried for sequence, annotated genes, patterns, and phenotypes. Automated

quality control is used to eliminate sequences from PCR contaminants, low-quality reads, and selection artifacts. Annotation is achieved using a tailor-made Java application that compares each sequence to the annotated *Arabidopsis* sequence in Genbank and updates the gene trap database with gene identifiers, similarity scores, and insertion orientation. We hope to disrupt more than half the genes in *Arabidopsis* in the next 2–3 years, each indexed by sequence as well as function.

Gene Trap Screens for Vascular and Secreted Proteins

A. Groover, R. Martienssen

The plant vascular system is an attractive model for studying diverse aspects of development, and it is of prime importance economically (e.g., in the development of woody tissues). The development of the vascular system involves patterning of the vascular tissues within organs (e.g., venation pattern within leaves) and the differentiation of highly specialized cell types. We have performed two genetic screens using *Arabidopsis* gene traps that identify genes involved in vascular development. The first screen identified gene trap insertions into genes encoding secreted and plasma-membrane-spanning proteins. This class of proteins includes key components of cell-to cell-signaling mechanisms, which are currently poorly understood in plants. The screen has identified numerous genes of interest, including membrane-spanning receptors and novel genes of unknown function. A second screen, also using *Arabidopsis* gene traps, has identified genes expressed at different stages of development and within specific cell types in the vasculature. Together, these genes give insight into the control of vascular development at the molecular level.

Maternal and Zygotic Roles of *PROLIFERA* in *Arabidopsis*

A. Groover, C. Yordan, J. Simorowski, R. Martienssen
[in collaboration with P. Springer, University of California, Riverside]

PROLIFERA (*PRL*) encodes a homolog of the DNA replication licensing factor Mcm7, a highly conserved protein found in all eukaryotes. Gene trap insertions in the *PRL* gene are lethal, resulting in decreased transmission through the female gametophyte and homozygous embryonic lethality. *PRL* is specifically expressed in populations of dividing cells in sporophytic tissues of the plant body, such as the palisade layer of the leaf and founder cells of initiating flower primordia. Gene fusions with the green fluorescent protein (GFP) reveal that the PRL protein accumulates during the G_1 phase of the cell cycle and is transiently localized to the nucleus. During mitosis, the fusion protein rapidly disappears, returning to daughter cell nuclei during G_1. PRL::GUS fusions are strongly expressed in the central cell nucleus of mature megagametophytes, which have a variety of arrest points reflecting leaky lethality. Expression is also observed in the endosperm of mutant *prl* embryo sacs that arrest following fertilization. Crosses with wild-type pollen result in occasional embryonic lethals that also stain for GUS activity. In contrast, embryos resulting from crosses of wild-type carpels with PRL::GUS pollen do not stain and are phenotypically normal. In situ hybridization of GUS fusion RNA indicates that transcription is equivalent from maternally and paternally derived alleles in these embryos. This rules out embryonic imprinting and indicates instead that accumulation of maternally derived protein and/or translation of maternal RNA is likely responsible for the "maternal" effect.

ramosa1 Controls Meristem Fate in the Maize Inflorescence

E. Vollbrecht, R. Martienssen

In plants, stem cell fate is perhaps the single most important determinant of growth and development, and meristem identity mutants potentially define the transition from indeterminate to determinate fate. *ramosa1* is such a mutation in maize. *ra1* mutants have indeterminate second-order meristems, resulting in a highly branched inflorescence, both in the ear and in the tassel. In some respects, *ra1* mutant inflorescences resemble those of related panicoid grasses. It is therefore possible that *ra1* may account for macroevolutionary change in grass inflorescence architecture. We identified four mutable alleles of *ra1* (Annual Report 1992), and we have now demonstrated that two of them are caused by the insertion of *Suppressor-muta-*

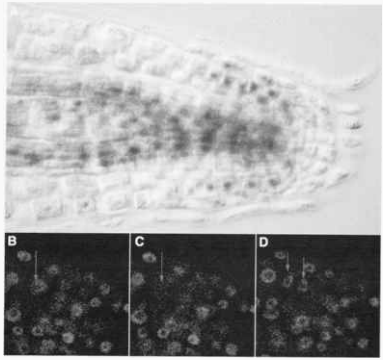

FIGURE 1 PROLIFERA is localized in the nucleus during the G_1 phase of the cell cycle. (*A*) Whole mount of root tip from *prl/+* plant stained with X-Gluc, showing GUS localized in the nucleus of individual files of cells. (*B–D*) Accumulation of PRL::GFP fusion in root tip nuclei. The time between images in *B* and *C* is 4 min. The time between the images in *C* and *D* is 28 min. The arrow marks a cell in *B* that has PRL::GFP localized to the nucleus. Localization is lost in *C* and returns in daughter nuclei (*D*) following mitosis.

tor (*Spm*) autonomous transposable elements. Cosegregating elements have been identified by Southern analysis. We will clone the *ra1* locus and analyze the expression of the gene in a variety of mutant backgrounds. We will then study the architecture of mutant inflorescences, and use a combination of double mutant and mosaic analysis to determine the function of the gene.

asymmetric leaves1 Controls Stem Cell Function in the *Arabidopsis* Apex

M. Byrne, J. Arroyo, R. Martienssen [in collaboration with A. Hudson, Edinburgh University]

Leaves arise as lateral organs from primordia initiated on the flank of the shoot apical meristem, an indeterminate group of stem cells located at the growing point of the plant. Fundamental to leaf development is the early establishment of two axes of growth, the dorsoventral axis and the proximodistal axis. Although many leaf developmental mutants have been described, the genetic basis of leaf initiation, determination, and patterning is largely unknown. One such mutant in *Arabidopsis* is *asymmetric leaves1* (*as1*). Wild-type *Arabidopsis* adult rosette leaves are spatulate in shape. In comparison, *as1* leaves are variably lobed with a drastically altered proximodistal pattern. We have used positional cloning to isolate the *AS1* gene. *AS1* is a *myb* transcription factor, closely related to *PHANTASTICA* in *Antirrhinum* and *ROUGH SHEATH2* in maize. These two genes are known to regulate homeobox genes negatively (Schneeberger et al., *Development 125:* 2857 [1998]; Timmermans et al., *Science 284:* 151 [1999]). Molecular and genetic analyses indicate that *AS1* interacts hierarchically with homoeobox genes that are principally expressed in the shoot apical meristem. *AS1* itself is expressed in lateral organ primordia. We have found that *as1* mutant plants misexpress two homeobox genes, *KNAT1* and *KNAT2*, which are likely to be contributing factors to defects in axis specification in *as1* leaf development. Another homeobox gene, *STM*, is not misexpressed in *as1* mutants. However, *as1* suppresses the vegetative shoot meristemless phenotype of strong *stm* mutants. This reveals a role of *STM* as a negative regulator of *AS1*. In turn, *AS1* serves to regulate *KNAT1* and *KNAT2* negatively. This genetic pathway may define an ancient mechanism for differentiating between the meristem and lateral organs.

daisy and *dandelion* Are Alleles of *argonaute* and Regulate Stem Cell Maintenance in *Arabidopsis*

C. Kidner, R. Martienssen [in collaboration with S. Grewal, Cold Spring Harbor Laboratory]

Last year, we reported that *dandelion* and *daisy* are alleles of the *argonaute* gene, which encodes a member of a large gene family required for proper formation of the dorsoventral axis in plant lateral organs (Bohmert et al., *EMBO J. 17:* 170 [1998]). We have now shown that strong alleles have premature stop codons, whereas weak alleles have changes in conserved amino acids and splicing defects. Using this allelic series and the expression patterns revealed by gene traps and enhancer traps, we have found genes in the same developmental pathway as *DANDELION*. Double mutants indicate that *DND* interacts with *UFO* (an F-box protein required for floral organ identity),

LEAFY (a transcription factor involved in organ and meristem identity), *ERECTA* (a receptor kinase involved in many aspects of plant development), and *STM* (a homeobox gene required for stem cell maintenance). Enhancer traps have identified several genes that are over- or misexpressed in a *dnd* background, including a gene with similarity to *cycloidea*, a transcription factor that controls dorsoventrality in flowers. There is also a representative of the *argonaute* gene family in the fission yeast *Schizosaccharomyces pombe*. We have made knockouts and GFP fusions of the fission yeast homolog *Pombago*. In *C. elegans* and *Drosophila*, related genes are required for stem cell maintenance and for the inhibition of gene expression by double-stranded RNA (RNAi), whereas similar proteins are associated with translational initiation factors in mammalian cells. We are investigating its role in stem cell function in plants and yeast.

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MAMMALIAN CELL GENETICS

M. Wigler J. Alexander, J. Brodsky, A. Buglino, K. Chang, D. Dong, J. Douglas, D. Esposito, E. Hatchwell, R. Lucito, V. Mittal, A. Reiner, M. Riggs, L. Rodgers, L. Serina, J. Stolarov, J. Troge, J. West, C. Yen

The pathophysiology of a cancer is mainly determined by its tissue of origin and the mutations it has accumulated. We do not have specific knowledge of the majority of genes that cause cancers. There are gaps in our knowledge of which pathways become deranged, and in the specific components of the known pathways that go awry. Without this knowledge, it is likely that our attempts to control cancer will be incomplete. Therefore, work in our group is centered upon cancer genomics. We have developed several methods for detecting genetic alterations in cancers, identifying the genes that are the targets of these alterations, and exploring the functional analysis of these genes by a variety of means. PTEN, a tumor suppressor gene isolated by our methods, has been the subject of our most intensive study. The major change in our program since last year is the inclusion of microarray analysis for both measuring gene copy number and profiling gene expression and the development and application of retrovirus-mediated gene induction systems for studying gene function. We are also applying our genomic methods to the analysis of spontaneous human genetic disease.

Discovery of Loci and Genes Altered in Cancer

C. Yen, R. Lucito, D. Esposito

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

In collaboration with Dr. Hamaguchi, two main candidate transcripts have been identified from one of the 8q22 loci. We are now in the process of obtaining full-length cDNAs and performing mutational analysis on these transcripts by a combination of techniques, including linked transcription-translation premature truncation and DNA sequencing. For a more comprehensive report on this locus, see Dr. Hamaguchi's research report in this section.

Development of Inducible Gene Expression Systems

J. Stolarov

In our early studies of the PTEN tumor suppressor, we used constitutively expressing retroviral vectors and immediately encountered the problem of great variability in the phenotypes of the clones that emerged. Clonal variability, and lingering questions about the cellular adaption to the chronic expression of PTEN,

prevented us from reaching clear conclusions. Thus, we sought to develop inducible expression systems in which, ideally, paired cultures could be compared for their acute responses to controlled levels of expression of the tumor suppressor.

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

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

Analysis of PTEN Function

J. Stolarov, K. Chang, D. Dong

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

We have utilized the retroviral system described above for further studies on PTEN. Expression of PTEN was clearly controllable in U87 hosts with muristirone, an ecdysone analog, with induction levels up to 50-fold above background. Cellular morphology changed radically, but not in vitro growth characteristics. Expression of several tumor markers were also affected, including thrombospondin, an inhibitor of angiogenesis, and TIMP3, an inhibitor of metalo-proteinases. Mutants of PTEN that lost either lipid phosphatase or all phosphatase activity were inert. Significantly, we observed virtually identical changes in cellular morphology with a small molecular inhibitor of the p110 PI3-kinase, the enzyme that generates PIP3,4,5, in keeping with the hypothesis that PTEN exerts its effects on cells through its lipid phosphatase activity. Additional details of the cellular responses of cells to PTEN are further discussed in the subsequent section on transcriptional profiling.

We have continued to study the properties of the PTEN homolog in the yeast *Saccharomyces cerevisiae*. In particular, we have shown that the yeast enzyme retains the biochemical activity of its mammalian homolog, and deletion of PTEN results in an impairment in sporulation.

Microarray Analysis of Expression

V. Mittal, J. Stolarov, R. Lucito, J. West, A. Reiner

We have begun implementing in our lab the technique of transcriptional profiling utilizing printed cDNA microarrays. In this technique, developed at Stanford in the laboratory of Pat Brown, arrays of cDNA probes are affixed to a glass surface, and the surface is hybridized to pairs of cDNA samples, prepared from the mRNA of two sources, each labeled with a different fluorescent dye (Schena et al., *Science 270:* 467

[1995]). The ratio of fluorescence detected at each probe gives a measure of the relative abundance of the mRNA corresponding to that probe. More than 25,000 expressed sequences, about one quarter of the estimated transcription units in the human genome, the IMAGE Consortium clones, are available for printing.

The reasons for implementing this technology in our lab are many. First, this is a potentially powerful tool for the functional characterization of new genes, especially for tumor suppressors and oncogenes, enabling their association with genes known to control certain pathways. Second, it is a powerful tool for the analysis of specific mutations, particularly for genes, such as RAS and PTEN, that encode proteins with multiple activities or multiple domains. Third, we can use this system in combination with our superb inducible expression system, and we are thus experimentally well positioned to carry out comparisons of well paired samples, allowing us to examine both up- and down-regulated genes, with time course and dosage controlled.

We printed moderate density (>6000 probes) cDNA microarrays within our own facility. We used as our paired samples U87MG cells induced or not induced to express PTEN and mutant PTEN genes, and treated or not with inhibitors of p110 PI3-kinase. The results of these experiments suggest that there are a very limited number of large changes in the transcriptional pattern when PTEN was induced. Of 6218 probes, 0.4% of transcripts appeared to be suppressed by greater than threefold upon PTEN induction, and 0.25% of transcripts appeared to be induced by greater than threefold upon PTEN induction. An overlapping pattern of change in transcription was observed when U87MG cells were treated with an inhibitor of PI3-kinase.

These results, although preliminary and requiring additional controls, strengthen our confidence that our model for the action of PTEN is at least partly correct and that our retroviral ecdysone-inducible system coupled with microarray analysis of transcription patterns is a powerful approach to the functional analysis of mammalian genes.

Detection of Gene Copy Number Fluctuations by Microarray Analysis of Genomic Representations

R. Lucito, J. West, A. Reiner

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

Microarrays, while widely utilized for expression profiling, cannot be effectively used for measuring gene copy numbers in a straightforward manner. Using the entire human genome for hybridization is inefficient due to its nucleotide complexity. To solve this problem, the microarray system we have developed is based on representations. A representation is a reproducible amplification of the genome with reduced complexity. In brief, a representation is produced by first cleaving the genome with a restriction enzyme, and then adaptors are ligated and used for a subsequent PCR amplification. The complexity of a representation based on *Bgl*II cleavage is approximately 3% of the genome, which is comparable to that of the complexity of transcripts within a cell. Single fragments from the representation are then cloned and arrayed on a slide. Representations from tumor and normal, prepared in the same manner as that of the array, are then hybridized to the array, and the results are analyzed to define regions that have undergone copy number changes such as deletion and amplification.

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

We also examined the reproducibility of our microarray measurements between multiple experiments. Multiple representations of two different human breast cancer cell lines, SKBR-3 and MDA-MB-415, were compared by hybridization. The ratios obtained

from one experiment were compared to those obtained from another parallel experiment. There was excellent concordance between independent microarray measurements, and excellent agreement with measurements of gene copy number by Southern blot hybridization. These experiments again attest to the reproducibility of representations and also to the reproducibility of printing, labeling, and hybridization.

Due to the use of representations, we are able to use minute amounts of starting material. Thus, we are able to measure genomic changes in the sorted tumor and normal nuclei of a cancer biopsy. The results of the microarray hybridization completely corroborate the results obtained by other methods. This will allow us to use primary tumors that have been microdissected by some means into tumor and normal fractions.

The project is currently in a stage of scaling up. We are subcloning 30,000 *Bgl*II representational fragments and are presently characterizing them. This will produce a microarray with coverage of 1 probe every 100 kb, on average. This microarray will be used to scan the genomes of breast cancers to uncover regions that have undergone copy number changes. Our hope is that our scan will be done at such precise coverage, and against such a large number of tumors, that we will uncover new candidate loci for the location of tumor suppressors and oncogenes. Furthermore, by analyzing many tumors, we should also be able to limit the size of the region needed to look for candidates. This method also has other potential uses, including measuring mutational load in cancers, monitoring DNA methylation patterns, genome-wide genetic typing, and detection of de novo mutations in humans.

Analysis of De Novo Mutation in the Human Germ Line

E. Hatchwell, L. Serina

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

In recent years, a number of syndromes have been shown to result from submicroscopic genomic alterations (i.e., deletions, duplications, or inversions). In the vast majority, the knowledge gained depended heavily on serendipity. It is likely that many of the syndromes that are currently undiagnosed result from a similar general mechanism.

No general method yet exists for the detection of submicroscopic genomic alterations. This project aims to develop such methods, using the techniques of subtractive hybridization and microarray analysis. The main goal is to modify existing techniques (including RDA), in order to be able to scan the genome of affected children for de novo genomic alterations. We have carefully selected children with sporadic disease (the majority of those who remain undiagnosed) and assumed that their mutation is de novo. By direct comparison, using RDA and representation-based microarrays, we can compare the genome of the child with genomes of the child's parents. A de novo genomic alteration will be detected if it creates a novel junction fragment.

We have successfully conducted experiments on artificial controls and are currently using families with known de novo translocations to test out our method. If successful, we aim to test "unknowns" in the near future.

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CELL BIOLOGY

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

- Shiv Grewal's laboratory studies the epigenetic control of gene expression. During the past year, they have shown that a member of the evolutionarily conserved chromodomain family of proteins has a crucial role in establishment and inheritance of an epigenetic imprint during mitosis and, remarkably, even through meiosis. That is, the persistence of macromolecular complexes associated with DNA contributes to cellular "memory" and maintenance of the gene expression state during cell division. Their work suggests that the unit of inheritance, the gene, occasionally comprises the DNA plus the associated chromatin protein complexes.
- David Helfman's group is studying how specific actin assemblies are organized and regulated and how alterations in actin filament assembly contribute to abnormal cell growth control in cancer. They have found that the actin cytoskeleton plays an essential part in signal transduction events and that alterations in the components of the cytoskeleton can play an important part in cellular transformation.
- Nouria Hernandez and her colleagues work on fundamental mechanisms of transcription using two model systems, the human snRNA genes and the HIV-1 LTR. Promoters are divided into a core region, which contains all the information required to assemble a transcription initiation complex and recruit the correct RNA polymerase, and a regulatory region, which recruits activators or repressors of transcription that modulate the activity of the core promoter. Their goal is to understand how core promoters ultimately recruit the correct RNA polymerase and how transcriptional activators perform their function.
- David Spector's laboratory continues to study the protein composition of interchromatin granule clusters (IGCs), nuclear structures that are thought to be staging grounds for the assembly of complexes of RNA processing factors and transcription factors prior to their recruitment to sites of transcription. Using mass spectrometry, 114 known proteins have been identified thus far, including splicing and other RNA processing factors. In addition, 12 new IGC protein candidates were identified and peptides were obtained that correspond to 69 different EST clones. Ongoing studies are aimed at revealing new aspects of the function of this nuclear structure.
- Tatsuya Hirano's laboratory studies higher-order chromosome dynamics with a major focus on the roles of SMC (structural maintenance of chromosome) proteins in chromosome condensation and segregation. They have characterized how eukaryotic SMC protein complexes interact with DNA and nucleosomes, and how their functions are regulated during the cell cycle. A systematic mutagenesis approach using a bacterial SMC protein is beginning to shed light on the basic mechanisms of action of this important class of chromosomal ATPases.
- Nick Tonks' laboratory is taking a variety of approaches to study the structure, regulation, and function of the protein tyrosine phosphatase (PTP) family of signal transducing enzymes. They have developed "substrate-trapping" mutant forms of these enzymes, which form stable complexes with target substrates in a cellular context, to identify the physiological substrates of members of the PTP family. This approach has illustrated that the PTPs display exquisite selectivity in a cellular context and function as specific regulators of cellular signaling events. Ongoing structural analyses are revealing how such selectivity is achieved.
- Linda Van Aelst's laboratory continues to study the function of the Ras and Rho family of small GTPases in cell growth and development, together with the mechanisms by which they exert their effects. Using cDNA-RDA strategies in combination with micro-arrays, they have identified targets of Rac, a Rho family GTPase, that are important for its effects on cell growth. They are also studying the role of Rho family members in neuronal development and metastasis. Their studies of Ras have focused on the junctional protein, AF-6, which they identified as a novel Ras-binding protein and which may be an effector of the Ras-related protein Rap1. They are using *Drosophila* to study the homolog of AF-6, known as Canoe, which is essential for dorsal closure, a process resembling wound healing in mammalian cells.

EPIGENETIC CONTROL OF GENE EXPRESSION

S. Grewal J. Nakayama
R. Krimper

Our research continues to focus on the fundamental question of how differentiated cells establish and maintain stable patterns of gene expression. Specifically, we are studying the molecular mechanism of epigenetic inheritance, whereby nonmutational alterations in chromosomes promote stable transmission of the gene-expression patterns during mitosis and, sometimes, even through meiosis. Such changes are known to have profound effects on mammalian development, as they regulate expression of developmentally important genes. Moreover, epigenetic changes affect other aspects of chromosome architecture such as recombination and chromosome segregation, which in turn have implications for the maintenance of the genome integrity. In addition, the abnormal gene expression caused by epigenetic changes is believed to play a causal part in cancer progression. To understand how epigenetic states are established and maintained through many rounds of cell division, we are studying the gene-repression mechanism known as "silencing" at the mating-type region of fission yeast *Schizosaccharomyces pombe.* Our earlier studies have revealed that the inheritance of the epigenetically maintained silenced state depends on the proper expression of genes that encode either chromatin proteins or factors that modify them. However, the underlying mechanism by which cells establish and maintain an epigenetic imprint affecting the inheritance of the silenced state has remained elusive. During the past year, considerable progress has been made toward understanding the nature of epigenetic imprint. We found that a member of the evolutionarily conserved chromodomain protein family plays a crucial part in imprinting at the *mat* locus. In addition, we have continued to investigate the role of putative histone deacetylase Clr6 in chromatin assembly and are following up on our recent finding that a mutation in the gene encoding DNA polymerase α (polα) affects heritability of the silenced state.

MECHANISM OF EPIGENETIC INHERITANCE

Our previous studies have revealed that an epigenetic imprint marking the mating-type region contributes to maintenance of a heterochromatin-like structure, which controls both efficiency of mating-type switching and silencing throughout the approximately 15-kb *mat2/3* interval. Moreover, expression of the *ura4*$^+$ marker gene inserted within the silent domain is variegated. The Ura$^-$ efficiently switching (*ura4-off*) and Ura$^+$ inefficiently switching (*ura4-on*) states are mitotically metastable and controlled by an epigenetic mechanism. Mutations in factors such as histone deacetylase homologs Clr3 and Clr6 and the chromodomain-containing proteins Clr4 and Swi6 affect propagation of the silenced state. According to a model, a protein-based imprint marking the *mat* locus might contribute to inheritance of the silenced state during cell division. In the past year, we have demonstrated that, indeed, chromatin proteins are important determinants of the imprint, i.e., persistence of macromolecular protein complexes associated with the mating-type region presumably contributes to the propagation of the silenced state. Our analyses suggest that a chromodomain-containing Swi6 protein is a dosage-critical component required for establishing and maintaining the imprint. Interestingly, Swi6 protein is present throughout the approximately 15-kb silent domain, and its levels at a particular location correlates with the stringency of silencing (Fig. 1). More importantly, the transient presence of three copies of *swi6*$^+$ altered the chromatin imprint at the *mat* locus, resulting in heritable change from the *ura4-on* state to *ura4-off* state. We found that *ura4-on* cells contain significantly lower levels of Swi6 at their *mat* locus than *ura4-off* cells. This differential Swi6 localization pattern is maintained not only during mitosis, but also remarkably through meiosis. Furthermore, transition from the *ura4-on* to the *ura4-off* state is tightly coupled to the increase in Swi6 levels at the *mat* locus. Once recruited, however, Swi6 remains associated with the mating-type region throughout the cell cycle, providing a molecular bookmark to propagate clonally a specific chromatin configuration, hence maintaining the silenced state during cell division.

In the previous year, we reported that transient inhibition of histone deacetylase activity by trichostatin A (TSA) removes the epigenetic imprint from

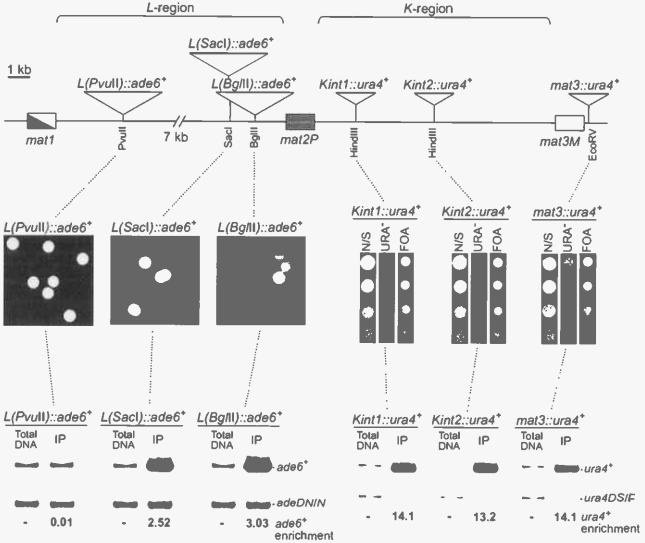

FIGURE 1 Swi6 is present throughout the silent mating-type region. (*Top*) The map of the mating-type interval and restriction enzyme positions where prototrophic markers were inserted. For cultures carrying the *ade6*+ insertion at *L(PvuI)*, *L(SacI)*, and *L(BglII)*, cells were grown on adenine-limiting media before photography. The representative colonies of each strain are shown (*middle, left three panels*). The gray or white color of colonies implies Ade- or Ade+ phenotypes, respectively. The expression of the *ura4*+ marker inserted at *Kint1*, *Kint2*, and *mat3* locations was examined by plating cells onto nonselective (N/S), AA-URA (URA-), and FOA medium. The URA- medium selects for growth of Ura+ cells, whereas the FOA medium selects for growth of Ura- cells (*middle, right three panels*). Chromatin immunoprecipitation (CHIP) assay with anti-Swi6 antibodies was employed to determine the presence of Swi6 at each marker gene location. The DNA recovered from immunoprecipitated (IP) fractions was analyzed quantitatively using a competitive polymerase chain reaction (PCR) strategy. In this approach, strains bearing *ade6*+ or *ura4*+ at the mating-type region and a small deletion of their corresponding loci (*ade6DN/N* and *ura4DS/E*, respectively) at the endogenous location were used. By using primers to give different size products from full-length and mutated alleles, the relative enrichment of the marker gene sequences in the immunoprecipitated fractions can be accurately determined.

DNA, resulting in heritable change from the *ura4-off* state to *ura4-on* state. We now have shown that TSA treatment alters the imprint by affecting localization of Swi6 at the mating-type region. Remarkably, a brief treatment by TSA for about ten generations induces heritable decrease in Swi6 levels at the *mat* locus, resulting in "erasure" of the imprint. On the basis of these studies, we suggest that the unit of inheritance, i.e., the "gene" at the *mat* locus, comprises DNA plus the associated Swi6 protein complex.

Chromodomain-containing proteins, such as polycomb group proteins in *Drosophila*, participate in heritable inactivation of gene expression in evolutionarily unrelated systems. Therefore, it is possible that protein-based mechanisms of epigenetic inheritance are responsible for phenotypic variations or increased risk to diseases within populations. Our recent results demonstrating the meiotic stability of the Swi6 association with the *mat* locus further supports this possibility. It can be imagined that certain carcinogens and

environmental factors affect gene expression by altering epigenetic imprints rather than causing genetic changes. Since chromatin proteins such as Swi6 also affect inter- and intrachromosomal recombination as well as chromosome segregation, it is also possible that genomic instability observed in certain cancer cells results from the loss of epigenetic imprints. Current efforts are directed to study this mode of inheritance further and to characterize other proteins that are essential for imprinting.

ROLE OF DNA POLYMERASE α IN SILENCING

A recent observation suggests that the connection between DNA replication and chromatin machinery may be more direct than previously assumed; i.e., DNA replication machinery may itself be involved in propagation of the silenced state. In collaboration with Amar Klar's laboratory (National Cancer Institute), we have shown that a mutation in *polα* results in 45-fold increase in the *ura4-off* to *ura4-on* transition and also causes derepression of the donor mating-type loci. Even more importantly, the mutant cells are defective in assembly of repressed chromatin structure at the silent mating-type interval. Genetic analysis revealed that Polα acts in the same silencing pathway as previously identified silencing factors such as Swi6. Further analysis revealed that mutation in *polα* affects silencing by altering the recruitment and maintenance of Swi6 at the mating-type region; i.e., significantly lower levels of Swi6 were found at the *mat* locus of *polα* mutant cells, when compared with wild-type cells. Additionally, we found that mutation in *polα* also affects Swi6 localization and transcriptional silencing at the centromeres. The biochemical basis of the Polα role in Swi6 localization at the mating-type and centromeric loci remains to be explored and is the focus of our current research.

PURIFICATION AND CHARACTERIZATION OF Clr6

In the previous year, we reported the identification of an essential gene called *clr6*, which affects silencing at heterochromatic loci. Interestingly, a mutation in Clr6, which shares similarity to histone deacetylases, also causes missegregation of chromosomes and UV light sensitivity, suggesting general chromatin assembly defects. Furthermore, Clr6 acts in a partially overlapping manner with another putative histone deacetylase Clr3, which affects silencing at the mating-type region and centromeres. We are now characterizing Clr6 to understand its precise function. In collaboration with Karl Ekwall's laboratory (Karolinska Institute), we showed that Clr6 is localized to the nucleus on chromatin. We also found that Clr6 levels remain constant throughout the cell cycle, and a majority of the protein remains associated with the chromatin. Purification of Clr6 revealed that it is part of a mutiprotein complex. Future studies are aimed at determining the identity of other subunits of the protein complex. In addition, we are investigating the effect of *clr6* mutation on steady-state levels of histone acetylation and exploring the possibility of whether lethality observed in mutant cells is due to defects in de novo nucleosome assembly.

In Press

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THE CYTOSKELETON IN NORMAL AND TRANSFORMED CELLS

D.M. Helfman E. Araya, C. Berthier, L. Connell, E. Kim, S. Lee, G. Pawlak, A.J. Rai

The actin cytoskeleton is involved in a wide range of motile activities such as cell movement, cell division, intracellular transport, phagocytosis, exocytosis, membrane ruffling, formation of microspikes and filopodia, growth cone formation, cell spreading, adhesion, changes and maintenance of cell shape, and signal transduction. In nonmuscle cells, such as fibroblasts, the formation of actin microfilaments and their assembly into a variety of structures are dynamic processes. We are studying how specific actin assemblies are organized and regulated and how alterations in actin filament assembly contribute to aberrant cell growth. We are also interested in understanding how the actin cytoskeleton 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.

The Cytoskeleton in Adhesion-dependent Signaling

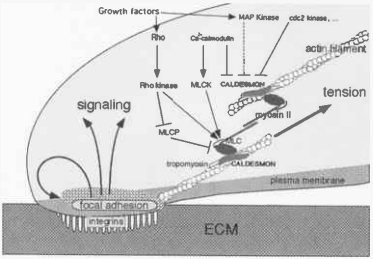
G. Pawlak, C. Berthier, S. Lee, L. Connell, E. Kim

Changes in the organization of actin filaments are highly correlated with both anchorage-independent growth and cellular tumorigenicity, suggesting a fundamental role for actin filaments in cell growth control. How the actin cytoskeleton participates in normal growth control and how changes in microfilament-associated proteins contribute to transformation are not understood. The activation of myosin II in 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 myosin II in adhesion-dependent signaling, we have studied the effects of nonmuscle caldesmon on myosin II, 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 of phosphorylated myosin II, and it can block myosin-II-driven motility (see Fig. 1). 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 with 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 further demonstrate the involvement of myosin 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 isoforms of tropomyosin 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 part tropomyosin and caldesmon plays in the signaling cascades associated with adhesion-mediated signal transduction events.

Tropomyosins (TMs) are a family of actin-filament-binding proteins that are essential for the integrity of actin filaments. Normal fibroblasts express three high-molecular-weight (HMW, 284 amino acids) TM isoforms, termed TM-1, TM-2, and TM-3, and three low-molecular-weight (LMW, 248 amino acids) TM isoforms, termed TM-4, TM-5(NM1), and TM-5a. In normal cells, TM-1 and TM-2 are the major HMW TMs and TM-4 is the major LMW TM. Since the first observations from Hal Weintraub's lab in the early 1980s, alterations in TM synthesis have been reported in a variety of transformed cells. Changes in TM expression have also been found in human breast cancer and prostate cancer. Although nonmuscle cells

express multiple isoforms of TM, the expression of only HMW TM isoforms is decreased during oncogenic transformation. HMW TMs protect actin filaments from severing proteins better than LMW TMs, consistent with their absence leading to a loss of stable actin filaments following transformation. We and others have found reversal of the transformed phenotype following forced expression of specific HMW TMs in cells, including restored formation of microfilament bundles and contact-inhibited cell growth, inability to grow on soft agar, and suppression of tumorigenicity in nude mice. These results show that changes in TM have a causal role in transformation. We hypothesize that specific isoforms of TM have a critical role in the regulation of cell growth by regulating myosin II. This is based on recent observations from our laboratory and others that activation of myosin II is essential for formation of focal adhesions and subsequent adhesion-dependent signaling, by promoting integrin clustering and the associated tyrosine phosphorylation and signaling events (see Fig. 1).

As diagrammed in the Figure 1, regulation of myosin is a key event in adhesion-dependent signaling. TM in association with caldesmon, bound along actin filaments, functions in regulating actomyosin-based contractility. Studies in smooth cell systems show that caldesmon acts cooperatively with TM to regulate (inhibit) actin-activated ATPase and movement of myosin. On the basis of our results in nonmuscle cells, we suggest that the interaction of caldesmon with specific TMs is important in maintaining the normal signaling in nonmuscle cells. A loss of specific isoforms of TM might lead to activation of adhesion-dependent signaling pathways as a result of increased actomyosin contractility. Furthermore, caldesmon is a target for various protein kinases, including MAP kinase, Cdc2 kinase, and protein kinase C. We propose that TM and caldesmon function as part of the cellular machinery that regulates various signal transduction pathways that act via activation of actomyosin contractility. Studies are in progress to determine how these actin-filament-associated proteins function in adhesion-dependent signaling.



Contractility and adhesion-dependent signaling

FIGURE 1 Proposed model for the involvement of actin filaments, myosin II, tropomyosin, and caldesmon in the regulation of cell contractility and adhesion-dependent signaling. Actomyosin contractility in nonmuscle cells involves a number of structural components including actin, myosin II, tropomyosin, and caldesmon. Tension on integrins at focal adhesions developed by the actin system affects adhesion-dependent signaling. This signaling in turn promotes further assembly of the focal adhesions and also induces downstream events culminating in cell proliferation. Tension is positively regulated by myosin light-chain kinase and Rho via Rho-kinase and negatively regulated by caldesmon. Caldesmon is regulated by calcium-calmodulin and by a number of kinases. Inhibition of the actin-activated myosin ATPase activity by caldesmon is dependent on tropomyosin. For simplicity, isolated copies of tropomyosin and caldesmon are shown, although in fact they are localized along the actin filaments. (*Pointed arrows*) Stimulation; (*blunt arrows*) inhibition. (ECM) Extracellular matrix; (MLCK) myosin light-chain kinase; (MLCP) myosin light-chain phosphatase.

Molecular Organization and Regulation of Actin Filaments

C. Berthier, E. Araya

In nonmuscle cells, the formation of actin filaments and their assembly into various structures, e.g., stress fibers, contractile ring, filopodia, and lamellipodia, are dynamic processes. How these different structures are formed and regulated within a single cell is not known. It is becoming apparent that in addition to actin and its associated motor molecules (myosin I and II), other actin filament-associated proteins, such as TM, have essential roles in the assembly, function, and regulation of these structures. To determine if distinct TMs are associated with distinct actin structures, we have developed and are using various reagents, including green fluorescent protein (GFP)-TM fusions, as well as hemagglutinin (HA)- and vesicular stomatitis virus (VSV)-epitope tagged TMs, to localize the different TM isoforms. These studies demonstrate that specific TM isoforms are involved in different subsets of actin filaments. Experiments are in progress to characterize the dynamic localization of the different TM isoforms during various cellular motility events

involving reorganization of the actin cytoskeleton, such as protrusion of filopodia, lamellipodia ruffling, forward movement, spreading and assembly of stress fibers, cytokinesis, and following stimulation via the small GTPases (Cdc42, Rac, and Rho).

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

A. Rai

We have identified a novel protein from human non-muscle cells. The protein is well conserved across evolution, as database searches have identified expressed sequence tags (ESTs) from a wide range of metazoan organisms that show homology with the human counterpart. These include rodents, zebrafish, flies, worms, and plants. Analysis of its domain structure indicates a hydrophobic region at its amino-terminal end, as well as a coiled-coil region toward the carboxyl terminus. Antibodies raised against this protein detect the existence of two isoelectric variants, and phosphatase sensitivity studies have demonstrated that one of these is a phosphorylated form.

Immunofluorescence studies show that there are two different populations of this protein. One of these is associated with free cytoplasmic vesicles, whereas another lies in a strong juxtanuclear region, similar to the Golgi compartment. Functional analysis of the protein reveals specific regions required for the movement through the Golgi, localization to vesicles, and fusion of vesicles to tubular membranes. Experiments are currently in progress to delineate the site of phosphorylation. Future experiments using mutational analysis will allow us to correlate protein modification with a functional role.

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

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We study fundamental mechanisms of transcription. Promoters can be divided into two regions, the core promoter region and the regulatory region. The core promoter region is sufficient to direct low levels of transcription in vitro and thus contains all the information required to assemble a transcription initiation complex and recruit the correct RNA polymerase. The regulatory region recruits activators or repressors of transcription that modulate the activity of the core promoter. We want to understand how core promoters ultimately recruit the correct RNA polymerase, and how transcriptional activators perform their function. As model systems, we use the human small nuclear RNA (snRNA) genes and the human immunodeficiency virus type-1 (HIV-1) promoter.

The human snRNA promoters have a number of features that make them especially suitable to address the broad questions mentioned above. First, the snRNA promoters recognized by RNA polymerase II are very similar in structure to those recognized by RNA polymerase III, and the RNA polymerase II and III snRNA promoters recruit several common transcription factors. Thus, any difference we observe in RNA polymerase II and III transcription initiation complexes is likely to be relevant to the determination of polymerase specificity. Second, the structure of snRNA promoters is relatively simple. The core RNA polymerase II snRNA promoters consist of just one element, the proximal sequence element or PSE. The core RNA polymerase III snRNA promoters consist of a PSE and a TATA box, and the regulatory region of snRNA promoters is interchangeable between RNA polymerase II and III snRNA promoters and virtually always contains an octamer sequence. Third, the transcription factors binding to the various snRNA promoter elements are well characterized. The TATA box and the PSE are recognized by TBP (TATA box-binding protein) and the snRNA activating protein complex ($SNAP_c$), respectively, whereas the octamer sequence is recognized by Oct-1. The crystal structures of the TBP and Oct-1 DNA-binding domains, both free and complexed with their respective DNA-binding sites, have been determined. And recently we have cloned all of the $SNAP_c$ subunits and reconstituted a recombinant complex.

One long-standing interest has been to identify the remaining factors required for both RNA polymerase II and III snRNA gene transcription. Another is to use the tools already in hand to characterize how the RNA polymerase II and III snRNA promoters assemble stable transcription initiation complexes. We find that basal transcription factors such as TBP and $SNAP_c$ have built-in mechanisms that down-regulate their binding to DNA. These "dampers" of DNA binding are deactivated by protein-protein interactions with a partner in binding. Thus, cooperative binding between TBP and $SNAP_c$ deactivates a damper of DNA binding in TBP, and cooperative binding between $SNAP_c$ and Oct-1 deactivates a damper of DNA binding in $SNAP_c$. These intricate mechanisms probably ensure that basal transcription factors, which are characterized by very slow off-rates, do not bind to random binding sites in the genome but rather are targeted specifically to promoters.

The HIV-1 promoter is subject to regulation at the level of transcription elongation. The promoter contains an element called the inducer of short transcripts (IST), which activates the synthesis of HIV-1 short RNA molecules. We have purified a factor that binds to the IST (FBI-1) and cloned a corresponding cDNA. One of our interests is to characterize this factor functionally.

Characterization of Partial $SNAP_c$s

V. Mittal, B. Ma, N. Hernandez

Core promoters usually recruit multisubunit complexes, and this may be because multisubunit complexes

confer the flexibility required to perform the very complicated task of core promoter-binding factors, namely, to nucleate the assembly of the correct transcription initiation complex in a regulated manner. This task requires a large number of protein-protein interactions, imposed in part by a combinatorial mechanism of transcription regulation. It is of great interest, therefore, to understand the functions of individual subunits that form a core promoter-binding factor. The PSE recruits the multisubunit complex $SNAP_c$. Because we can assemble $SNAP_c$ from recombinant subunits, and because $SNAP_c$ is involved in the assembly of both an RNA polymerase II and an RNA polymerase III initiation complex, it provides us with an exceptionally interesting case in which to understand the function of the individual subunits of a core promoter-binding factor.

To study this question, we first determined the network of protein-protein interactions among the five $SNAP_c$ subunits, SNAP190, SNAP50, SNAP45, SNAP43, and SNAP19. For this purpose, individual $SNAP_c$ subunits, or deleted versions thereof, were translated in vitro and tested for their ability to associate with other subunits of the complex in immunoprecipitation experiments. These studies indicated that SNAP43 and SNAP19 associate directly with the amino-terminal portion of SNAP190, whereas SNAP45 associates with the carboxy-terminal portion of SNAP190. SNAP50 joins the complex through association with SNAP43.

Guided by this map of protein-protein interactions within $SNAP_c$, we then used the baculovirus expression system to assemble subcomplexes missing one or several subunits and characterized the properties of the resulting partial complexes. The smallest complex we assembled that was still capable of binding to the PSE consisted of the amino-terminal third of SNAP190, SNAP43, and SNAP50 and was named "mini-$SNAP_c$." $SNAP_c$ is capable of binding cooperatively with TBP. We tested whether mini-$SNAP_c$ could do the same and showed that indeed, mini-$SNAP_c$ and TBP bind cooperatively to their respective binding sites on an RNA polymerase III snRNA promoter. Like cooperative binding of $SNAP_c$ and TBP, cooperative binding of mini-$SNAP_c$ and TBP required the nonconserved amino-terminal domain of TBP. This raised the possibility that mini-$SNAP_c$ was still capable of directing basal snRNA gene transcription. Indeed, mini-$SNAP_c$ could mediate both RNA polymerase II and III snRNA gene transcription.

$SNAP_c$ and the Oct-1 POU domain bind cooperatively to their respective targets on the DNA. In previous work, we had shown that cooperative binding required a direct protein-protein contact involving a glutamic acid at position 7 in the Oct-1 POU domain and a lysine at position 900 within the largest $SNAP_c$ subunit, SNAP190. Mini-$SNAP_c$ is missing the carboxy-terminal two thirds of SNAP190, including the region of SNAP190 required for protein-protein contact with the Oct-1 POU domain. We therefore expected that the complex would not be able to bind cooperatively with the Oct-1 POU domain and would bind poorly to DNA. To our surprise, however, mini-$SNAP_c$ indeed could not bind cooperatively with the Oct-1 POU domain, but it bound very efficiently to the DNA on its own, as efficiently, in fact, as the full complex together with the Oct-1 POU domain. Thus, in a sense, mini-$SNAP_c$ is already activated, and indeed in transcription assays, it behaves like the full complex in the presence of the Oct-1 POU domain.

These results indicate that $SNAP_c$ contains a built-in inhibitor of DNA binding, which is located somewhere in the carboxy-terminal two thirds of SNAP190 or, perhaps, within the SNAP45 subunit. This region of the complex is the same region that contains Lys-900 and is required for cooperative binding with the Oct-1 POU domain. Cooperative binding with the Oct-1 POU domain serves to deactivate the inhibitor of $SNAP_c$ binding and to promote very efficient binding of the two factors. This is very reminiscent of what we observed previously with $SNAP_c$ and TBP. In this case, cooperative binding of the two factors depends on the nonconserved amino-terminal domain of TBP. A truncated TBP missing the amino-terminal domain binds much more efficiently to a TATA box than full-length TBP, but cannot bind cooperatively with $SNAP_c$. Thus, in this case also, the domain that down-regulates binding of the protein is also the target for cooperative interactions that counteract the inhibition of binding and promote very efficient binding.

Why devise such complicated mechanisms of binding, in which a negative regulator must be deactivated before efficient binding can take place? The answer may have to do with a general property of basal transcription factors, namely, their very slow off-rates. It may be important for the cell to ensure that such factors do not bind to the wrong target, for example, that TBP not bind to random A/T-rich sequences in the genome, since once such factors are bound, they do not come off the DNA easily. A built-in damper of DNA binding, which is deactivated by protein-protein contact with a partner binding to the same promoter, ensures that the factor does not bind to isolated sites and also ensures very efficient binding to sites located

within promoters, i.e., close to the partner's binding site.

Unlike $SNAP_c$ and TBP, Oct-1 has a very fast on- and off-rate. Thus, one can imagine that Oct-1 could serve as a sensor, constantly probing the DNA for the presence of octamer sequences. If Oct-1 finds an octamer sequence, and if the sequence is located within an snRNA promoter, i.e., close to a PSE, then Oct-1 will recruit $SNAP_c$ to the PSE through cooperative binding, and in RNA polymerase III snRNA promoters, $SNAP_c$ will then in turn recruit TBP to the nearby TATA box. The cooperative interactions thus result in the assembly of a very stable transcription initiation complex.

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. We are introducing point mutations in the various partners and testing their effect on complex assembly. Such information will allow us to build a "minimal" $SNAP_c$ lacking all sequences dispensable for the integrity of the complex, which will then be tested for DNA binding and transcription activity. We are also testing such complexes for cooperative binding with TBP, with the aim of defining the target within $SNAP_c$ for cooperative interactions with the amino-terminal domain of TBP.

Mechanism of $SNAP_c$ Binding to the PSE

B. Trumbull, N. Hernandez

The largest subunit of $SNAP_c$, SNAP190, is a Myb domain protein. The SNAP190 Myb domain consists of four repeats, and we have shown that deletion of the two most carboxy-terminal repeats strongly reduces binding of $SNAP_c$, whereas deletion of the two most amino-terminal repeats has only a slight effect. SNAP190 is probably not the only $SNAP_c$ subunit involved in DNA binding. Indeed, we can cross-link SNAP50 to the DNA, suggesting that SNAP50 also contacts the PSE. To study how $SNAP_c$ recognizes the PSE, we are generating probes with photosensitive groups at various positions along the PSE and determining which $SNAP_c$ subunit can be cross-linked to which part of the PSE.

Identification of $SNAP_c$-associated Factors

F. Emran, N. Hernandez

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

Characterization of a Human RNA Polymerase III Holoenzyme

S. Sepehri, N. Hernandez

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

Factors Required for snRNA Transcription by RNA Polymerase III

L. Schramm, N. Hernandez

Basal RNA polymerase III transcription of the human snRNA genes requires $SNAP_c$, RNA polymerase III, and the TBP component of TFIIIB. We are determining which other TFIIIB component is required for U6 transcription.

Transcription from Chromatin Templates

X. Zhao, N. Hernandez

Our studies so far have used naked DNA templates. The goal of this project is to study the transcription of snRNA genes wrapped into chromatin templates and to determine the part played by cooperative interactions between the Oct-1 POU domain and $SNAP_c$, and $SNAP_c$ and TBP, for transcription from such templates.

Factors Required for snRNA Transcription by RNA Polymerase II

P. Hu, N. Hernandez

Previous work has shown that RNA polymerase II transcription from snRNA promoters requires many of the general transcription factors required for RNA polymerase II transcription from mRNA promoters, namely, TFIIA, TFIIB, TFIIF, and TFIIE. It is not yet clear whether TFIIH is required, but we know that all of the general transcription factors together with $SNAP_c$ and TBP are not sufficient to promote RNA polymerase II transcription from snRNA promoters. The goal of this project is to determine whether TFIIH is required for RNA polymerase II snRNA gene transcription and to isolate the missing factors required.

Regulation of snRNA Gene Transcription

M. Shanmugam, N. Hernandez

snRNA genes are very actively transcribed in proliferating cells. It is not known, however, whether snRNA gene transcription is down-regulated in resting cells, nor is it known when during the cell cycle snRNA gene transcription is most active. We will address these questions.

Localization of FBI-1, $SNAP_c$, Oct-1, and Other Factors In Vivo

P.S. Pendergrast, N. Hernandez

FBI-1 binds to the HIV-1 IST, but we have not been able to demonstrate a role of FBI-1 in short transcript formation. Instead, FBI-1 synergizes with the HIV-1 *trans*-activator Tat to produce very high levels of full-length transcripts. We are now using chromatin immunoprecipitation experiments to determine whether FBI-1 can be found bound to the HIV-1 promoter in vivo. We have also used the chromatin immunoprecipitation method to determine that all five $SNAP_c$ subunits as well as Oct-1 are bound to snRNA promoters in vivo.

Function of FBI-1

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

Through a combination of transfection and coimmunoprecipitation experiments, we are deciphering the function of FBI-1 in HIV-1 transcription. We are also characterizing how it binds to DNA.

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HIGHER-ORDER CHROMOSOME DYNAMICS

T. Hirano M. Hirano D. MacCallum
K. Kimura T. Yokochi
A. Losada

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. By using a cell-free extract derived from *Xenopus laevis* eggs, we previously identified two protein complexes, 13S condensin and 14S cohesin, that have central roles in chromosome condensation and sister chromatid cohesion, respectively. Although the two complexes have different cellular functions and are differently regulated during the cell cycle, they share a similar subunit organization: Each of the complexes contains a heterodimeric pair of SMC (structural maintenance of chromosomes) subunits and two or three non-SMC subunits. The goal in our laboratory is to understand how the two eukaryotic SMC protein complexes work at a mechanistic level and how they interact with chromatin and other proteins to fulfill their essential functions. We also use a bacterial SMC homodimer as a model system to dissect the basic mechanisms of SMC action.

Action of Condensin on Nucleosomal DNA

K. Kimura, T. Hirano

We found previously that 13S condensin reconfigures DNA structure in an ATP-dependent manner. In the presence of a type I topoisomerase and ATP, the complex introduces positive supercoils into a closed circular DNA. In the presence of a type II topoisomerase and ATP, 13S condensin promotes the formation of positive knots into a nicked circular DNA. The two activities are regulated by mitosis-specific, Cdc2-dependent phosphorylation of condensin subunits. On the basis of these results, we proposed that 13S condensin actively compacts and organizes DNA by introducing global positive writhe. Although these findings provided fundamental insights into the basic mechanism of condensin's action, little was known about how the complex interacts with its physiological substrate, nucleosomal DNA. To address this question, we first assembled nucleosomes on a plasmid DNA in condensin-depleted *Xenopus* egg extracts and separated them from free proteins by sucrose gradient centrifugation. Removal of histones from the isolated nucleosomes resulted in negatively supercoiled DNA. However, if the nucleosomes were preincubated with 13S condensin and topoisomerase I before deproteinization, the linking number of DNA shifted to a less negative (thereby positive) direction. The change in the linking number was ATP-dependent, and it was not observed when the nucleosomes were treated with either 13S condensin or topoisomerase I alone. Mitosis-specific phosphorylation of condensin subunits appeared to be important to support this activity. One of the most straightforward explanations for these results is that the mitotic form of 13S condensin introduces positive writhe into nucleosomal DNA as it does so into naked DNA. We cannot rule out the possibility, however, that condensin may have an ability to "remodel" nucleosomes either by altering histone structure or by dissociating histones from the DNA. Experiments are now in progress to distinguish between the two possibilities.

Cell Cycle Regulation of Condensin-Chromatin Interactions

D. MacCallum, K. Kimura, T. Hirano

How does 13S condensin initiate chromosome condensation specifically at the onset of mitosis? In the cell-free extracts of *Xenopus* eggs, the association of 13S condensin with chromatin is mitosis-specific. In contrast, the mitotic and interphase forms of 13S condensin, once purified from the extracts, display a comparable level of DNA-binding activity. Thus, Cdc2-dependent activation of the supercoiling and knotting activities does not account for the whole mechanism of mitotic regulation of condensin. There must be an additional regulatory mechanism at the level of chromosomal targeting.

To address this issue, we have developed an in vitro assay to test the interactions between purified condensin and isolated chromatin. Sperm chromatin was incubated with either mitotic or interphase extract that had been depleted of condensin, and it was then isolat-

ed by centrifugation through a sucrose cushion. This structure, termed remodeled sperm chromatin (RSC), contains cell-cycle-specific chromatin components, including core histones, and thereby represents the most physiologically relevant substrate for a condensin-binding assay. The mitotic or interphase form of RSC was incubated with 13S condensin purified from either mitotic or interphase extracts, and their interactions were assayed by immunofluorescent staining of chromatin with an anti-condensin antibody. We found that mitotic condensin was able to bind to mitotic and interphase RSC in an equal efficiency. Likewise, interphase condensin bound to both mitotic and interphase RSC. Thus, the cell cycle specificity normally observed in the cell-free extracts was abolished in this purified system. However, the addition of an interphase extract to the reaction prevented condensin from binding to the RSC. These results suggest that there is a soluble inhibitor in interphase extracts that is necessary to prevent the interaction between condensin and chromatin, thus conferring the cell cycle specificity. This putative inhibitory factor is currently being purified by conventional biochemical fractionation.

We have also established an assay to test the interactions between purified condensin and nucleosomes immobilized on magnetic beads. In this assay, a linear DNA was coupled to magnetic beads via a biotin-streptoavidin linkage, and then incubated with condensin-depleted extracts. Nucleosomes assembled on the immobilized DNA were purified in a magnetic field and tested for their ability to interact with purified condensin by quantitative immunoblotting. Consistent with the results from the RSC-binding assay, we did not observe any cell-cycle-specific interactions between the purified nucleosomes and purified condensin. Although this magnetic bead assay seems to be less physiological than the RSC assay, the former is technically easier and more quantitative than the latter. We anticipate that the two approaches will complement with each other, facilitating the identification of factors that determine the cell-cycle-specific behavior of condensin.

Search for Additional Condensation Factors

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

Accumulating lines of evidence from our own and other laboratories strongly suggest that the condensin complex has a central role in chromosome condensation both in vitro and in vivo. Nevertheless, it is possible that additional factors cooperate with condensin to promote full condensation. Recent evidence from our reconstitution experiments supports this idea. When isolated mitotic RSC (see above) was incubated with purified mitotic condensin, some condensation occurred, but no discrete condensed chromosomes were observed. When a mitotic extract was added back into the reaction, full condensation was restored. These observations, together with other fractionation data, suggest that an additional soluble factor present in the extract, other than topoisomerase II, is required for condensation and resolution of mitotic chromosomes. As a complementary approach, we have also started a reevaluation of protein components that are copurified with chromosomes assembled in *Xenopus* egg extracts.

Molecular Actions of the Cohesin Complex

A. Losada, T. Hirano

The cohesion of duplicated chromosomes (sister chromatids) is established at the time of DNA replication and maintained until the metaphase-anaphase transition in mitosis. We showed previously that eukaryotic cells contain a second SMC protein complex (termed 14S cohesin) that plays an essential part in the establishment of sister chromatid cohesion during interphase. In vertebrates, but not in yeast, most of the cohesin complex dissociates from the chromatin at the onset of mitosis. It remains to be determined whether a small amount of cohesin left on the condensed chromosomes is sufficient to hold sister chromatids together until the metaphase-anaphase transition, or whether other noncohesin factors are involved in this process. We are currently trying to determine the mechanisms by which the chromatin targeting and release of cohesin are regulated at different stages of the cell cycle.

How does cohesin work at a molecular level? Do cohesin and condensin share a common mechanism of action? To address these questions, we have purified the cohesin complex from both *Xenopus* egg extracts and human tissue culture cells and assayed its biochemical activities in vitro. We found that cohesin possessed a weak ATPase activity that was stimulated in the presence of DNA. The complex bound to single-stranded DNA (ssDNA) but did not support reannealing of complementary DNA strands, an activity displayed by other SMC complexes such as the bacterial BsSMC dimer. Cohesin also bound to double-stranded DNA with a preference for its supercoiled form. When incubated with a linear DNA template in the presence of DNA lig-

ase, cohesin strongly favored intermolecular end-to-end joining (concatemer formation) over intramolecular ligation (recircularization). Furthermore, in the presence of topoisomerase II, cohesin promoted catenation between nicked circular DNA molecules. In a striking contrast, condensin directed only intramolecular knotting, but not intermolecular catenation, in the same assay. Results from gel-shift experiments also suggested that cohesin and condensin interact with DNA in different ways. We hypothesize that cohesin functions as an intermolecular DNA cross-linker, with the two putative DNA-binding ends of the complex acting on noncontiguous DNA segments. Condensin, however, appears to behave as an intramolecular DNA cross-linker, with both ends of the complex bound to a contiguous DNA segment. Although this is probably an oversimplified model, the cross-linker hypothesis provides a framework for our understanding of how cohesin, condensin, and other SMC protein complexes might work in the dynamic organization of chromosomes in the cell.

Identification of Additional Cohesion Factors

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

The *Xenopus* cohesin complex consists of two SMC subunits (SMC1-type and SMC3-type) and at least three non-SMC subunits. Microsequencing of one of the previously uncharacterized subunits, p155, revealed that it is homologous to Scc3p, a gene product recently shown to be a component of the yeast cohesin complex. A cohesin complex purified from HeLa cell extracts also contained an Scc3p homolog, providing additional evidence that subunit composition of the cohesin complex is highly conserved from yeast to humans. To get more insights into the biochemical mechanisms of sister chromatid cohesion, we have started characterization of *Xenopus* homologs of BimD, a protein that is known to interact with a cohesin subunit in the fungus *Aspergillus nidulans*.

Structural and Functional Dissection of a Bacterial SMC Homodimer

M. Hirano, T. Hirano

Mutations in the single *smc* gene of the gram-positive bacterium *Bacillus subtilis* cause defects in chromosome condensation and segregation, suggesting that the basic function of SMC proteins may be in common from bacteria to humans. We showed previously that the *B. subtilis* gene product (termed BsSMC) is a DNA-stimulated ATPase and is able to form large nucleoprotein assemblies with ssDNA in an ATP-dependent manner. A recent electron microscopy study from another group showed that BsSMC is dimerized via antiparallel coiled-coil interactions, forming a "two-armed" symmetrical structure with a central flexible hinge. To understand how this unique structure of BsSMC might contribute to its function at a mechanistic level, we have started a systematic mutagenesis approach. We are particularly interested in constructing two different classes of mutant derivatives. The first class is the so-called "single-armed" mutant that is composed of an amino-terminal and carboxy-terminal halves of a BsSMC polypeptide. The second class is a "stiff-hinge" mutant in which two functional arms are connected by an inflexible hinge. To this end, multiple glycine residues highly conserved in the hinge domain were replaced with alanines. We found that the hydrodynamic properties of the expressed proteins were consistent with those expected for the single-armed and stiff-hinge mutants. These mutants failed to interact with ssDNA properly and displayed unusual characteristics of ATPase activity. Further characterization of these mutants should provide fundamental insights into how the ATPase cycle of BsSMC might be coupled with its mechanical cycle. We also anticipate that this approach will help us to understand the different actions of condensin and cohesin in eukaryotic cells and will shed light on the evolutionary origin of condensation and cohesion.

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CELL BIOLOGY OF THE NUCLEUS

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

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 over the past year, and numerous collaborations are under way with the excellent technical expertise of Tamara Howard.

Organization of Interchromatin Granule Clusters

P. Sacco-Bubulya

The majority of RNAs synthesized by RNA polymerase II contain introns that must be spliced out prior to transport of the RNA to the cytoplasm where it functions by providing protein-coding information to the translational apparatus. Interchromatin granule clusters (IGCs) are regions within mammalian cell nuclei that are the sites of localization for splicing factors and other proteins required for pre-mRNA synthesis and processing. Splicing factors are recruited from these regions to active transcription sites, where they function in cotranscriptional pre-mRNA processing. Many splicing factors contain an RS domain that, when hyperphosphorylated, results in the release of these factors from IGCs. Splicing factors that contain such a domain are referred to as SR proteins. The release of splicing factors from IGCs is regulated by SR protein kinases. When SR protein kinases are overexpressed in cultured cells, they alter the organization of nuclear speckles and can ultimately cause complete redistribution of splicing factors into a diffuse nuclear localization.

We are using one of these SR protein kinases, *c*dc2-*l*ike *k*inase (CLK/STY), as a tool to disrupt IGCs in vivo. Overexpression of CLK/STY in A-431 cells

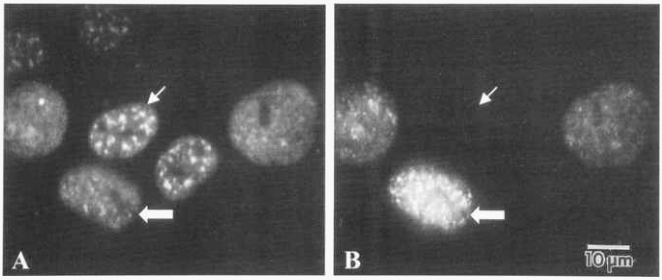

FIGURE 1 Overexpression of FLAG-CLK/STY in A-431 cells. (*A–B*) Coimmunofluorescence of SR proteins (panel *A*, 3C5 antibody) and wild-type FLAG-CLK/STY (panel *B*, M2 anti-FLAG antibody). Transient overexpression of the kinase CLK/STY causes a dramatic redistribution of SR proteins (*thick arrow*) as compared to the typical speckled SR protein localization in untransfected neighboring cells that are not overexpressing CLK/STY (*thin arrow*).

causes a dramatic reorganization of splicing factors within the nucleus (Fig. 1). It is not known if IGCs occupy specific regions of the nucleus or if they contain organizational elements such as filaments or SR protein receptors. At the ultrastructural level, the IGCs of normal nuclei appear as clusters of electron-dense granules each measuring 20–25 nm in diameter. We are currently evaluating nuclear ultrastructure in cells that overexpress CLK/STY, as the splicing factors will be released from IGCs in these cells and the underlying structural elements may be revealed. In addition, we would like to determine the importance of the integrity of these clusters for supplying factors involved in RNA metabolism to active genes. For example, splicing factors may arrive at transcription sites in preassembled complexes that are functional only if they originate from IGCs. This study aims to reveal the structural organization of the IGCs, and it will address the significance of the clustered arrangement of RNA synthesis and processing factors for supporting gene expression.

An In Vitro System to Study Splicing Factor Dynamics

N. Saitoh

Previous studies from our laboratory have shown that pre-mRNA splicing factors are recruited from IGCs to sites of transcription. Furthermore, we have shown that this recruitment is dependent on phosphorylation of the RS domain of SR splicing factors. To determine the molecular mechanism of this nuclear dynamics, we began to establish an in vitro reconstitution system by using biochemically isolated IGCs. We isolated IGCs by a modified method so that the morphology of these structures is maintained in a physiological buffer. The purified fraction contains large clusters of interchromatin granules (Fig. 2). Immunofluorescence microscopy with an antibody that recognizes a phospho-epitope on the SR family of splicing factors was used to label the purified structures. The positive immunolabeling (Fig. 2) implies that the bulk phosphorylation status of these proteins is maintained during the purification. One of the major goals of this project is to recapitulate the recruitment of splicing factors to the site of active transcription in a cell-free system. We will place the fraction in an in vitro transcription system and address whether splicing factors leave the granule-shaped structures for a DNA template. Furthermore, we will fractionate nuclear extract to identify proteins responsible for the recruitment. An additional goal of this project is to reconstitute the peripheral movement of the interchromatin granule clusters observed in vivo. We will record the movement of isolated clusters mixed with an ATP regenerating system and nuclear extract by time-lapse microscopy. We will then utilize the fractionated nuclear extract to identify proteins responsible for the movement. Other morphological changes of the isolated IGCs are expected upon treatment with certain reagents such as α-amanitin (rounding up), Clk/Sty kinase (disperse), phosphatase 1

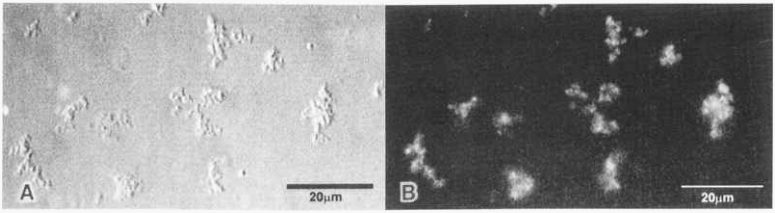

FIGURE 2 Fractionated interchromatin granule clusters were adhered to a glass slide and immunolabeled with an antibody that recognizes the SR family of pre-mRNA splicing factors. Shown are differential interference contrast (*A*) and fluorescence (*B*) microscopy of the same isolated structures.

inhibitor (enlarged), and mitotic extract (disperse). These experiments will address the mechanism of splicing factor recruitment and will bring us closer to understanding the spatial organization of gene expression.

Proteome Analysis of a Nuclear Domain Involved in Gene Expression

N. Saitoh, P. Sacco-Bubulya, R. Kobayashi [in collaboration with S. Patterson and C.S. Spahr, Amgen Inc.]

We have continued to use two complementary approaches toward identification of the protein composition of IGCs. In the first approach, we resolved IGC protein constituents on two-dimensional gels as performed previously and excised spots enriched in the IGC fraction for peptide microsequencing. This method is advantageous because it allows us to directly identify proteins enriched in the IGCs as well as to obtain preliminary information about the proteins, such as their pIs and molecular weights. Using this approach, we have identified 14 known proteins and one peptide that corresponds to a sequence in the expressed sequence tag (EST) library. Peptide sequences obtained from one of the most abundant of the enriched proteins on the two-dimensional gel were a precise match to the protein KIAA0111, also called eukaryotic initiation factor 4A-like protein (eIF-4A-like). KIAA0111 was fused to yellow fluorescent protein (YFP) and expressed transiently in HeLa cells, where it colocalized with endogenous splicing factor SC35 in nuclear speckles. The precise role of this IGC protein is currently being investigated.

In the second approach, we applied the whole IGC fraction to proteome analysis by mass spectrometry. This method allows us to identify the entire protein composition of the isolated IGC fraction by combining peptide sequence analysis and database searching. The advantages of this method are that it is rapid, it does not require separation of each component prior to mass spectrometry, and it is sensitive enough to identify less abundant, yet important proteins that other methods may not detect. On the basis of our previous studies, we anticipate identifying (1) new splicing factors, (2) architectural proteins such as molecular chaperones that are responsible for assembly and maintenance of the IGCs or may be responsible for preassembly of splicing factor complexes, (3) proteins involved in nuclear signal transduction which regulate movements of splicing factors, (4) receptors for the SR proteins, (5) a hypothetical molecular motor that may carry splicing factors to the site of active transcription, and (6) a series of proteins involved in human disease such as the two examples related to cancer that have already been identified (skip, TLS-associated protein with SR repeats).

We utilized automated micro-column liquid chromatography electrospray ionization tandem quadrupole mass spectrometry (LC/MS/MS). Using this method, we identified 114 known proteins that have previously been localized in the nuclear speckles, or whose functions have been associated with nuclear speckles, such as splicing factors and other RNA processing factors. We have identified 12 new IGC protein candidates, and in addition, we obtained peptides that correspond to 69 different accession codes for EST clones. We are using information provided by the primary sequence of the identified proteins, such as functional analysis for the known proteins and domain analysis for the less characterized proteins, to screen for constituents that may be critical for the function of nuclear speckles. Particular emphasis is being placed on domain and motif analyses that strongly indicate a structural component or components that will reveal an altogether novel function for nuclear speckles. Identification of the IGC components will lead us to a better understanding of the function of the IGCs in coupling transcription and pre-mRNA splicing and to understanding the functional/structural organization of the nucleus.

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

S. Janicki

Transcription and RNA processing are believed to be functionally linked in vivo as RNA processing factors have been found in association with the carboxy-terminal domain of RNA polymerase II. To understand how these processes are coordinated within the struc-

tural framework of the nucleus, we are studying the function of SCAF10, a protein that may regulate interactions between factors known to be essential for gene expression. SCAF10 localizes to both nuclear speckles and the nucleoplasm and was identified in yeast two-hybrid screens for proteins that interact with the CTD of RNA polymerase II (Bourquin et al., *Nucleic Acid Res. 52:* 2055 [1997]) and Clk/Sty kinase (Nestel et al., *Gene 180:* 151 [1996]). SCAF10 is a 106-kD protein with an amino-terminal cyclophilin domain and a carboxy-terminal serine/arginine-rich RS domain. Cyclophilins have been reported to act as chaperones in protein trafficking and macromolecular assembly, and they contain peptidyl-prolyl *cis-trans* isomerase (PPIase) domains that catalyze *cis-trans* isomerizations about Xaa-Pro peptide bonds. The peptide folding activity of this protein may potentially regulate interactions between RNA processing factors and the CTD or play a part in the assembly and disassembly of the macromolecular complexes involved in splicing. The carboxy-terminal RS domain of SCAF10 is essential for its association with the RNA polymerase II CTD. We are currently making antibodies to SCAF10 in order to identify a broader range of proteins with which SCAF10 is associated. Antisense treatment will also be used as a means of determining the effects of knocking out SCAF10 on complex formation, nuclear structure, and nuclear functions, including transcription and splicing. Additionally, we are studying the dynamics of SCAF10 movement in living cells through the use of green fluorescent protein fusion proteins. We are investigating whether SCAF10 is recruited to active transcription sites and whether splicing factors move to these sites from the nuclear speckles in association with SCAF10 and/or RNA polymerase II.

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PROTEIN TYROSINE PHOSPHATASES AND THE CONTROL OF CELLULAR SIGNALING RESPONSES

N.K. Tonks H.H. Chen, R.L. Del Vecchio, T. Fukada, J.P. Liu, T.C. Meng, M.P. Myers, H.L. Palka, K.A. Pennino, 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 and differentiation. Protein phosphorylation is a reversible, dynamic process in which the net level of phosphate observed in a target substrate reflects not only the activity of the kinases that phosphorylate it, but also the competing action of protein phosphatases that catalyze the dephosphorylation reaction. We study the expanding family of protein tyrosine phosphatases (PTPs) which, like the kinases, comprise both transmembrane receptor-linked forms and nontransmembrane cytosolic species and represent a major family of signaling enzymes. The structures of the PTPs indicate important roles in the control of key cellular functions. We are utilizing a variety of strategies to characterize the normal physiological function of several members of the PTP family. Disruption of normal patterns of tyrosine phosphorylation has been implicated as a contributor to several human diseases. Therefore, insights into the mechanisms involved in modulating PTP function may ultimately yield important information to help counter such diseases.

During the last year, Shao-Hui Zhang completed his postdoctoral studies and accepted a position at Tanabe Research Laboratories. He and J.P. Liu have now moved to San Diego. We were joined by Toshiyuki Fukada and T.C. Meng as postdocs, and Hsu-Hsin (May) Chen has begun her work as a graduate student in the lab.

IDENTIFICATION OF PTP SUBSTRATES USING "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. We have developed a method by which we can examine PTP substrate specificity in a cellular context. By mutating the invariant catalytic acid residue (Asp-181 in PTP1B) to alanine, we generate a form of the PTP that maintains a high affinity for substrate but does not catalyze dephosphorylation effectively, i.e., we convert an extremely active enzyme into a "substrate trap." 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 identified by immunoblotting or, on a larger scale, by primary sequence determination. Importantly, the residue that is mutated to generate "substrate-trapping" mutants is conserved in all members of the PTP family. Therefore, the approach should be applicable to all members of the family. The major take-home message from this work to date is that members of the PTP family display exquisite substrate specificity in a cellular context.

During the past year, our work on characterization of the cellular substrate specificity of PTP-PEST and PTPH1 was published. We showed that through specific dephosphorylation of $p130^{cas}$, PTP-PEST regulates the rate of cell migration. In addition, we identified VCP (valosin-containing protein, also known as p97 or yeast Cdc48), which is an ATPase involved in cell cycle regulation, protein degredation, organelle biogenesis, and vesicle-mediated protein transport, as a major cellular substrate of PTPH1. It appears that PTPH1 may exert profound inhibitory effects on cell proliferation through its dephosphorylation of VCP. The exact mechanism by which VCP function is regulated by tyrosine phosphorylation remains to be established.

We have continued our study of the role of TCPTP in regulating epidermal growth factor (EGF)-induced signaling events. TCPTP occurs in two forms generated by alternative mRNA splicing: a 48kD form (TC48) that is associated with the endoplasmic reticulum (ER) and a 45-kD form (TC45) that in the basal state is nuclear. Both forms share the same catalytic domain. Previously, we had shown that the TC48-D182A substrate-trapping mutant formed a complex in the ER with the tyrosine-phosphorylated EGF receptor (EGFR), suggesting that TC48 may regulate the state

of phosphorylation of nascent EGFR that is in transit through the ER on the way to the plasma membrane. In contrast, TC45 displayed a distinct substrate specificity. In response to EGF stimulation, TC45 exits the nucleus and recognizes the EGFR, one of the isoforms of the adapter protein Shc, and two, as yet unidentified, pTyr proteins (p50 and p64) as substrates. Trapping mutant forms of TC45 displayed preferential recognition of Shc phosphorylated on Y239, compared to the protein phosphorylated on Y240 or Y317. These observations illustrated a remarkable degree of substrate specificity for TC45 in a cellular context, which encouraged us to investigate further the effects of this PTP on EGF-induced signaling events. We noted that expression of TC45 in COS cells inhibited EGF-dependent activation of the c-Jun amino-terminal kinase (JNK) but did not effect the activation of extracellular signal-regulated kinase 2 (ERK), thus illustrating that TC45 can regulate selectively mitogen-activated protein kinase (MAPK) signaling pathways emanating from the EGFR. We observed that wild-type and substrate-trapping mutant forms of TC45 inhibit almost completely the EGF-dependent activation of PI3-kinase and PKB/Akt. TC45 and TC45-D182A act upstream of PI3-kinase, most likely by inhibiting the recruitment of the p85 regulatory subunit of PI3-kinase by the EGFR. Recent studies have indicated that the EGFR can be activated in the absence of EGF following integrin ligation. We found that the integrin-mediated activation of PKB/Akt in COS1 cells is abrogated by the specific EGFR PTK inhibitor tyrphostin AG1478 and that TC45 and TC45-D182A inhibit activation of PKB/Akt following the attachment of COS1 cells to fibronectin. Thus, TC45 may serve as a negative regulator of growth factor or integrin-induced, EGF receptor-mediated PI3-kinase signaling. In this respect, the function of TC45 is reminiscent of that of Smad7, an inhibitor of transforming frowth factor-β (TGF-β) signaling (Itoh et al., *J. Biol Chem. 273:* 29195 [1998]). In the absence of ligand, Smad7 is predominantly localized in the nucleus. Stimulation of cells with TGF-β induces Smad7 to exit the nucleus, associate with the TGF-β receptor at the plasma membrane, and inhibit TGF-β-induced signaling. Whether or not TC45 (and for that matter Smad7) has a regulatory function in the nucleus remains to be established.

STRUCTURAL ANALYSES OF PTP FAMILY MEMBERS

We have also continued our collaboration with David Barford (now at the Institute of Cancer Research, Chester Beatty Labs, London, UK) in which we are undertaking a structural analysis of members of the PTP family. Our most recent work has focused on the determination of the structure of complexes between trapping mutant forms of PTP1B and phosphotyrosyl peptides modeled on physiological sites of tyrosine phosphorylation. In 1999, a paper was published [Elchebly et al., *Science 283:* 1544 [1999]) which reported that disruption of the PTP1B gene yielded apparently normal mice that displayed enhanced sensitivity to insulin and resistance to weight gain when fed a high-fat diet. These observations suggest that PTP1B is a major regulator of insulin-induced signaling and fuel metabolism and may be an important player in type-2 diabetes and obesity. To understand how PTP1B may exert specific effects on insulin-dependent signaling, we solved the structure of PTP1B in a complex with a phosphotyrosyl peptide modeled on two of the autophosphorylation sites (Y1150, Y1151) from the activation loop of the insulin receptor. The structure reveals that of the two adjacent phosphotyrosyl residues, pY1150, which is a key regulator of the activity of the insulin receptor, is engaged in the active site of the enzyme, whereas pY1151 binds to a distinct crevice on the surface of the protein. At this second site, the phosphate moiety of the pTyr residue interacts with a basic residue on the surface of PTP1B that is unique to PTP1B and its closest homolog TCPTP. We are currently undertaking further structural analyses in combination with testing the effects of mutation of key surface residues of PTP1B on substrate recognition. These studies may yield structural insights into the molecular mechanism underlying the substrate specificity of this important PTP.

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SIGNAL TRANSDUCTION

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

Our laboratory continues to study the function of the Ras and Rho family members of small GTPases and the mechanisms by which they exert their effects. These proteins have been implicated in a wide variety of important cellular activities, including cell growth control and morphogenesis. Their genes encode low-molecular-weight guanine nucleotide-binding proteins that function as binary switches by cycling between an active GTP-bound state and an inactive GDP-bound state. It is only in their GTP-bound state that they are able to interact with downstream effector molecules which mediate their effects. The ratio of the two forms is regulated by the opposing effects of guanine nucleotide exchange factors (GEFs) which promote the exchange of bound GDP for GTP, and the GTPase-activating proteins (GAPs) which stimulate hydrolysis of bound GTP.

Of all the GTPases, the Ras proteins have been studied most, since activating mutations in *ras* genes are commonly found in a wide variety of human tumors. As outlined below, our research efforts have been focused mainly on defining the downstream effector pathways of Ras. In particular, we have continued the further analysis of a protein, AF-6, which we had identified as a novel Ras-binding protein. In addition, we focused on the functional characterization of $p62^{dok}$, a Ras-GAP-associated protein, which was found to be constitutively tyrosine-phosphorylated in chronic myelogenous leukemia (CML) progenitor cells.

Members of the Rho family, namely, RhoA, Rac, and Cdc42, are best known as regulators of the actin cytoskeleton. It is believed that alterations in the actin cytoskeleton triggered by the Rho proteins contribute to their effects observed on adhesion, invasion, membrane trafficking, and, more recently, neuronal development. We previously demonstrated, for example, that the effect of Rac on T-cell adhesion and spreading is integrin-dependent and requires an intact actin cytoskeleton. Currently, we initiated studies examining the role of the Rho GTPases and their regulators on neuronal development. In particular, we are assessing the physiological role of Oligophrenin, a Rho-GAP involved in mental retardation. In addition to their role in controlling the actin cytoskeleton, the Rho GTPases are also known to regulate gene expression, which may contribute to their effects on cell growth. To identify genes whose transcription is regulated by Rac, we made use of representational difference analysis (cDNA-RDA).

RAS SIGNALING PATHWAYS

Previous studies have shown that oncogenic Ras proteins signal through the well-established Raf, RalGDS, and PI3-kinase pathways and that all these pathways participate in the cell-transforming activity of oncogenic Ras. In addition, we demonstrated that whereas Ras-mediated tumorigenicity can arise independently from MAPK activation, experimental metastasis requires constitutive activation of the Raf/MEK/MAPK pathway. However, we cannot exclude that other effectors may contribute to the metastatic phenotype, in addition to Raf. Moreover, 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 early steps (e.g., intravasation) in which the Rho-GTPases are likely to have a role. We initiated studies in collaboration with G. Webb and G. Vande Woude (Van Andel Research Institute, Michigan) to assess the contribution of the Rac GTPase to metastasis.

The importance of cell adhesion and cell shape for the control of cell cycle progression has been well established by now. Oncogenic transformation by Ras is usually accompanied by a loss in cell-cell adhesive properties and morphological changes. However, the precise mechanism by which Ras exerts this morphological phenotype remains elusive. In light of this, we identified AF-6 as a novel Ras-binding protein and found that AF-6 accumulates at cell/cell contact sites.

RAS AND RAP1 INTERACTION WITH THE AF-6 EFFECTOR TARGET

In addition to the ability of the AF-6 protein to bind Ras, the AF-6 gene was also found to be fused to the ALL-1 gene in a subset of acute lymphoblastic leukemias caused by chromosomal (t6;11) translocation events; hence, the name *A*LL-1 *f*used gene on chromosome *6*. Database analysis led to the prediction of an array of motifs, such as two amino-terminal Ras-binding domains (RBD), U104 and DIL motifs, that were initially found in microtubule and actin-based motor proteins, respectively, and a PDZ domain followed by an extended carboxy-terminal tail interspersed with proline-enriched patches. As mentioned previously, various subcellular localization experiments performed in polarized epithelial cells and tissue sections of intestinal epithelia suggest its distinct residency in cell-cell junctional complexes. We observed that in MDCK and MCF7 cells, AF-6 colocalizes with zona occludens (ZO-1), an integral component of tight junctional complexes. Furthermore, we isolated profilin as an AF-6-associated protein. Profilin has a critical role in actin polymerization events, and hence the AF-6/profilin interaction might provide another more dynamic link between junctional complexes and the actin cytoskeleton. Given AF-6 localization at sites of cell/cell contacts, we investigated whether oncogenic Ras could perturb AF-6 localization. We observed that the introduction of oncogenic Ras into MDCK cells causes a dramatic alteration in their morphology: The typical epithelial cell shape is lost and is transformed into a fibroblastic one. In these cells, AF-6 does not accumulate at the membrane anymore and ZO-1 also disperses into the cytoplasm. A central question in this context, which we are currently addressing, is whether activation of Ras dissociates AF-6 directly from junctional complexes or if this may be a secondary effect provoked by events downstream from other signaling pathways.

In addition to its ability to bind Ras, we recently found that AF-6 also interacts with the Ras-related GTPase Rap1, both in vitro and in vivo. The first Rap GTPase was originally identified in a screen for revertants of the morphology exhibited by Ki-Ras-transformed cells. However, recent studies underline fundamental differences between Ras and Rap signaling pathways. Although Rap1 has been implicated in *Drosophila* morphogenesis, its function in mammalian cells remains unclear. Rap1A does exhibit some effect on cell adhesion, though, as yet shown only in integrin-based adhesion complexes that link cells to the extracellular matrix. We are presently investigating the role of Rap1 and AF-6 in cell/cell adhesion. In addition, to examine the Ras/Rap1-AF-6 interactions in a more comprehensive system, we turned to *Drosophila* as a model system. A *Drosophila* homolog of AF-6 known as Canoe was first identified by virtue of its severe rough eye phenotype, and it has genetically been placed in the Notch signaling pathway, a pathway that determines various cell fates in a multitude of developmental processes. Furthermore, Canoe is involved in a process called dorsal closure, the dorsalward movement of the lateral and ventral epidermis to enclose the embryo. We initiated studies to assess genetic interactions among DrRas, DrRap1, and Canoe. The latter is being done in collaboration with Ulrike Gaul (Rockefeller University, New York).

FUNCTIONAL CHARACTERIZATION OF P62^{DOK}

p62^{dok} is an adapter protein that is found to be highly phosphorylated in bone marrow samples of CML patients and cell lines expressing p210$^{bcr\text{-}abl}$, and it was isolated by virtue of its ability to bind Ras-GAP. It contains a PH and a PTB domain at its amino terminus, and it harbors 15 tyrosines, 9 proline-rich domains, and numerous serine/threonine residues at its carboxy-terminal tail. In addition to bcr-abl, p62^{dok} has been demonstrated to be a substrate of numerous cytoplasmic and receptor tyrosine kinases, including the platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR), and src family members. We observed that p62^{dok} becomes phosphorylated and recruited to the membrane after PDGF stimulation or introduction of bcr-abl and that this event requires an intact PH domain.

To further address the role of p62^{dok}, we initiated a collaboration with P.P. Pandolfi and colleagues (Memorial-Sloan Kettering Cancer Center, New York), who generated a null mutation in the *dok* gene. *dok*–/– mice did not reveal gross aberrant phenotypes. However, in vitro analysis of p62^{dok}–/– primary cells from different origins showed growth alterations in response to several cytokines and growth factors. For example, p62^{dok}–/– primary bone marrow cells and mouse embryonic fibroblasts show a higher proliferation rate compared to wild-type cells when treated with interleukin-3 (IL-3) and PDGF, respectively, suggesting that p62^{dok} is a negative regulator of cytokine or growth factor signaling. Since p62^{dok} associates

with Ras-GAP upon PDGF treatment, we assessed for altered levels of Ras and MAPK activation in $p62^{dok}$–/– cells in response to growth factors. We observed prolonged Ras and MAPK activation after removal of PDGF in $p62^{dok}$–/– MEF cells when compared to wild-type cells, suggesting that $p62^{dok}$ is a negative regulator of the Ras/MAPK pathway. Further experiments assessing the involvement of $p62^{dok}$ in CML are ongoing.

RHO SIGNALING PATHWAYS

Most of the identified Rho GTPases' effector molecules are proteins mediating their effects on actin cytoskeleton organization. Among them, we identified POR1 as a Rac1-interacting protein. Further characterization of these effector proteins is likely to shed light on the mechanisms by which the Rho GTPases exert their effects on processes such as adhesion and neuronal development. Additional roles for the Rho GTPases, including the regulation of gene expression, invasion, and proliferation have recently been revealed. Although large efforts have been taken, including two-hybrid and biochemical approaches, the target molecules mediating the effects of Rho GTPases on cell growth control still remain elusive. Hence, as alternative approach, we used representational difference analysis (cDNA-RDA) in combination with a micro-array technique to identify genes whose expression is altered as a consequence of activated Rac expression.

POR1, A RAC-INTERACTING PROTEIN INVOLVED IN ACTIN REMODELING

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

ROLE OF RHO AND THEIR REGULATORS IN NEURONAL DEVELOPMENT

The involvement of the Rho GTPases in neuronal development has become more apparent during the past year. There is compelling evidence that perturbing the activity of the Rho GTPases results in alterations in the formation of neuronal processes. In collaboration with Z. Li and H. Cline here at the Laboratory, we investigated whether Rho GTPases regulate branch dynamics and branch extensions in optic tectal neurons in live *Xenopus* tadpoles. These studies indicated that the three members of the Rho GTPases have distinct effects on dendritic arbor development; namely, Rac and Cdc42 regulate branch additions and retractions, whereas RhoA regulates the elongation of existing branches. The importance of the fine-tuned regulation of the neuronal dendritic arbor is emphasized by the reports suggesting that children with mental retardation have reduced dendritic arbors. We have been focusing our research on a protein called Oligophrenin-1, which was originally isolated by positional cloning methods to identify genes responsible for MRX (primary or nonspecific X-linked mental retardation). Oligophrenin contains a domain that is typical of Rho-GTPase-activating proteins (Rho-GAP), and this Oligophrenin GAP domain acts as a GAP for Rac, Rho, and Cdc42 in vitro, thus down-regulating the activity of these molecules. Oligophrenin also contains a conserved amino-terminal domain of unknown function, a PH domain, and a unique carboxy-terminal domain. The role that Oligophrenin-1 has in a cellular and in vivo context remains to be determined, in particular, its role in neuronal development and how absence of this GTPase regulatory molecule may lead to mental retardation on a molecular and morphological level. Morphological changes as a result of activation of particular Rho GTPases have been well-characterized for numerous cell lines, including Swiss-3T3 cells and neuronal cells. To determine which GTPase(s) Oligophrenin acts upon in a cell, we made use of these morphological changes as a readout. Preliminary studies hint that it may be spe-

cific for RhoA. To assess in a more natural environment what effect Oligophrenin has on neuronal development and ultimately synaptic transmission, we turned to hippocampal slices. Currently, we are examining the effect(s) of overexpressing a green fluorescent protein (GFP)-tagged Oligophrenin construct in these slices. Furthermore, we are setting up antisense versions to mimic a loss of Oligophrenin gene function and to correlate basic cellular and molecular biology with the disease phenotype of mental retardation.

IDENTIFICATION OF RAC TARGET GENES USING CDNA-RDA

The aforementioned effects of Rac on cell growth, such as proliferation and invasion, are likely to involve changes in gene expression patterns. However, specific genes whose expression is regulated by Rac remain largely unknown. Toward this end, we have used cDNA-RDA, which is a modified form of genomic RDA, a subtractive hybridization technique, which incorporates kinetic enrichment during PCR. More specifically, in this technique, one cDNA population (called the driver) is hybridized in excess against a second population (the tester) to remove common hybridizing sequences, thereby enriching target sequences unique to the tester. We isolated cDNAs from fibroblasts and epithelial cell lines expressing the constitutively activated RacV12 mutant under an inducible promoter. To obtain genes that are either up- or down-regulated as a result of RacV12 expression, we used cDNAs from cells induced for RacV12 as both tester and driver against cDNAs from uninduced cells. The difference products obtained after two rounds of cDNA-RDA were subcloned in a bacterial expression vector for analysis. Approximately 400 colonies from each library were selected at random, and their individual inserts were PCR-amplified. To confirm the differential expression of the isolated clones, we made use of micro-array technology (in collaboration with R. Lucito and M.Wigler at CSHL). The PCR-amplified inserts were printed on a glass slide and then driver and tester cDNAs, each labeled with a different fluorophore, were hybridized simultaneously to this chip. The fluorescence intensities of each of the two labels were quantified for each clone, and as such we were able to discern which clones were up-regulated or down-regulated as a consequence of RacV12 expression. At present, 300 clones out of a total of about 3500 were sequenced, resulting in 85 independent gene fragments, of which 23 are novel. From both cell lines investigated, we found a total of 37 apparently up-regulated and 48 down-regulated candidate genes. The obtained genes could be categorized in different groups: genes encoding nuclear, ribosomal, mitochondrial, membrane-associated, secreted, and cytoskeletal proteins. We are currently performing Northern blots and other tests for further validation.

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STRUCTURE AND GENOMICS

Members of the Structure and Genomics group at Cold Spring Harbor continue to interact with a large number of the Laboratory community. As computational and "wet-lab" methods develop in parallel, more and more they coalesce in a single research area. In fact, this distinction is largely artificial. The members of this group combine in vitro, in vivo, and in silico approaches to provide powerful tools to understand the correlation between structure and function in complex biological processes.

- On the computational front, Andy Neuwald has begun applying his computational approaches to studying protein structure to the study of repeated motifs within a protein. This has allowed him to detect structural repeats in proteins where they had not yet been detected.
- Lincoln Stein's group has developed an AcePerl program to allow the ACEDB database popular among biologists to be linked to a Web interface. They have used this to develop a Web-based version of the *C. elegans* database. The Stein lab has also been chosen as the database developers for the human Single Nucleotide Polymorphism Consortium (SNP) database.
- Michael Zhang's lab has continued their work in predicting gene structure. In the past year, they have made considerable progress on the difficult problem of predicting the 3′ end of genes from genomic sequence and have a working version of software to do this.
- From the more "wet lab" perspective, Ryuji Kobayashi's protein chemistry group has acquired a new mass spectrometer that will greatly increase their ability to sequence small amounts of protein. They have been using mass spectrometry to analyze the role of phosphorylation in the Bcr-Abl pathway.
- The McCombie lab, along with their collaborators, completed one of the two first chromosomes to be sequenced from a higher plant. The region they completed contained a heterochromatic knob, similar to a structure first described in maize by Barbara McClintock nearly 80 years ago. This was the first region of heterochromatin sequenced.
- The lab of Leemor Joshua-Tor has continued work on bleomycin hydrolase. In the past year, they have determined the structure of an active site mutant of this enzyme and can now begin studying that mutant and designing inhibitors of the enzyme.
- Rui-Ming Xu has continued his structural studies of the interaction of protein and DNA. His lab has determined the structure of a fragment of the yeast splicing complex and developed a model for its interaction with other elements of the complex. They have also begun structural studies on components of DNA replication in archaebacteria as a model of eukaryotic DNA replication.

STRUCTURAL BIOLOGY

L. Joshua-Tor A. Auster, E. Enemark, T. Messick, P.R. Kumar, P. O'Farrell, J. Rosenbaum, D. van Aalten, D. Vaughn, H. Zhou, F. St. Pierre (URP)

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

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

Caspase Activation

D. Vaughn [in collaboration with Y. Lazebnik, Cold Spring Harbor Laboratory]

Caspases are cysteine proteases that have a central role in programmed cell death (apoptosis). They are expressed as inactive zymogens or precursors which when activated are part of an irreversible proteolytic cascade. In response to a variety of pro-apoptotic signals, initiator caspases, such as caspase-8 and caspase-9, are activated through an autocatalytic event and then activate effector caspases by proteolysis. Activation of caspase-9 occurs through binding of the regulatory protein Apaf-1 in the presence of cytochrome *c* and dATP. Recently, Joe Rodriguez and Yuri Lazebnik here at Cold Spring Harbor Laboratory showed that the proteolytic activity of caspase-9 is much higher when it is in complex with Apaf-1. Therefore, it appears that Apaf-1 not only is required for the activation of caspase-9, but also acts as a regulatory subunit of the protease. Apaf-1 contains a caspase recruitment domain (CARD) at its amino terminus, which binds to the CARD of caspase-9. The CARD is one of several families of small protein interaction domains identified in proteins involved in programmed cell death and are grouped into three families: CARDs, death effector domains (DEDs), and death domains (DDs).

We determined the crystal structure of the Apaf-1 CARD at 1.3 Å resolution using a two-element multiwavelength anomalous dispersion (MAD) X-ray diffraction experiment. The Apaf-1 CARD adopts a six-helix bundle fold with Greek key topology (Fig. 1). The six helices are arranged like a six-pack of beverage cans, and when viewed from the top, as in the figure, they form three stacked pairs of helices. The helices enclose a tightly packed hydrophobic core and form a globular shape that provides a structural scaffold for potential binding surfaces which includes both hydrophobic and electrostatic patches. This fold, which we call the "death fold," is found in other domains, other CARDs, DEDs, and DDs that mediate interactions in apoptotic signaling despite very low

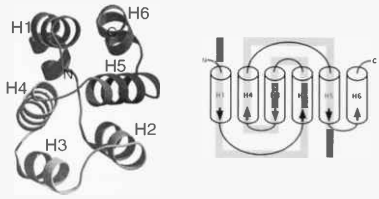

FIGURE 1 Six-helix fold with Greek key topology.

sequence identity. Indeed, the principal interactions occur on different surfaces for the different modules. Even for the interaction between Apaf-1 and caspase-9, the two molecules use different surfaces.

This utilization of the death fold (a Greek key six-helix bundle) for protein-protein recognition in apoptotic signal transduction is analogous to the use of the immunoglobulin (Ig) fold (a Greek key β-barrel) in antigen-protein and cell-cell recognition functions. Both the death fold and the Ig fold are small (~90 amino acid residues) rigid domains whose secondary structural elements surround a hydrophobic core. In each case, there are individual families of domains (CARD, DED, and DD for death fold and V, I, C1, and C2 for the Ig fold) which share additional sequence and structural characteristics that are common to the larger superfamily. Both folds occur as specific modules that are found as components of multidomain proteins with a wide range of functions.

Bleomycin Hydrolases

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

Bleomycin hydrolase (BH) is a 300-kD cysteine protease with unusual structural and biological features. It was discovered due to its ability to deactivate the glycopeptide antibiotic, bleomycin, which is used as a therapeutic agent in the treatment of a number of different forms of cancer. The clinical use of bleomycin is limited due to drug resistance and dose-dependent production of pulmonary fibrosis. The endogenous enzyme, BH, is overexpressed in some tumor cells and is thought to be a major cause of tumor cell resistance to bleomycin therapy. It was shown that in both yeast and mammalian cells, BH is the only enzyme with bleomycin deamidation activity. Interest in its clinical relevance was heightened by a recent report of the genetic linkage of an allelic variant to the nonfamilial form of Alzheimer's disease. BH was also found to bind amyloid precursor protein (APP).

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

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

In the last year, we designed and solved the structure of an active-site mutant of the enzyme that would enable us to study the complex between the enzyme and the anticancer drug bleomycin. In contrast to previously designed mutants, the active site remains open for binding to the drug. In collaboration with Dan Rich's laboratory at the University of Wisconsin, Madison, we embarked on an iterative study to design specific, tight-binding active site inhibitors of BH activity to increase the efficacy of bleomycin, and perhaps reduce dosage levels in cancer treatment. The emerging link between hBH and Alzheimer's disease is another incentive, since an inhibitor to hBH may be useful in understanding this connection and may also be useful in therapy. We are using our crystal structure to create novel inhibitors by use of computerized structure-generating programs. Using this approach, we can create both peptide-like and nonpeptide inhibitors by a combinatorial process. We identified four compounds in our initial screens that are good inhibitors, which we are currently using for structural studies.

The DNA-binding Domain of the Papillomavirus Initiator Protein E1

E. Enemark, G. Chen, A. Auster [in collabroation with A. Stenlund, Cold Spring Harbor Laboratory]

Papillomaviruses are a large family of closely related viruses that give rise to warts in their hosts. Infection of the genital tract by the human papillomaviruses (HPVs) from this group represents one of the few firmly established links between viral infection and the development of cervical cancer, as HPV DNA is found in practically all cervical carcinomas. Although the progression to malignancy represents a low-frequency event, due to the high frequency of infection, this disease affects a large number of individuals. Bovine papillomavirus (BPV) has served as a prototype for this group especially regarding viral DNA replication. The E1 protein belongs to a family of multifunctional viral proteins whose main function is related to viral DNA replication. These proteins bind to the origin of DNA replication and also have other activities related to DNA replication, including a DNA distortion and a DNA helicase activity. Furthermore, these proteins interact with cellular replication proteins such as DNA polymerase α and RPA. Thus, this group of proteins is intimately involved with initiation of DNA replication. In collaboration with Arne Stenlund's group, we embarked on structural studies to provide high-resolution structural information about E1 and its DNA-binding activity, which would provide general insight into the biochemical events that are involved in viral DNA replication. They can also provide a basis for the development of clinical intervention strategies. Second, the viral DNA replication machinery itself represents an obvious target for antiviral therapy, and detailed information such as high-resolution structures of viral proteins required for replication will greatly facilitate the development and testing of antiviral agents.

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PROTEIN CHEMISTRY

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A. Lin D. Wurtz
S. Lopez

The year 1999 was one of major transition to my lab. Kimbery Wanat left in January to join a pharmaceutical company. Nobuhiro Kashige returned to Fukuoka University, Japan, in March, and Nora Poppitto moved to CSHL Press in August. David Sargent joined the lab in March, Stacy Lopez in August, and Andrew Lin and Denise Wurtz in October, and Dr. Daniel Bogenhagen started his sabbatical 6-month visit in December. In the summer, Cold Spring Harbor Laboratory purchased an electrospray ionization ion-trap mass spectrometer with funds from the Howard Hughes Medical Institute. This new mass spectrometer is able to sequence protein with higher sensitivity than the traditional Edman sequencer if protein is in the database and it was set up for the post-genome era.

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

The t(9,22) chromosomal translocation is found in almost all patients with chromic myelogenous leukemia (CML). The resultant *bcr-abl* fusion gene expresses a chimeric fusion protein p210$^{bcr\text{-}abl}$ with

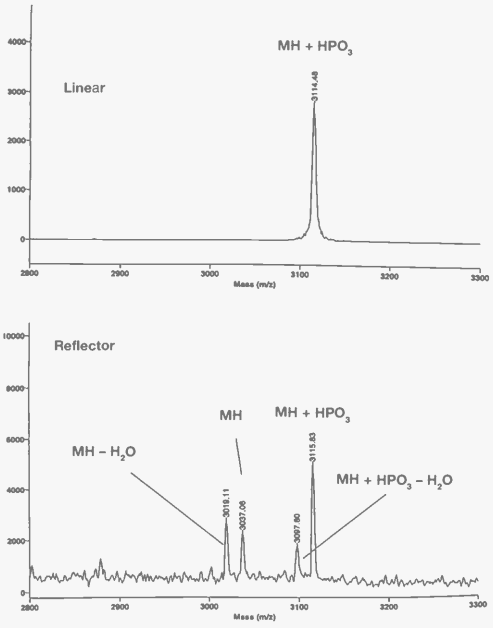

FIGURE 1 In vivo tyrosine phosphorylation site analysis of p62^{dok} by p210$^{bcr\text{-}abl}$ using MALDI TOF mass spectrometry. p62^{dok} purified from cell lysates expressing p210$^{bcr\text{-}abl}$ was digested by lysylendopeptidase and separated by high-performance liquid chromatography (HPLC). One HPLC peptide fraction was found to contain a peptide whose mass was 3114.48, which matches a combined mass of the peptide EDPIYDEPEGLAPVPPQGLYDLPREPK [3035.33] and a phosphate [79.98]. The linear mode (*upper panel*) of MALDI TOF mass spectrometry showed only intact phosphopeptide, whereas the reflector mode (*lower panel*) separated metastable ions such as the dephosphorylated form and the dehydrated form of the phosphopeptide, and dehydrated form of the dephosphorylated peptide. This reflection spectra pattern is typical for a tyrosine-phosphorylated peptide. Since the MALDI TOF mass spectrometry analysis only showed the number of phosphate molecules in a peptide and the peptide contained two tyrosines, further analysis by peptide sequencer was used to determine the phosphorylation site.

increased tyrosine kinase activity. Hematopoietic progenitors isolated from CML patients in the chronic phase contain constitutively tyrosine-phosphorylated $p62^{dok}$ protein. We investigated the role of tyrosine phosphorylation of $p62^{dok}$ in CML. $p62^{dok}$ is directly tyrosine-phosphorylated by $p210^{bcr\text{-}abl}$ and is associated with the Ras GTPase-activating protein (RasGAP), but only when $p62^{dok}$ is tyrosine-phosphorylated. By using MALDI-TOF mass spectrometry, the truncation construct, and mutants of $p62^{dok}$, we located the sites of tyrosine phosphorylation in the carboxy-terminal half of the $p62^{dok}$ molecule. We have found that $p62^{dok}$ is heavily phosphorylated by $p210^{bcr\text{-}abl}$ and among them, we identified five tyrosine residues that are involved in in vivo RasGAP binding. We have investigated the consequences of $p62^{dok}$ tyrosine phosphorylation on the activity of RasGAP and have found that tyrosine-phosphorylated $p62^{dok}$ inhibits RasGAP activity. In contrast, we observed no diminution of RasGAP activity when the assay was conducted in the presence of nontyrosine-phosphorylated $p62^{dok}$ or in the presence of a five Tyr→Phe point mutation ($p62^{dok}$) unable to bind RasGAP. Since RasGAP is a negative regulator of Ras, our results suggest that $p210^{bcr\text{-}abl}$ might lead to the activation of the Ras signaling pathway through tyrosine phosphorylation of $p62^{dok}$.

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SEQUENCE-BASED ANALYSIS OF COMPLEX GENOMES

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In 1998, the major changes that we made in our sequencing operation led to a large increase in our sequencing capability, and these changes continued in 1999, resulting in another year of major increases in sequencing throughput. Our application of this capacity to several ongoing projects has continued, and we have begun two major new genomics initiatives.

In 1999, we nearly doubled the output of finished sequence submitted to GenBank when compared to 1998. This was in addition to redirecting a substantial part of our sequencing capacity toward the so-called "rough draft" sequence of the human genome. We have continued our *Arabidopsis* and cancer genetics sequencing projects and have initiated new programs in both rice and mouse genome sequencing.

ARABIDOPSIS GENOME SEQUENCING

This work was done in collaboration with Rob Martienssen (CSHL), Richard K. Wilson and Marco Marra (Washington University), European Scientists Sequencing *Arabidopsis* (ESSA), and Daphne Pruess (University of Chicago). In 1999, we reached a major milestone in our efforts to sequence the *Arabidopsis* genome. We and our collaborators have completed the sequence of *Arabidopsis* chromosome IV, which was published in December along with the accompanying paper describing the sequencing of chromosome II (The *Arabidopsis* Consortium, *Nature 402:* 769 [1999]). These represent the first ever plant chromosome sequences to be resolved. The region sequenced by the CSHL/WU group contained much of the centromeric regions of chromosome IV and the complete region of heterochromatin found on the short arm of chromosome IV.

The *Arabidopsis* Consortium (1999) paper described a number of interesting findings in the chromosome IV sequence. The sequence of chromosome IV is composed of 17,380,000 base pairs, and analysis of this region predicted the presence of 3744 protein-coding genes. Interestingly, only about 35% of these predicted genes are present in the large, public expressed sequence tag (EST) database. This is due to huge representation of some common genes in the public database. For instance, 6% of the predicted genes from chromosome IV accounted for 75% of the ESTs that matched chromosome IV (The *Arabidopsis* Consortium 1999).

More interesting than the discrepancy between ESTs and the genomic sequence is the comparison of the chromosome IV sequence with the rest of the *Arabidopsis* genome. Surprisingly, this comparison showed that large regions of chromosome IV are duplicated on other parts of chromosome IV or in others parts of the *Arabidopsis* genome (e.g., chromosomes II and V). These results indicated that a minimum of 15% of the *Arabidopsis* genome may be present as large duplications in the genome (The *Arabidopsis* Consortium 1999).

The centromere of *Arabidopsis* chromosome IV was more complex than we expected. Although we were not able to sequence the entire centromeric region, we were able to sequence substantial contiguous regions of the centromere. These results showed a relatively complex structure that has genes containing unique sequence present between regions of highly repetitive DNA (The *Arabidopsis* Consortium 1999; Copenhaver et al. 1999). This sequence will provide the tools (probes, information for computational analysis) to understand further the structure and function of the centromeres of high eukaryotes.

In addition to the centromeric region, Paul Fransz (Fransz et al. 2000) observed a region of condensed chromatin on the short arm of chromosome IV, which was being sequenced by our group (CSHL/WUGSC/PEB *Arabidopsis* Sequencing Consortium 2000). This region appeared to resemble the heterochromatic knobs described previously in maize by McClintock (*Science 69:* 629 [1929]). This region of heterochromatin was completely sequenced and analyzed. It shows an incredible density of repeat DNA including multiple copies of

a large tandomly arrayed sequence. The availability of this sequencing will be invaluable in trying to understand the nature of chromosome condensation and its associated properties.

Following completion of chromosome IV, we will finish working on our region of chromosome V including the chromosome V centromere. We are scheduled to complete the majority of this sequencing by July 2000. We estimate that all of the euchromatic region of the *Arabidopsis* genome will be completed by the end of 2000.

RICE GENOME SEQUENCING

This work was done in collaboration with Rob Martienssen (CSHL), Rod Wing (Clemson University), and Elaine Mardis and Richard K. Wilson (Washington University). The monocot plant rice is one of the most important crop plants in the world. In addition, it has the most compact genome among the cereals (corn, wheat, barley, etc.). Hence, coupled with the compact dicot genome of *Arabidopsis*, rice represents one of the most tractable targets for plant genome sequencing. The rice genome has recently been targeted for an international sequencing effort. Our group, in collaboration with Clemson University, was funded to begin sequencing chromosome 10 of rice in late 1999.

Since beginning this project in October 1999, we have already started sequencing a large number of BACs. We currently have about 2.5 Mb, one half of our scheduled sequencing for the period from October 1999 to September 2000, in progress.

CANCER GENETICS

This work was done in collaboration with Mike Wigler, Masaki Hamaguchi, and Robert Lucito here at the Laboratory. We have been collaborating with the Wigler lab to carry out gene discovery by sequencing areas of the genome deleted in breast cancer. The first three areas of focus are chromosomes 4, 8, and 20. We have completed sequence skimming of a 120-kb region on chromosome 8, and a more complete sequence of another 120 kb from this region is nearing completion. We have completed sequencing a 269-kb region on chromosome 20 and have significant sequence data from an additional 206-kb-region from chromosome 20 and are completing this sequence. We have completed a partial sequence of about 360 kb from chromosome 4. Another roughly 800 kb from different areas of the genome are being sequenced as part of this project. We have also begun pilot studies to prepare for the sequence of probes to be used in microarray studies.

The generation of this much sequence data and its analysis present considerable challenges to our informatics capabilities. To use this data effectively, we have begun developing a number of tools both to track the progress of projects and to analyze the resultant data. We have set an intranet-based project tracking system that allows the status of projects to be monitored by staff in both the Wigler and McCombie labs. This system also allows appropriate personnel in both labs to enter data and change the priority of projects. Status information such as how many sequence reads have been done for a project is automatically retrieved from a relational database and displayed on the web page. We have developed a data analysis pipeline that relies largely on BLAST searches at the present time.

For data analysis, the thousands of reads that are generated are used to search public sequence databases. This is done through a Web-based multiple BLAST server developed for this project (with partial support from the Cancer Center Informatics Facility). After BLAST searches are done, the sequences and the search results are copied to computer directories where they can be accessed by the Wigler lab. The data analysis pipeline will soon have automated searches that are carried out as sequences are generated. The results will be parsed for significant matches to the databases and notifications sent to appropriate personnel to check the results of the searches. In addition, the facility to automatically re-search the databases using old data to look for new matches will be built into the process. This is quite important given the rapid pace of DNA sequence generation worldwide. Importantly, many of these same tools will be usable by members of the Cancer Center as a whole and not just those involved in this specific project.

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SEQUENCE-BASED PREDICTION OF PROTEIN STRUCTURE AND FUNCTION

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T. Carlson
K. Christie (URP)

Our goal is to derive important clues to the cellular roles of various proteins by examining conserved patterns present in related sequences. This can be compared to deciphering the meaning of an unknown language, where the genome projects perform the role of the archeologist by unearthing volumes of books written in that language. Experimental laboratories serve as the Rosetta stone by providing insight into the language's meaning through direct observation. We perform the role of the linguist who attempts to better understand the language by combining translated material with observations about the patterns of letters in the untranslated material; i.e., in the light of the available experimental evidence, we search for conserved patterns in protein sequences corresponding to similarities in structure and function. This year, a major focus has been the characterization of sequence motifs corresponding to repetitive structural features. On a larger scale, we have also studied the types of domains shared by functionally related proteins.

DETECTION, ALIGNMENT, AND MODELING OF REPETITIVE PROTEIN STRUCTURES

The repetitive structures we have been studying include bihelical repeats, β-stranded repeats, and more complex repeats such as are found, for example, in DNA polymerase sliding clamp proteins. Bihelical repeats can be described as molecular curlicues that function as flexible joints, which wrap around and bind to target substrates, and as scaffolding, on which other molecular structures may assemble. Thus, these repeats appear to function in the recognition of target sequences and in the assembly of cytoskeleton-like structures. This class includes HEAT, Armadillo, tetratricopeptide, and ankyrin repeats. Examples of β-stranded repeats include β-propeller and β-barrel repeats, which form repetitive structures resembling the blades of a propeller and the staves of a barrel, respectively.

We have been studying repetitive motifs for several reasons. First, many repeat-containing proteins are biologically and medically important, and we aim to make some key predictions regarding their functions. Several HEAT repeat proteins described below, for example, are associated with cohesins and condensins—complexes that perform key roles in sister chromosome cohesion and chromosome condensation during cell division. HEAT repeats also occur in the Huntingtin protein, which is associated with a progressive neurodegenerative disorder called Huntington's disease.

Another motivation for this research is that even though repetitive structures are quite common, the corresponding sequence repeats are typically very subtle, and as a result, the repeats are often only discovered after experimental determination of a protein's three-dimensional structure. It would be helpful to be able to predict these repeats based on a protein's sequence alone, but there has been a lack of adequate tools for this purpose because repeats add an additional layer of algorithmic and statistical complexity to fundamental sequence analysis problems. Through the development and application of several new approaches, however, we can now predict the presence of very subtle repeats in many proteins.

This was achieved by attacking the problem on three fronts using a combination of computational, statistical, and biological analyses. First, the use and continued development of C'' libraries containing a large repertoire of basic sequence analysis routines have provided us with the necessary computational tools. Second, improved statistical models were developed and, within various programs, are now automatically fitted to the characteristics of a particular repeat family. Insertions and deletions within sequences, for example, can now be detected more readily by using information about the secondary structure, sequence conservation, and hydrophobicity associated with a particular class of repeats. Similarly, repeat alignment procedures now make use of clusters of closely related sequences to better locate insertions and deletions. Finally, rather than developing these methods based on theory alone, each of these computational and statisti-

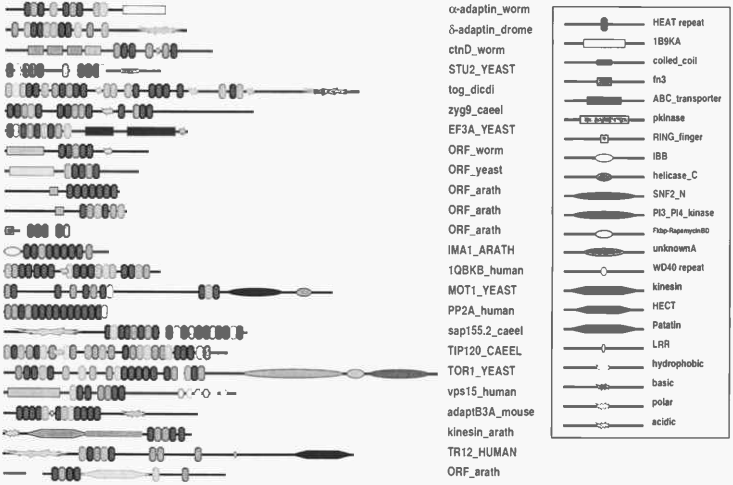

FIGURE 1 Domains present in representative HEAT repeat proteins.

cal improvements stems from extensive testing on actual repeat families, such as the HEAT repeat proteins.

ANALYSIS OF HEAT REPEATS

HEAT repeats correspond to tandemly arranged structural elements consisting of two conserved α helices connected by a loop region. The name "HEAT" repeat is an acronym derived from four characterized proteins with these repeats, namely the *H*untingtin protein, *e*longation factor 3, the *A* subunit of PP2A phosphatase, and *T*OR kinases. In searches of the protein database for HEAT repeats, we detected matches in diverse proteins, many of which had not been previously reported to contain these repeats. These include several families of condensin- and cohesin-associated proteins.

In all, we found four families of condensin- and cohesin-associated proteins with HEAT repeats. These include members of two proteins families corresponding to the XCAP-D2 and XCAP-G subunits of the *Xenopus laevis* 13S condensin complex, which Dr. Hirano here at the Laboratory has been studying for several years now. Because we also detected the HEAT repeat in vesicle coat proteins that assemble into scaffold structures, an intriguing possibility is that these XCAP-G, XCAP-D2 condensin subunits perform a role in nuclear scaffold assembly. HEAT repeats also occur in another family of proteins that possibly interact with the cohesin complex; this family includes *Sordaria macrospora* Spo76p, which is required for sister chromatid cohesiveness and chromatin condensation in meiosis. A fourth family includes the budding yeast Scc2p, which is responsible for sister chromatid cohesion, and the *Drosophila* Nipped-B protein, which participates in transcriptional activation by remote enhancers. In the same analysis, we discovered HEAT repeats in other chromosome-associated proteins, including the TBP-associated protein TIP120, which globally enhances transcription by all three RNA polymerases, and the budding yeast Mot1p. Mot1p is a member of the SWI2/SNF2 family, which consists of helicases that perform diverse roles in transcription control, DNA repair, and chromosome segregation and that often form ATP-dependent chromatin remodeling complexes.

ANALYSIS OF DOMAINS PRESENT IN FUNCTIONALLY RELATED PROTEINS

We have also examined protein function on a grander scale by looking at the various domains associated with groups of functionally related proteins. Figure 1, for example, shows a number of domains associated with HEAT repeat proteins. This sort of figure can be generated automatically using our own and the publicly available protein domain databases, along with various programs developed in our group. As a result, we can now very quickly look for domain associations within a particular protein family and between various families. There are numerous applications for these tools. One application, which stems from a collaboration with Dr. Spector's laboratory, is to characterize proteins associated with internal nuclear domains called interchromatin granule clusters (IGCs). By analyzing IGC components identified by Dr. Spector's laboratory in this way, we aim to better understand the functions associated with these nuclear domains.

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STRUCTURAL BIOLOGY

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During 1999, our focus has continued to be on structural studies aimed at understanding the molecular mechanisms of pre-mRNA splicing and initiation of DNA replication. In collaboration with Adrian Krainer here at the Laboratory, we are continuing our efforts of crystallizing and solving the crystal structure of various complexes of heterogeneous nuclear ribonucleoprotein (hnRNP) A1 and SR proteins. We completed the crystal structure of the functional domain of Prp18p, a yeast protein involved in the second step of splicing, in collaboration with David Horowitz at Uniformed Services University of the Health Sciences. We are investigating the structure and function of the origin recognition complex (ORC) and minichromosome maintenance complex (MCM) proteins in collaboration with the Stillman lab here at the Laboratory. Two areas of research are highlighted below.

CRYSTAL STRUCTURE OF THE FUNCTIONAL DOMAIN OF THE SPLICING FACTOR PRP18P

Splicing of mRNA precursors takes place in two sequential transesterification reactions. The splicing reactions occur within a large RNP particle called the spliceosome, which assembles in an ordered pathway on the pre-mRNA. Once the spliceosome is assembled, the two splicing reactions appear to require only the U2, U5, and U6 small nuclear RNP particles (snRNPs), as well as a large number of proteins. In the second reaction, nucleophilic attack by the 3′-hydroxyl group of the first exon on the phosphate at the 3′ splice site in the lariat intermediate yields the spliced mRNA and the lariat intron.

In *Saccharomyces cerevisiae*, Prp18p is involved only in the second step of splicing and is associated with the U5 snRNP. We have isolated and determined the crystal structure of a large fragment of the *S. cerevisiae* Prp18p that lacks the amino-terminal 79 amino acids. This fragment, called Prp18Δ79, is fully active in yeast splicing in vitro and includes the sequences of Prp18p that have been conserved evolutionarily. The core structure of Prp18Δ79 is compact and globular, consisting of five α helices, which adopt a novel fold that we have designated the five-helix X-bundle. The structure suggests that the interaction between Prp18p and Slu7p occur on one face of Prp18p, whereas the more evolutionarily conserved amino acids in Prp18p form the opposite face. The most highly conserved region of Prp18p, a nearly invariant stretch of 19 amino acids, forms part of a loop between two α helices and may interact with the U5 snRNP. The structure is consistent with a model in which Prp18p forms a bridge between Slu7p and the U5 snRNP.

PREREPLICATIVE COMPLEX IN EUKARYOTIC DNA REPLICATION

Eukaryotic initiation of DNA replication requires the assembly of a multicomponent prereplicative complex (pre-RC) around the origin of replication. The pre-RC is composed of the ORC, the MCM complex, and the CDC6 and CDC45 proteins. Structural information of any of the pre-RC components will provide important insights into the mechanism of pre-RC assembly and function.

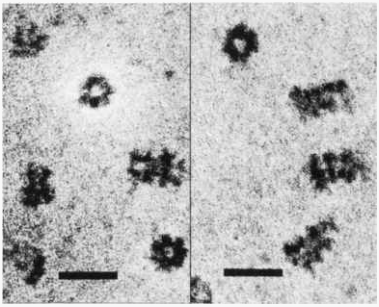

FIGURE 1 Scanning transmission electron microscopy (STEM) showing that mtMCM forms a double hexamer. Bars, 200 Å.

Complete genome sequencing of a number of archaea has revealed that the initiation of DNA replication in archaea may resemble that in eukaryotes. ORC/CDC6 and MCM-like open reading frames (ORFs) have been identified in archaea. It is likely that archaea can serve as a simplified system for studying eukaryotic DNA replication. In collaboration with James Chong in Bruce Stillman's lab, we have cloned several MCM-like genes and have overexpressed and purified the proteins for structural and functional studies. The thermophilic methanogen, *Methanobacterium theroautotrophicum*, has a single MCM-like gene (MtMCM), and we have shown that purified protein forms a complex that is consistent with it being a double hexamer (Fig. 1). The protein binds to DNA in an ATP-independent manner, and it has a DNA-stimulated ATPase activity and a helicase activity. We are currently optimizing crystallization conditions for obtaining diffraction quality crystals.

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COMMUNITY ACCESS TO GENOME RESOURCES

D. Stein

During the past year, my lab has developed two software tools that are key to the visualization and annotation of the *Caenorhabditis elegans* genome. AcePerl provides a programmatic interface to remote and local ACEDB databases in the popular Perl programming language. AceBrowser is layered on top of AcePerl to provide Web access to the *C. elegans* data. These software tools were released in October of 1998 and are currently in use at dozens of laboratories around the world. In addition, the tools have proven to be of use in nonbiological domains as well. For example, the tools are in use in the University of Pennsylvania's library catalog system and at Lawrence Berkeley National Laboratory to map and manage network topologies. This general usefulness won the recognition of the Long Island Software Award organization, which awarded the software the top prize in the Large Business category. AcePerl can be found at:

http://stein.cshl.org/AcePerl

AcePerl is now being used as the basis on which to build a distributed sequence annotation system, which will allow laboratories around the world to add sequence annotation information to the reference *C. elegans* sequence. This system will work hand-in-glove with the formal curatorial system installed at Sanger Centre and Washington University. A prototype of this system is available at:

http://stein.cshl.org/wormbase

In the past year, we received a contract from The SNP Consortium, Ltd., to serve as the bioinformatics component of a large effort to identify 300,000 new human single-base-pair polymorphisms (SNPs). We have designed information workflow systems, analysis tools, and a database to display and manipulate the data. Currently, we have mapped and released more than 7000 SNPs from a total of approximately 40,000. The first data release occurred on November 11, 1999, and a subsequent release occurred on December 23. Regular releases will continue on a bimonthly basis until the entire set of 300,000 SNPs has been placed in the public domain.

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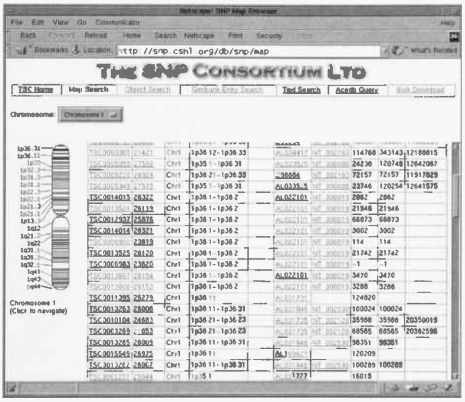

FIGURE 1 More than 7000 mapped human single nucleotide polymorphisms are available at Dr. Stein's Web site.

COMPUTATIONAL GENOMICS

M.Q. Zhang J. Tabaska I. Ioshikhes J. Zhu
R.V. Davuluri T. Zhang A.C. de Alcantara

Our research interest continues to be identification and characterization of the genetic elements in nucleic acid sequences by computational means. As the Human Genome Project entered its large-scale sequencing phase, developing efficient computational methods for identification of genes and their control/regulatory elements has become extremely important. Knowing the organization of a gene often becomes the prerequisite for further functional studies. In the past, we studied statistical characteristics of exons and introns in protein-coding regions and developed coding-exon prediction programs by applying multivariate statistical pattern recognition techniques. We then began looking into more difficult problems of finding regulatory *cis* elements in noncoding regions. This year, we continue to focus on splicing enhancers, promoters, and transcriptional start site and polyadenylation site regions in order to develop new computational methods for identification of these functional elements. To facilitate large-scale gene expression data mining, we have also developed several computational tools for clustering and promoter analysis.

Dr. Tabaska has joined Monsanto as a software engineer, Dr. Ioshikhes went on to an instructor's position at Albert Einstein College of Medicine, and Dr. Zhu started a new staff investigator position at Koffmann La Roche.

Large-scale Human Promoter Mapping Using CpG Islands

I. Ioshikhes, M.Q. Zhang

Approximately 50% of mammalian gene promoters is known to be associated with CpG islands. We have devised features that can discriminate the promoter-associated CpG islands from the nonassociated islands, which led to an effective algorithm for large-scale promoter mapping (with 2-kb resolution). Statistical tests showed that approximately 85% of the CpG islands within an interval from −500 to +1500 around the TSS (transcriptional start site) were correctly identified and that about 93% of the CpG-island-containing promoters were correctly mapped.

3′-Terminal Exon Recognition

J. Tabaska, M.Q. Zhang

Reliable detection of gene termini is critical to the success of automated gene discovery in genomic sequences. To resolve a string of exons into individual genes, one needs to know the location of the 5′- and 3′-terminal exons. Terminal exon detection has remained a weak point for many gene prediction methods; these programs are frequently criticized for their propensity to skip terminal exons, thereby fusing several genes together. To help fill this gap in gene prediction technology, we have developed a 3′-terminal exon recognition module for the MZEF exon finding program.

The major obstacle to detecting 3′ terminal exons is the signal-to-noise ratio: only about 10% of coding exons are 3′ termini, and since the consensus splice donor sequence (G|GTRAGT) contains a stop codon, which is in the best coding frame one third of the time, roughly this proportion of internal exons will look like 3′-terminal exons. To help overcome this problem, our terminal exon finder uses a two-pass approach. First, the program scans input sequences for splice acceptor sites. Putative splice acceptors are evaluated using a quadratic discriminant function (QDF) of eight variables. In addition, for each acceptor site, the best corresponding stop codon is determined using a novel Bayesian scoring function that allows coding fragments of widely disparate lengths to be evaluated and compared. During this scan, no attempt is made to differentiate between internal and terminal exon acceptor sites; all acceptor stop codon pairs are simply considered to be terminal exon candidates. An intermediate filtering step performed after the acceptor site scan then reduces the candidate list by removing overlapping exons. A second pass through the sequence is then performed to find splice donors and polyadenylation signals. This scan makes use of a QDF of six variables for the donor sites and a QDF of three variables for poly(A) signals. When a donor site isfound, it is matched up with an acceptor site to form internal exons; these candidate exons are then removed from

consideration. When a poly(A) signal is detected, it is matched up with an acceptor site and evaluated using a QDF of four variables. High-scoring terminal exons are then reported to the user.

In a test using 42 genomic sequences containing 97 known genes, our terminal exon finder correctly identified 70 3′ termini (72.2% sensitivity) with 54 false positives (56.5% specificity). Including cases where the program predicted a 3′-terminal exon that overlapped a true terminus, our program achieved 86.6% sensitivity with 60.9% specificity. On the same test set, GENSCAN, which is currently regarded as the best human gene finder, finds 59.8% of the 3′-terminal exons with 61.7% specificity. Our exon finder thus has a higher sensitivity level with comparable specificity. Significantly, though, in cases where there are two or more adjacent genes on the same strand, GENSCAN fuses the genes together more than three fourths of the time, i.e., it has a sensitivity of less than 25% in such cases. In contrast, our exon finder's sensitivity remains approximately the same (75.9%) in these cases as in the overall test. We are therefore confident that our terminal exon finder will prove to become a valuable addition to the gene-finding repertoire.

Computational Tools for Large-scale Gene Expression Analysis

J. Zhu, M.Q. Zhang

Coregulated gene clusters could be derived based on common expression profiles, some function or pathway information, or sharing promoter regulatory elements. To obtain a thorough understanding of large-scale gene expression and global regulation networks, these three aspects should be combined in an organic manner. In the process of studying various microarray gene expression data, we explored possible ways of analyzing large-scale gene expression data. Three approaches were used to analyze yeast temporal expression data: (1) start from clustering on the expression profiles, followed by function categorization and promoter analysis; (2) start from function categorization, followed by clustering on expression profiles and promoter analysis; and (3) start from clustering on the promoter region, followed by clustering on expression profiles. For clustering analysis on the time-series data, we developed a largest-first algorithm, which can provide a mechanism for quality control on clusters. For promoter analysis, we developed a core-extension algorithm which can systematically detect a motif core and then try to extend it into the flanking regions by χ^2 tests. We have also implemented our own version of Kohonen self-organized map (SOM) clustering algorithm and coupled its result with a χ^2-based regulatory motif-finding algorithm that can detect significant *cis* elements in the promoter region of each cluster of coregulated genes.

Toward Detection of 5′ and 3′ Ends of Protein Coding Genes in the Genome

R. Davuluri, M.Q. Zhang

We continue the process of developing a complete gene prediction system with a current focus on detecting the ends. As one of the steps toward this goal, a 3′-terminal exon recognition module has recently been added to MZEF (internal exon finding program). Our next step in achieving this task is the development of a 5′-terminal exon detection module. Toward this end, we have prepared a database of human 5′-untranslated regions (UTR) of mRNA which are complete from the transcription start site to the translation start site. We are characterizing the important features of these 5′UTRs by data-mining techniques.

Development of a 5′-terminal exon finder involves some special challenges, especially with regard to the detection of transcription start site. Most of the existing gene-finding programs predict the protein-coding regions and ignore the untranslated regions of mRNA. These programs define the 5′ end of the gene as the translation start site. In fact, to our knowledge, no prediction program is available for finding the 5′UTR of the mRNA. In most of the 5′-terminal exons, there is very little or no coding information left, and hence we are statistically characterizing the 5′UTRs to find important features present in this part of the gene. These regions of the gene are known to contain translational regulatory elements, and the predicted 5′-terminal exon will include this valuable information.

To ensure that we use the best possible data, we have prepared a nonredundant data set of 2300 full-length (transcription start site to translation start site) human 5′UTR sequences. We obtained these sequences from two different sources: (1) Sugano's Laboratory, University of Tokyo, Japan and (2)UTRdb (http://bigarea.area.ba.cnr.it:8000/BioWWW/-UTRdb). Each of the sequences in the database has been checked for their completion at both 5′ and 3′ ends. We have collected a number of examples on regulatory elements in the 5′UTR from published litera-

ture. This information along with computational approaches allowed us to classify these 5′UTRs into two categories (1) genes with low translation efficiency and (2) genes with high translation efficiency. This will help us to find the feature variables for the gene modeler. The database of 5′UTRs is being extended so that each sequence in the database would contain flanking regions of 500 nucleotides at both ends.

We currently have a poly(A) signal finder, called Polyadq, which was developed by Tabaska and Zhang. We improved this program by adding a new version for mouse. We call this new version "Polyadq2." Polyadq2 performed substantially better than Polyadq with a correlation coefficient of 0.601. We are in the process of adopting this new version for human sequences, and it will be made available through the World Wide Web.

Computational Characterization of Liver-specific Promoters

T. Zhang, M.Q. Zhang

Our work focuses on developing computational tools to detect regulatory regions (i.e., promoters) that confer tissue-specific gene expression. Our approach uses liver-specific gene promoters, mainly because of the recent advances in the studies of the liver-specific transcription factors. First, we collect all liver-specific transcription-factor-binding sites that are experimentally verified in literature. Second, we construct PWMs (positional weighted matrices) that summarize the binding sites in our collection. Third, we apply linear regression analysis to combine matches to multiple binding sites into a single model. For various test sets, our models have achieved the state-of-art performance.

We are currently investigating ways to enhance the performance of our models. The most promising direction is to incorporate additional regulatory elements into our models. Such elements could bind to transcription factors other than the four factors already examined in building the existing models, or they could be related to secondary structures. To identify such elements, we have developed new algorithms to search for motifs that are significantly over/underrepresented in the given promoter sequences. In contrast to the previous motif-finding methods, we now allow both mismatches and gaps in our motifs, which reflect more realistically the actual DNA and protein interaction. Another approach to discover putative motifs is by comparing promoters from distantly related species. In general, functional regions are more likely to be conserved over evolution than nonfunctional regions. By carefully selecting the organisms under examination, we can identify conserved regions, which are the putative functional regions.

Identification of Splicing Enhancer Motifs and Splicing Factors

M.Q. Zhang [in collaboration with A. Krainer and A. Mayeda, Cold Spring Harbor Laboratory]

We have continued the work of identifying splicing enhancers by acombination of biochemical and computational means. After charactering SF2/ASF, SRp40, and SRp55 functional enhancers, we also studied the SC35 enhancer motif. We have also purified and characterized the human RNPS1 (a general activator of pre-mRNA splicing) and found that it is conserved in metazoans.

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NEUROSCIENCE

As the twentieth century draws to a close, hindsight clearly reveals that we are in the midst of a genetic revolution. Its historical roots, of course, reach back in the previous century to the seminal insights of Darwin and Mendel. The modern revolution, however, began with discovery of the double helix. From that point on, "molecular biology" has come to encompass all of the natural sciences, including neurobiology. The neurogenetic perspective first emerged with simple invertebrate model systems in the 1960s and exploded into mammals in the 1990s. In the next few years, as the Human Genome Sequence comes on-line, the genetic revolution will sweep full force into human cognition.

Relatively quickly, the DNA sequence will help to identify and categorize various forms of heritable cognitive dysfunction. To develop useful therapies for cognitive dysfunction, however, we will have to gain biological insight to how the DNA sequence gives rise to the emergent properties of brain function. This is the problem of *vertical integration* of gene function to cognition. In no other organ is the problem as complex. The brain is unique, evolutionarily designed to sense its internal and external environment, to perceive causal relations among stimuli, and to change its response adaptively. To accomplish this incredible task, a self-regulating network of 70 thousand genes directs the development of a cellular network of 1 trillion neurons with 70 trillion connections among them. To add to such staggering complexity, the computational activity generated by this neural network continually feeds back on the underlying gene network by regulating the expression of "plasticity" genes, which then alter neural connectivity.

To accomplish a vertical integration, neurogenetic experiments must be pursued at genetic, cellular, systems and behavioral levels of analysis. To this end, CSHL was joined by three new faculty this year: Dmitri Chklovskii, Zach Mainen, and Tony Zador, who focus their research on computational aspects of brain circuitry. In this manner, the cellular functions (and dysfunctions) of neural genes are translated into behavioral responses via dynamic properties of the underlying neural circuits. Next stop, human cognition!

ACTIVITY-DEPENDENT NEURONAL DEVELOPMENT

H.T. Cline C. Aizenman, K. Bronson, I. Cantallops, M. Davenne, L. Foa, K. Haas, A. Javaherian, K. Jensen, Z. Li, E. Ruthazer, W.C. Sin, D.-J. Zou

The structure of the dendritic arbor of neurons is a critical determinant of the neuron's function in terms of the inputs it receives and the integration of the synaptic inputs. For instance, a neuron within the visual system that has a large tangential spread of the dendritic arbor can receive inputs from more visual afferents, leading to a larger receptive field over which information is processed. Similarly, neurons that extend their dendritic arbor into superficial laminae are capable of receiving inputs and processing information from afferents in those laminae. Consequently, the factors that regulate the development and plasticity of the dendritic arbor control both the structure and function of the neuron and likely have an impact on circuit properties.

The molecular mechanisms that underlie the development of the dendritic arbor are not yet clear. In my lab, we use in vivo time-lapse imaging to make direct observations of dendritic arbor development. We combine this with viral gene transfer to introduce genes of interest into optic tectal neurons. Time-lapse images of optic tectal neurons collected at intervals ranging from minutes to days in living *Xenopus* tadpoles show that dendritic branches are very dynamic during dendritic arbor formation. The dynamic processes include addition of new branches, retraction of branches, and selective extension or shortening of existing branches. These events can be observed and quantified by collecting repeated images over several hours. The data suggest that the net growth of dendritic arbor occurs as a result of several distinct events: the emergence of a new branch, the selective maintenance of the new branch, and the extension of the branch length. It is possible that each of these events is individually regulated within the dendrite. One goal of our research is to determine the mechanisms controlling dendritic arbor development.

Neuronal dendritic arbors do not develop in a vacuum. As part of a circuit controlling brain function, they develop in a coordinated fashion with presynaptic axonal inputs, and these pre- and postsynaptic partners also work together during the development and plasticity of synaptic connections. Several projects in the lab examine coordinated development of these multiple aspects of circuit formation.

Control of Dendritic Structure In Vivo during Normal Development and by CaMKII

G.-Y. Wu, D.-J. Zou, 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 both the maturation of retinotectal glutamatergic synapses in *Xenopus* and the morphological maturation of optic tectal neurons using in vivo time-lapse imaging of single neurons over periods up to 5 days. Gang-Yi Wu carried out a thorough quantitative analysis of the morphological development of efferent optic tectal cells. We distinguish the neuronal development into three stages: Stage-1 neurons are newly differentiated; they undergo axonogenesis with little elaboration of the dendritic arbor. Stage-2 neurons are in a phase of rapid dendritic growth. After 2 days, neurons enter stage 3, a period of slower dendritic arbor growth. Short-interval observations indicate that stage-2 neurons have branch additions and retractions twice that of the more mature stage-3 neurons.

Our previous experiments demonstrated that CaMKII expression in tectal neurons correlates with the transition from rapidly growing stage-2 neurons to more stable stage-3 neurons. D.-J. Zou tested whether endogenous CaMKII activity in postsynaptic tectal cells regulates the elaboration of neuronal processes in the *Xenopus* tadpole retinotectal projection by selectively infecting postsynaptic tectal cells with recombinant vaccinia viruses that express CaMKII-specific inhibitory peptides. Using in vivo time-lapse imaging, he found that CaMKII inhibitor autoinhibitory peptide (AIP) expression 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.

Role of Glutamate Receptor Activity in Development of Tectal Cell Dendrites and Retinal Axons In Vivo

I. Rajan, K. Bronson, H.T. Cline

Previous studies by O'Rourke, Cline, and Fraser (*Neuron 12:* 921 [1994]) suggested that blocking retinotectal synaptic activity might increase the elaboration of retinal axon arbors, whereas experiments by Rajan and Cline (*J. Neurosci. 18:* 7836 [1998]) indicate that blocking activity can limit elaboration of the dendritic arbor. I. Rajan investigated the role of *N*-methyl-D-aspartate (NMDA) receptor 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 receptor antagonist, amino phosphono valeric acid (APC). Retinotectal axons or tectal neurons were imaged at 30-minute intervals over 2 hours or twice over 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 receptor activity in postsynaptic neurons promotes the initial development of the dendritic arbor and stabilizes presynaptic axon arbor morphology.

Structural Plasticity of Retinal Axon Arbors

E. Ruthazer, H.T. Cline

The goal of E. Ruthazer's experiments is to analyze the role of coordinated activity in the structural plasticity of retinal axon arbors. To do this, he has developed an experimental system that allows him to compare the structural dynamics of retinal axons in an environment in which their activity is either largely coordinated or largely not coordinated with the activity of the other retinal axons in the target area. The experimental system that allows this comparison is one in which both retinal projections are forced to converge on the same optic tectal neuropil. The activity patterns from the two eyes differ from each other. Initially, the inputs from the two eyes overlap in the tectal neuropil, but they gradually segregate out during a period of 1 week. E. Ruthazer has collected time-lapse images of single retinal axons early in the segregation process, when coactivity levels are relatively low, and later in the segregation process, when coactivity is likely to be higher. Comparison of the dynamics of the structure under these conditions will allow us to test the effects of different degrees of coactivity on axon arbor dynamics.

Imaging Synaptic Activity in the Intact Brain

K. Haas, H.T. Cline [in collaboration with A.R. Kay, University of Iowa]

The fluorescent probe FM1-43 has been used extensively for imaging vesicle recycling; however, high background fluorescent levels have precluded its use in most experiments using brain slices of intact tissues. We have found that a sulfobutylated derivative of β-cyclodextrin has a higher affinity for FM1-43 than the plasma membrane and can therefore act as a carrier to remove FM1-43 nonspecifically bound to the outer leaflet of the plasma membrane. This has enabled us to visualize synaptic vesicle recycling in an isolated brain preparation from *Xenopus*. We also found that FM1-43 labeling can work well in the translucent tadpole brain tissue without the addition of the cyclodextrin.

Candidate Plasticity Genes

I. Cantallops, M. Davenne, L. Foa, K. Haas, K. Jensen, A. Javaherian, K. Bronson, H.T. Cline [in collaboration with P. Worley, Johns Hopkins University, and E. Nedivi, Massachusetts Institute of Technology]

Our experiments have demonstrated the important role of synaptic activity in promoting the development of the brain. One potential effect of synaptic activity is to

induce gene transcription. The activity-induced genes may then promote the further development of the brain. We have begun to investigate several activity-induced genes to test their potential function in brain development.

CPG15 Expression in the Tectal Cell Increases Presynaptic Retinal Axon Arbor Growth In Vivo

I. Cantallops, K. Bronson, H.T. Cline

E. Nedivi, G.-Y. Wu, and H.T. Cline had previously shown that viral expression of CPG15 in optic tectal neurons selectively increases the dendritic arbor elaboration of tectal projection neurons but has no effect on the elaboration of tectal interneurons. We have also reported that CPG15 functions in a non-cell autonomous fashion to promote the growth of neighboring neurons. This suggests that CPG15 may act as a signaling molecule. I. Cantallops tested the effect of viral expression of CPG15 in tectal neurons on the development of presynaptic retinotectal axons. She collected images of single labeled retinal ganglion cell axons over a 2-day period in vivo. CPG15 expression in tectal cells increases the length of retinal axons in comparison with axons imaged in *lacZ* vaccinia-virus-infected controls. Overexpression in tectal cells of a truncated form of the protein lacking the GPI link domain blocks the growth-promoting effect seen with full-length CPG15, indicating that GPI linkage of CPG15 to the membrane is necessary to mediate this effect. These results together with those of K. Haas (below) suggest that CPG15 may coordinate multiple aspects of circuit formation in the visual system.

CPG15 Promotes Maturation of Retinotectal Synapses

K. Haas, H.T. Cline

Based on the studies mentioned above, we postulated that CPG15 affects the synaptic development in the visual system. K. Haas recorded synaptic responses from neurons in animals infected with the CPG15 vaccinia virus to test whether the enlarged dendritic and axonal arbors are accompanied by an increase in the strength of retinotectal synaptic transmission. He found that expression of CPG15 promotes synapse maturation, whereas expression of a truncated form of the protein, lacking the GPI link, deters synaptic maturation. The frequency of spontaneous synaptic currents also increases with CPG15 expression, as we have seen with synaptic maturation in control animals. The strong correlation between the increased maturation of synaptic responses and increased morphological complexity in dendritic and axonal arbors suggests that CPG15 may be a key player in the regulation of both neuronal growth and synaptic function.

Developmental Regulation of *cpg15* Gene and Protein Expression

A. Javaherian, H.T. Cline

To determine how CPG15 expression is regulated in the developing nervous system, A. Javaherian cloned *cpg15* from *Xenopus*. The gene from *Xenopus* is highly homologous to *cpg15* from rats, cats, and humans. The *cpg15* message is highly expressed in retinal ganglion cells, but no other cells in the retina. Within the brain, the *cpg15* message is widely expressed in differentiated neurons. Levels of *cpg15* expression can be decreased by exposure to the NMDA-R antagonist, APV, supporting the idea that transcription is controlled by glutmate receptor activity.

Analysis of Aberrant Axon Trajectories in Homer Expressing Neurons

L. Foa, K. Jensen, H.T. Cline [in collaboration with P. Worley, Johns Hopkins University]

Axonal growth cone pathfinding during early stages of central nervous system development is essential to establish the fundamental connectivity of the brain. The plethora of molecular mechanisms that guide axons to their targets operate to minimize axon pathfinding errors. We have studied axon pathfinding by rostrally projecting optic tectal neurons in albino *Xenopus* tadpoles using time-lapse imaging over periods of several days. We find that axons do make errors in their projection pattern, but the errors are typically corrected over a period of 1 day by retraction of the errant axon. Furthermore, we find that viral expression of an activity-regulated gene, *homer1a*, in tectal neurons significantly increases the numbers of axons with aberrant

projection patterns. Time-lapse imaging demonstrates that the increase in numbers of aberrant projections results from a failure to correct aberrant axon projections. The results suggest that normal activity-regulated expression of Homer in developing tectal neurons may provide a mechanism that maintains axons within their target area and thereby limits the ability of the system to either detect or correct aberrant axons. The results further suggest that there is a period during nervous system development when axon errors can be corrected, after which such plasticity is limited.

Function of the Rho GTPases in Regulating Dendritic Arbor Elaboration and Plasticity

Z. Li, W.C. Sin, K. Bronson, H.T. Cline [in collaboration with L. Van Aelst, Cold Spring Harbor Laboratory]

The Rho family of small GTPases regulates actin cytoskeletal structure. The signal transduction pathways that lead to structural changes in the dendritic arbor likely include components that regulate the cytoskeleton. Z. Li tested whether the GTPases, Rac, Cdc42, and RhoA, regulate dendritic arbor development in the intact animal by collecting time-lapse images over periods of 20 hours of single optic tectal neurons in albino *Xenopus* tadpoles expressing dominant negative (DN) or constitutively active (CA) forms of Rac, Cdc42, or RhoA. Analysis of images collected at 2-hour intervals over 8 hours indicated that enhanced Rac activity selectively increased branch additions and retractions. Cdc42 increased branch dynamics, but to a lesser extent than Rac. Activation of endogenous RhoA by LPA dramatically decreased branch extension without affecting branch additions and retractions, whereas DN RhoA expression increased branch extension. These data support a model in which Rac and RhoA regulate distinct, yet interdependent events in dendritic arbor development: Enhanced Rac activity increases new branch additions to the arbor; a decrease in endogenous RhoA activity promotes the extension of branches within the arbor. Finally, we provide data suggesting that RhoA activity is involved in mediating the effect of the NMDA receptor in promoting normal dendritic arbor development. W.C. Sin is investigating the function of the Rho GTPases in short-term dynamics in optic tectal neurons.

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SIGNAL TRANSDUCTION AND DIFFERENTIATION

G. Enikolopov J. Hemish, S. John, P. Krasnov, T. Michurina, J. Mignone, B. Mish, N. Nakaya, M. Packer, N. Peunova, V. Scheinker, Y. Stasiv, A. Vaahtokari, M. Verzi

Stem and progenitor cells receive specific cues to initiate their differentiation pathways. We are interested in how cells progress along these differentiation pathways and how this is linked to their activity. Our efforts have been focused mostly on nitric oxide (NO), a multifunctional second messenger. This signaling molecule acts in a variety of ways with both short- and long-term consequences. The former have been well characterized in the last decade. The latter, including cessation of proliferation, differentiation, and programmed cell death, are now beginning to be more fully appreciated. We have found strong evidence that NO acts as an essential negative regulator of cell proliferation in several systems. We are now focusing on the molecular mechanisms of this action of NO during tissue differentiation and organism development, using a variety of systems and animal models.

NO and Gene Expression

N. Nakaya, J. Hemish

Much of the action of NO in the organism is mediated by fast-acting signaling cascades, which rapidly subside after the disappearance of the original stimulus (e.g., calcium influx). NO can also stimulate signaling pathways that elicit long-lasting changes in the cell, such as cessation of cell proliferation, apoptosis, or acquisition of differentiated phenotype. This action of NO is presumably due to its ability to affect gene expression. The molecular mechanisms of gene activation by NO are poorly understood, and only a few NO-inducible genes have so far been described. Identifying an extended set of genes whose expression is affected by NO will provide an indication of the direct and indirect targets of NO-related signaling cascades. We have used a cDNA array hybridization approach to obtain NO-induced transcript profiles and to identify genes differentially affected by NO. Our results demonstrate that NO is a potent activator of gene expression in mammalian cells. More than 7% of the tested genes are affected by prolonged exposure to any of three different chemical donors of NO. A subset of genes affected by NO is involved in cell cycle progression. Another partially overlapping subset of genes is dependent on p53 for activation by NO, as revealed by using cells from knockout mice deficient in p53. Yet other subsets depend on guanylate cyclase, protein kinase C, and PI3-kinase activity, as revealed by using selective inhibitors of these enzymes. These data demonstrate the potential of combining cDNA array transcript analysis, RNA from normal and transgenic animals with defined genetic lesions, and specific chemical inhibitors to study the long-term effects of NO action. Our results suggest that NO uses multiple signaling pathways to induce gene activity in mammalian cells

NO and the p53 Pathway

N. Nakaya [in collaboration with S. Lowe, Cold Spring Harbor Laboratory, and Y. Taya]

NO is an efficient inhibitor of cell proliferation. We have studied the mechanisms of the antiproliferative activity of NO and found that it is partially mediated through the p53 signaling pathway.

We have found that NO strongly induces expression of genes that are known targets of the transcription factor activity of p53. Using cells from transgenic mice deficient in p53, we have demonstrated that some of the activated genes depend on p53 for their induction by NO. We have further found that NO strongly increases the cellular level of p53 protein. Finally, we have found that p53-deficient cells are compromised in their ability to stop dividing in response to NO. Together, these data strongly implicate p53 and p53-dependent pathways in NO-induced cell cycle arrest.

Since the activity of p53 often correlates with site-specific modifications of the p53 molecule, we have compared the changes in p53 induced by NO and by other known activators of p53. An extensive overlap between the telltale signatures of different inducers may point to commonalties in the signaling pathways mediating their action. We have studied the temporal pattern of phosphorylation of certain residues of p53 protein and compared this molecular signature of the signaling pathway leading from NO to induction of

p53 with the signatures of other well-characterized inducers of p53: γ-irradiation, UV irradiation, and the chemotherapeutic agent adriamycin. Our results suggest that NO activates specific signaling pathways that are unique, but may share some elements in common with signaling pathways activated by other known inducers of p53 activity.

Xenopus laevis NOS Gene

V. Scheinker, Y. Stasiv, N. Peunova

We have recently cloned NO synthase (NOS) cDNA from *Xenopus laevis* (*XNOS1*). Its primary structure, enzymatic properties, and distribution in the tissues of the tadpole and adult frog suggest that it is an ortholog of the mammalian neuronal isoform of NOS.

We have now demonstrated that at least five different transcripts originate from the *XNOS1* gene. They arise through the use of multiple promoters and alternative splicing, and their expression is tissue- and developmental-stage-specific. For instance, the appearance of some of the transcripts mark the zygotic induction of the genome and of the others—neurulation and organogenesis. These findings suggest that the biological activity of NO is tightly and specifically regulated during development by a complex pattern of alternative transcription initiation and splicing. We are studying the appearance of new *XNOS1* transcripts in the context of the properties of NO as a strong inducer of gene activity and a potent antiproliferative agent.

NO and *Drosophila* Development

Y. Stasiv

We have recently identified numerous alternatively spliced transcripts of the *Drosophila* NOS gene. One of them, *DNOS4*, has an extra exon inserted in the open reading frame which codes for a truncated protein with a unique carboxyl terminus. This protein lacks important domains and does not produce NO; however, it strongly inhibits *DNOS1* activity in cotransfection experiments, suggesting that it may act as a negative-dominant inhibitor of NOS activity. To test the physiological role of *DNOS4*, we have generated transgenic flies where this gene is expressed under the control of an eye-specific GMR promoter. Transgenic flies have a profound eye phenotype, with many extra cells per ommatidium, and a decreased activity of NOS. Furthermore, the *DNOS4* transgene shows genetic interactions with the components of the retinoblastoma pathway, thus supporting the hypothesis that *DNOS4* codes for an endogenous negative regulator of the antiproliferative activity of NOS in *Drosophila*. We have now raised *DNOS4*-specific antibodies and are using them to study *DNOS4* expression during fly development and its relation to other proteins arising from the *DNOS* locus.

NO and Mouse Hematopoiesis

T. Michurina, P. Krasnov

We study the potential of NO to regulate hematopoiesis in bone marrow. We have found that cells of mouse bone marrow express various isoforms of NOS. Moreover, we have found that by regulating the synthesis of NO, we can regulate hematopoietic maturation along both erythroid and myeloid lineages, change the proportion of cells with early progenitor markers in bone marrow, and alter the proportion of stem and early progenitor cells in retransplantation assays. Our data suggest that manipulation of NOS activity and NO levels during hematopoiesis may be potentially used for therapeutic purposes. Indeed, we have begun experiments with human hematopoietic cells and found that we can change the extent of their proliferation and differentiation by manipulating levels of NO.

NO and *Xenopus* Brain Development

N. Peunova [in collaboration with H. Cline, Cold Spring Harbor Laboratory]

The developing brain of *Xenopus laevis* represents an excellent system in which to study the role of NO in organogenesis. Division of neural precursors, their migration, differentiation, and synaptogenesis occur in a spatially distinct pattern throughout development of the tadpole. For instance, in the optic tectum, new cells are generated in the narrow germinal zone at the caudomedial border of the tectum and are displaced laterally and rostrally from the germinal zone as they differentiate and mature. Consequently, interfering with the normal course of cell proliferation would be recognized as a disruption in the spatiotemporal pattern of development in the optic tectum.

We found that the gene encoding the neuronal isoform of *Xenopus* NO synthase (*XNOS1*) is strongly expressed in the developing brain of the *Xenopus* tadpoles. We combined in situ hybridization with an

XNOS1-specific RNA probe and detection of proliferating cells after BrdU labeling to show that a narrow zone of *XNOS1*-expressing cells lies adjacent to the zone of dividing neuronal precursors; however, the germinal zone itself is free of NOS. This suggests that NO may act as an antiproliferative agent to suppress division of neuronal precursors in the tadpole brain. We have previously shown that by suppressing NOS activity, we can dramatically increase the number of proliferating cells in the optic tectum, the total number of cells, and the overall size of the optic tectum. We have now found that the converse is also true: We can decrease the number of proliferating cells, the total number of cells, and size of the tectum by supplying exogenous NO to the developing tadpole brain. Our results strongly implicate NO as an essential negative regulator of neuronal precursor cell proliferation during vertebrate brain development.

NO in Neuronal Differentiation and Cell Death in the Mouse Brain

M. Packer

We study the potential of NO to change the proliferation of neuronal precursors in the adult brain. We have generated transgenic mouse lines where expression of neuronal NO synthase is placed under the control of the calcium/calmodulin IIa promoter. This promoter directs strong expression of the transgene in specific regions of the adult mouse brain. In particular, it directs the expression of the *nNOS* transgene in the subgranule zone of the dentate gyrus of the hippocampus, one of the regions of adult neurogenesis in the rodent brain. These transgenic mice therefore are good models to test the role of NO in the control of neuronal precursor proliferation. We are also using this mouse model to assess the role of NO in death protection pathways by studying the expression of apoptosis-related genes in the brains of transgenic animals.

Neural Stem and Progenitor Cells in the Developing and Adult Brain

J. Mignone

To detect stem and progenitor cells in the developing and adult brain, we generated transgenic mice which express green fluorescent protein (GFP) under the control of nestin gene regulatory sequences. These sequences, as determined by R. McKay and co-workers, direct highly specific expression of nestin in the neuroepithelial stem and progenitor cells of the developing and adult nervous system. We have obtained several transgenic lines with very strong and region-specific expression of the GFP transgene, such that green fluorescence marks neuronal stem and progenitor cells in the developing nervous system of the embryo and in the brain of adult animals. In the adult brain, highly selective expression of GFP marks the limited areas of neurogenesis: subventricular zone, rostral migratory path, and the dentate gyrus. We are now using these transgenic mice to visualize stem cells in the developing and adult brain and to study their proliferation. We are also isolating GFP-expressing cells with the help of fluorescence-activated cell sorting, and using them for transcript and protein expression profiling of neural stem cells.

Dividing Progenitor Cell Groups in the Developing and Adult Brain

A. Vaahtokari

We are interested in visualizing clusters and groups of dividing neural stem and progenitor cells in the developing and adult brain. We have generated transgenic mice where expression of GFP marks cells at a specific phase of the cell cycle. We are now analyzing the pattern of the marker gene expression in these transgenic animals at different stages of development.

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TRANSMISSION AND PLASTICITY IN MAMMALIAN CENTRAL SYNAPSES

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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.

The main result of this year was to determine that glutamate receptor trafficking is a major contributor to synaptic plasticity. Our results have led to a model in which transient (regulated) delivery of receptors is maintained by a subsequent constitutive receptor replacement. We have identified α-amino-3-hydroxy-5-methyl-4-isoxazole receptor (AMPA-R) subunits that may participate in different aspects of this process. This model can explain how a transient event (e.g., induction of long-term potentiation or LTP) may produce a long-lasting change in transmission despite protein turnover.

Delivery of Glutamate Receptors to Synapses during LTP

Y. Hayashi, S. Shi, J. Esteban, A. Piccini, J.-C. Poncer

To elucidate mechanisms controlling and executing activity-dependent synaptic plasticity, AMPA-Rs with an electrophysiological tag were expressed in hippocampal neurons. LTP or increased activity of the calcium/calmodulin-dependent protein kinase II (CaMKII) induced delivery of tagged AMPA-Rs into synapses. This effect was not diminished by mutating the CaMKII phosphorylation site on the GluR1 AMPA-R subunit, but it was blocked by mutating a predicted PDZ domain interaction site. These results show that LTP and CaMKII activity drive AMPA-Rs to synapses by a mechanism that requires the association between GluR1 and a PDZ domain protein.

Dendritic Trafficking, Clustering, and Synaptic Delivery of AMPA-R in Hippocampal Neurons

S. Shi, Y. Hayashi, J. Esteban [in collaboration with K. Svoboda, Cold Spring Harbor Laboratory; R. Petralia and R. Wenthold, National Institutes of Health]

To monitor optically 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 *N*-methyl-D-aspartate receptor (NMDA-R) activation and may contribute to the enhanced AMPA-R-mediated transmission observed during LTP and activity-dependent synaptic maturation.

Spontaneous Oscillatory Activity Delivers GluR4-containing AMPA-R into Silent Synapses during Early Development

J. Zhu, J. Esteban, Y. Hayashi

Spontaneous activity early in postnatal development is crucial for the formation of functional neuronal circuits. During this early period, many glutamatergic synapses contain only NMDA-Rs and are largely "silent," becoming functional by acquiring AMPA-Rs. In this series of studies, we show that spontaneous oscillatory activity in the hippocampus makes silent synapses functional by selectively delivering GluR4-containing AMPA-Rs. This differs from LTP seen in older animals, which delivers GluR1-containing AMPA-Rs and requires CaMKII activity. Consistent with this special role, GluR4 expression in the hippocampus is largely restricted to the first postnatal week. Once delivered by activity, synaptic GluR4-AMPA-Rs are exchanged with GluR2-containing AMPA-Rs in a manner that requires little neuronal activity and maintains the enhanced strength of the synapse despite protein turnover. This "delivery and exchange" represents a new form of activity-dependent long-term synaptic plasticity that may be responsible for the initial establishment of functional neuronal circuitry.

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, one can also see an increase 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, molecular biological, and pharmacological methods.

Transient Oxygen/Glucose Deprivation Induces Rapid Morphological Changes in Rat Hippocampal Dendrites

A. Piccini

Previous studies on ischemia-induced cell damage have revealed that pyramidal neurons in the CA1 region of the hippocampus are extremely susceptible and undergo selective degeneration 2-4 days after the insult. Nothing is known about early morphological changes occurring immediately after the insult in neurons in the organotypic hippocampal slice preparation. Using two-photon laser scanning microscopy, we monitored dendritic morphology of cells expressing GFP in response to a transient hypoxic-ischemic episode. This type of vital imaging provides direct evidence of growth of dendritic filopodia in rat CA1 pyramidal neurons occurring as soon as 20 minutes after oxygen-glucose deprivation. We propose that dendritic reorganization may be an early-stage response to compensate the loss of synapses caused by ischemia-induced neuronal injury.

β-amyloid as a Homestatic Negative Regulator of Synaptic Transmission

F. Kamenetz [in collaboration with S. Sisodia, University of Chicago, D. Borchelt, Johns Hopkins Medical School, T. Iwatsubo, University of Tokyo]

We are testing the hypothesis that β-amyloid acts as a homestatic negative regulator of synaptic transmission. In support of this, we find that organotypic slices secrete less β-amyloid if transmission is depressed, and they secrete more β-amyloid if transmission is enhanced. Furthermore, we find that acute expression of amyloid precursor protein (APP) in neurons leads to a secretion of β-amyloid and depression of synaptic transmission onto those neurons. However, if transmission is chronically pharmacologically depressed onto these APP-expressing neurons, then normal transmission is preserved. We plan to continue to test this view by expressing mutant APP molecules that

perturb the secretion of β-amyloid in predictable ways. If this hypothesis is correct, it will have a significant impact on the proposed therapeutic use of agents that reduce β-amyloid secretion for Alzheimer's disease. In conclusion, we are continuing to elucidate the basic mechanisms involved in central synaptic transmission and plasticity.

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PLASTICITY OF CORTICAL NEURONS AND THEIR CIRCUITS

K. Svoboda B. Burbach, B. Chen, K. Greenwood, I. Koh, B. Lendvai, Z.F. Mainen, M. Maravall, E. Nimchinski, A. Oberlander, P. O'Brien, B. Sabatini, E. Stern, J. Sugrim

The neocortex underlies most cognitive functions in mammals. Even though these functions are extremely diverse, the underlying anatomy 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. It is unclear how electrical and chemical activity in 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, and how they change in response to experience.

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 Ca^{++} levels at high resolution. Optical stimulation by 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 monitoring of the electrical membrane potential and synaptic currents of neurons, and intrinsic signal imaging allows monitoring of the dynamics of a network of neurons in vivo. We have also begun to use powerful molecular methods to label neurons for imaging and to introduce transgenes to perturb signal transduction cascades in very specific ways.

Despite the complexity of the problem, there is some reason to hope that an understanding might not be too far off of 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 neocortex is decomposable into a simpler problem, namely, understanding the function of the elemental module. During the last year, we have been focusing on the function and plasticity of neocotical neurons and their synapses in isolation. Over the next several years, we plan to work our way up to the level of the module.

Quantitative Cellular [Ca^{++}] Imaging at the Micrometer Length Scale

M. Maravall, B. Sabatini, Z.F. Mainen, K. Svoboda

Intracellular Ca^{++} concentration, [Ca^{++}], is an important state variable encoding neuronal activity. In addition, [Ca^{++}] couples electrical excitation to a myriad of important intracellular biochemical processes. During the last few years, it has become clear that detailed features of the spatiotemporal dynamics of [Ca^{++}] signals determine their biochemical meanings. To understand [Ca^{++}] signaling, it is therefore important to obtain quantitative estimates of [Ca^{++}] at high spatial and temporal resolutions. However, traditional approaches, such as those based on wavelength ratioing, are not useful for rapid imaging in small structures.

We combined TPLSM fluorescence imaging with indicators that change their brightness upon binding to Ca^{++}. We developed new calibration procedures that allow absolute [Ca^{++}] measurements and do not depend on wavelength ratioing. These protocols can be used routinely in situ and are applicable to micrometer-sized neuronal compartments. To test the quality of the estimates delivered by our method, we measured the sizes of action-potential-evoked [Ca^{++}] changes in the apical dendrites of CA1 pyramidal neurons and determined the dendritic buffer capacity, the ability of the cytoplasm to bind Ca^{++}. We are now using this method to explore the intracellular [Ca^{++}] signaling underlying synaptic plasticity.

Optical Studies of Single Synapses

B. Sabatini, Z.F. Mainen

Long-term changes in synaptic efficacy occur when a postsynaptic neuron consistently fires an action potential within 10 msec of the arrival of a particular synaptic input. It is known that increases in [Ca^{++}] in the postsynaptic neuron trigger these changes. We have used TPLSM and whole-cell patch clamp recordings to study the [Ca^{++}] signals that arise in spines and small dendrites of CA1 pyramidal neurons in response to back-propagating action potentials and synaptic stimuli. By contrasting the properties of action potential evoked [Ca^{++}] transients in the main apical dendrite with those of distal dendrites and spines, we were able to define functionally distinct neuronal compartments for Ca^{++} signaling. We find that action potential-evoked [Ca^{++}] transients in spines reach about 1 μM and return to baseline in less than 20 msec, much larger and faster than previously thought. These transients could account for the short integration time that is observed for the induction of synaptic plasticity. In addition, we show that spine heads can operate as isolated compartments such that gradients in [Ca^{++}] can be maintained between spine head and dendrite. Our measurements indicate that buffer capacity in the spine is lower than in the apical dendrite and soma, facilitating large local increases in [Ca^{++}]. Finally, we use a novel analysis of response fluctuations to estimate the number, location, and properties of the voltage-sensitive calcium channels and synaptic receptors contributing to synaptic Ca^{++} signals.

Spine Morphogenesis and the Cellular Basis of Fragile X Syndrome

E. Nimchinsky, A. Oberlander

The fragile X mental retardation syndrome is the most common form of inherited mental retardation. The molecular basis of the syndrome is a large expansion of a triple repeat (CGG) in the 5′-untranslated region (UTR) of the *FMR1* gene that renders it transcriptionally silent. The protein coded by this gene, called FMRP (fragile X mental retardation protein) is an RNA-binding protein of unknown function. One suggested function is the transport of mRNA out of the nucleus and into neuronal dendrites. Interestingly, the only cellular abnormality in the brains of affected individuals appears to be restricted to dendritic spines, the targets of excitatory synapses. These spines have been described as unusually long, thin, and tortuous, and more numerous than in normal brains. Since the gene is functionally knocked out in humans suffering from fragile X syndrome, a reasonable animal model of this disease is a mouse lacking *FMR1*. In these mice, as in patients, dendritic spines have been described as being longer and more numerous than in control mice. Long thin spines are suggestive of immature spines, found early in development, that are highly motile and presumably are in search of the correct presynaptic partners.

To understand the nature of this abnormality, we characterized the developmental aspects of structural defects. We used in vivo transfection of mouse barrel cortex neurons with green fluorescent protein (GFP)-

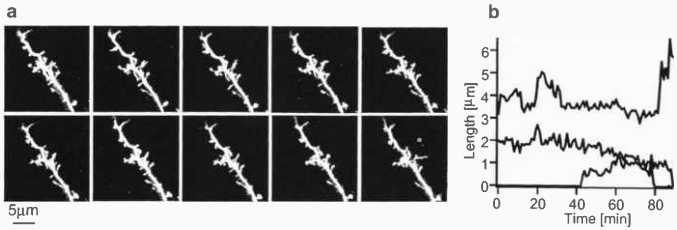

FIGURE 1 Motility of dendritic protrusions in the developing barrel cortex in vivo. (*a*) Time-lapse image sequences showing growth, retraction, and other shape changes of dendritic protrusions (time stamps are in minutes). Colored arrows point to protrusions that are analyzed further in panel *b*. (*b*) Time courses of length changes of selected protrusions.

expressing virus. Fluorescent neurons were analyzed *postmortem*. We find that there is a transient large abnormality in spine structure and density in the developing brain (PND 14) that decreases as the brain matures. To investigate the dynamic aspects of these spines, we are studying the motility of spines in slice cultures prepared from mutant mice at a time when dendritic spines are known normally to be highly motile. Finally, we are investigating the effects of reintroduction of FMRP into knockout mice on both morphology and motility.

Morphological Basis of Experience-dependent Plasticity In Vivo

B. Chen, B. Lendvai, B. Burbach, K. Svoboda

The rodent somatosensory cortex contains a map of the facial whiskers (barrel cortex) where the neuronal responses in each barrel column are dominated by a particular principal whisker. Whisker deprivation changes the responses in the whisker map. It has long been debated whether changes in neuronal structure underlie such experience-dependent cortical plasticity. We used time-lapse TPLSM of layer 2/3 pyramidal neurons in the developing rat barrel cortex to image the structural dynamics of dendritic spines and filopodia. Surprisingly, these protrusions were found to be highly motile throughout development: Spines and filopodia appeared, disappeared, or changed shape over tens of minutes, suggesting that synaptic lifetimes might be relatively short in vivo (~10–100 hours). To test whether sensory experience plays a part in driving this motility, we trimmed whiskers 1–3 days before imaging. Sensory deprivation dramatically (~40%) reduced protrusive motility in deprived regions of the barrel cortex during a critical period around PND 11–13, but had no effect in younger (PND 8–10) or older (PND 14–16) animals. Surprisingly, whisker trimming did not change the density, lengths, or shapes of spines and filopodia. These results show that sensory experience may drive the structural plasticity of dendrites that underlies the reorganization of neural circuits. More recently, we developed a technique to image the same dendritic segment over chronic time scales of several days. This will allow us to address long-standing questions regarding the effects of sensory experience on the large-scale structural plasticity of neural circuits.

Neocortical Experience-dependent Plasticity In Vivo

E. Stern, K. Greenwood

In parallel with our imaging studies of experience-dependent morphogenesis, we are exploring the physiological properties of neocortical neurons. In particular, we are interested in the effects of sensory experience on the development of topographic maps in the barrel cortex. In contrast to previous experiments that have employed extracellular recordings, we are using intracellular recordings in vivo. Such measurements inform us not only about neuronal spiking activity, but also about synaptic potentials. We find that brief whisker trimming (a couple of days) has a profound effect on the structure of sensory maps.

Experience-dependent Changes in the Properties of Neocortical Pyramidal Neurons

M. Maravall

Some subtle experience-dependent changes in cellular properties are difficult to study in the intact brain. For example, the sizes of quantal synaptic currents cannot be measured in vivo, but they can be measured in brain slices. We are comparing the properties of neocortical pyramidal neurons in brain slices derived from animals that were deprived and from control animals. To discover possibly diverse modes of plasticity, we are using a barrage of electrophysiological and imaging approaches to look for effects of sensory experience on neuronal Ca^{++} dynamics, excitability, and synaptic currents.

Algorithms for Automated Morphometric Analysis

I. Koh, E. Nimchinsky [in collaboration with Brent Lindquist, State University of New York, Stony Brook]

Changes in the structure of neurons are intimately related to neural development and plasticity and its dysfunctions. It is of interest to find genes that control aspects of neuronal shape. A conceptually simple

approach might be to use the gene gun to transfect neurons with candidate genes together with GFP and subsequently analyze the structure of transfected cells. An underappreciated problem with this approach is that structural data are extremely noisy and difficult to analyze. Mind-numbing laborious manual analysis invariably limits the throughput in such studies. For these reasons, we have invested considerable effort in developing software that allows essentially automated analysis of neuronal structure. Our approach consists of three steps: digital filtering by deconvolution, image segmentation, and image analysis. A prototype of a program that reliably detects changes at the level of spines in time-lapse movies is now running and is ready to extract useful data.

Instrumentation

K. Svoboda, P. O'Brien, J. Sugrim, Z.F. Mainen

Our instrumentation efforts during the last year have focused on getting further microscopy/physiology stations working and others updated. We also set up a simple intrinsic signal imaging microscope that will in the future be combined with TPLSM. Finally, we designed and built an A/D board that allows us to acquire multiple fluorescence wavelengths simultaneously. This board was implemented in a printed circuit design, substantially reducing maintenance requirements.

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MOLECULAR NEUROBIOLOGY OF LEARNING AND MEMORY IN *DROSOPHILA*

T. Tully C. Alexander, J. Christensen, J. Connolly, J. DeZazzo, J. DiLeo, J. Dubnau, R. Gasperini, S. Gossweiler, L. Grady, R. Jones, H. Li, S. Pinto, M. Regulski, M. Saitoe, P. Smith, M. Sullivan, S. Xia, L. Zhang

Jeff Hall, professor of biology at Brandeis University once said, "We're all working on the same genes, we just don't know it yet." *Latheo*, a gene identified in a behavioral screen for learning mutants, encodes the *Drosophila* homolog of ORC3, a protein component of the origin recognition complex involved in DNA replication during cell proliferation. In terminally differentiated, nonproliferating motor neurons of the larval nervous system, *latheo* is expressed in presynaptic terminals. What exactly *latheo* is doing at the synapse will be the subject of future research. The fact that this connection was made between two rather disparate cellular functions of a gene, however, is the purpose of "forward" genetics. Classical mutagenesis makes no assumptions about the molecular mechanism(s) underlying a particular phenotype. In our context, this approach also allows the identification of behaviorally relevant mutations in multifunctional (pleiotropic) genes.

Identification of New Genes Involved with Associative Learning

T. Tully, S. Pinto, J. Christensen, J. DeZazzo, M. Saitoe, P. Smith, C. Alexander, H. Li, L. Zhang, L. Grady
[in collaboration with M. Ramaswami, University of Arizona; K. Broadie, University of Utah]

The Hartford Screen: The behavioral screen (forward genetics) for new Pavlovian learning/memory mutants is complete. In all, more than 6700 transposant lines were generated and assayed for 1-day memory after spaced training using a 48-array of Robotrainers. From this screen, we have identified more than 60 candidate mutants. From two replicate experiments, one before and one after outcrossing and egg-bleaching (for virus; see below), these candidate mutants yielded average scores less than or equal to 50% of normal. We now are assessing initial learning, olfactory acuity, and shock reactivity in these candidate mutants. Some will show lower-than-normal performance because of a sensorimotor defect. Most, however, promise to result from bona fide defects in associative learning or subsequent memory processing. One such mutant already has been characterized and named. As shown in Figure 1, *ikar* shows normal learning but no 1-day memory after spaced training. With luck, this screen will double the number of learning and memory mutants known in *Drosophila*.

latheo: *latheo* encodes the fly homolog of ORC3, an integral member of the origin recognition complex (ORC). This molecular identification certainly explains the cell proliferation defect and pupal lethality of severe (amorphic) *latheo* mutants. We wondered, however, if this cellular function was responsible for the adult learning defect of less severe (hypomorphic) mutants. To address this issue, we raised a polyclonal antibody and a monoclonal antibody against bacterially expressed LAT antigen and then, in collaboration with K. Broadie's group, looked at the larval neuromuscular junction (NMJ) for LAT expression. Surprisingly, we detected LAT immunoreactivity at this peripheral synapse in wild-type larvae but not in null mutants, thereby demonstrating that LAT is expressed at the synapse in nonproliferating larval motor neurons. Moreover, *latheo* (null and hypomorphic) mutants showed defects in basal transmission and synaptic plasticity at the NMJ. Taken together, these results suggest an acute role for *latheo* in synaptic plasticity, a cellular function distinct from its role as a member of ORC. More generally, these are the first observations to suggest that an ORC subunit may function outside of the cell nucleus.

linotte: LIO is expressed postsynaptically at the NMJ and preferentially in both axonal and dendritic processes in the adult central nervous system (CNS). We have discovered defects in synaptic plasticity at the NMJ of *linotte* mutants that can be rescued by expression of

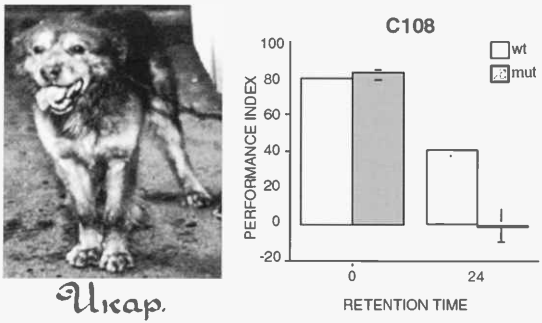

FIGURE 1 (*Left panel*) *Ikar*, one of Pavlov's dogs (from a photo album presented to Pavlov by his students on his 83rd birthday). (*Right panel*) Performance indices (PIs) for *ikar* (strain C108). Immediately after one training session (0) of an olfactory classical conditioning task, "learning" is normal, whereas long-lasting memory is defective 24 hr after spaced training (24).

(two independent) *hs-lio*$^{+}$ transgenes. Biochemically, we have detected reduced levels of cAMP in third-instar larval CNS. Behaviorally, we have determined that *rut;lio* double mutants yield no olfactory associative learning despite the fact that their underlying sensorimotor responses are normal. Together, these observations suggest that *linotte* is a novel molecule involved in the modulation of cAMP signaling in neurons.

nalyot: In collaboration with M. Ramaswami's group, we also have shown that *nalyot* is involved in the maturation of synaptic structure—but not function—at the NMJ. This observation is in striking contrast to studies on the cAMP response element-binding protein (CREB) by G. Davis and colleagues (then at the University of California, Berkeley), where they showed that CREB was involved in the development of synaptic function but not structure. Thus, ADF1 and CREB appear to define two distinct transcription factor cascades involved in activity-dependent synaptic plasticity. Since long-term memory (LTM) formation also involves synaptic plasticity and CREB, we assessed LTM in *nalyot* mutants. As predicted, we have discovered that LTM is abolished, but ARM, an earlier memory phase, is normal in *nalyot* mutants. Hence, *nalyot* is the first mutation of an endogenous gene known to disrupt LTM preferentially.

Characterization of Extant Mutants

T. Tully, M. Regulski

Although our initial attempts to generate a mutation by jumping a nearby P element into the NOS (nitric oxide synthase) transcription unit have failed, we did identify a small deletion of the NOS region produced by imprecise excision of the P element. Homozygotes of this deletion are lethal, but heterozygotes survive. Using our newly developed enzymatic assay for NOS activity in *Drosophila* tissue, we have determined that NOS activity in deletion heterozygotes is 50% of normal. Molecular analysis of the genomic region suggests that this deletion eliminates only the NOS and porin transcripts. Using these deletion heterozygotes, we have initiated a chemical (EMS) mutagenesis to identify point mutations in NOS, based on the assumptions that such mutations will be lethal over deletion. To date, several mutations have been identified, and they fall into only two lethal complementation groups. Hence, we finally may have identified point mutations in NOS, thereby allowing future developmental and behavioral analysis of NOS and synaptic plasticity.

Molecular Search for CREB-dependent LTM Genes

T. Tully, J. Dubnau, S. Gossweiler [in collaboration with Helicon Therapeutics, Inc.]

In our attempts to search a portion of the fly genome for transcriptional responses to LTM formation, we have discovered a significant signal-to-noise issue when analyzing data from DNA chips. To validate such analyses biologically, we have expanded our chip analyses to include three distinct types of chip comparisons: (1) LTM vs. no LTM, (2) drug-enhanced cAMP signaling vs. no drug, and (3) mutant vs. wild-type. With the latter comparison (of *nalyot* vs. wild type), we already knew that ADF1 transcript levels were reduced in *nalyot* mutants. ADF1 was included on the Affymetrix fly chip and thus constituted an internal positive control for signal-to-noise analysis. With this approach, we have determined an appropriate statistical method to identify candidate transcripts that are differentially regulated. Importantly, not all statistical approaches returned our positive control as a statistical candidate. Once the appropriate quantitative method was empirically validated, we analyzed data from our LTM vs. no LTM comparison. One statistical candidate that has been confirmed with Q-PCR (polymerase chain reaction) is C/EBP—an immediate early gene that is transcriptionally regulated by CREB during LTM formation in *Aplysia*.

The Virus Hypothesis

T. Tully, R. Gasperini [in collaboration with B. Stillman and T. Howard, Cold Spring Harbor Laboratory]

The viral pathogen that has infected our fly colony is under control. We identified the virus to be *Drosophila* C virus (DCV) and have developed a sensitive reverse transcriptase (RT)-PCR method to detect its presence. Using RT-PCR, we then developed a stringent egg-bleaching protocol, which can significantly reduce the virus titer carried by a fly strain. With this decontamination method, combined with sterile culturing of fly stocks, we now can maintain stocks for several generations before virus titers again become problematic. We currently are studying a curative method to eliminate DCV infections altogether.

Behavioral Analyses

T. Tully, S. Xia

Recently, T. Preat's group published a claim that most of the learning and memory mutants are not defective for associative learning but rather perform poorly in the Pavlovian assay because of a nonassociative effect on the perception of odors. We do not agree with this interpretation of these experiments. To address this issue, we have (1) replicated the published results, (2) produced data suggesting that this "Preat effect" actually reflects an associative process, and (3) shown that the Preat effect does not influence the calculation of an associative Performance Index.

From these behavioral experiments, we suggest that the Preat effect can influence Pavlovian performance only if it changes the flies' ability to *discriminate* between two odors simultaneously delivered. To this end, we have developed three behavioral assays of discriminability, each of which appears normal in the learning and memory mutants. Thus, we find no defects in sensorimotor responses of learning and memory mutants that are sufficient to explain the mutants' performance in the olfactory associative task.

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LONG-TERM MEMORY FORMATION IN *DROSOPHILA*

J. Yin M. Cowan, E. Drier, R. Filipkowski, E. Friedman, J.. Horiuchi, W. Jiang, K. La Vine, Z. Lin, C. Margulies, K. Seidenman, M. Stebbins, M. Tello, P. Wu, H. Zhou

Regulation of dCREB2 DNA-binding Activity

J. Horiuchi, W. Jiang, P. Wu, H. Zhou

Endogenous and transgenic dCREB2 protein is totally inhibited from binding DNA in crude, cell-free extracts. Inhibition is seen in extracts made from embryonic, pupal, and adult stage tissue, as well as from Schneider (tissue culture) cells. Phosphatase treatment of extracts unmasks the DNA-binding activity. Four apparent casein kinase phosphorylation sites, amino-terminal to the well-characterized S133 residue, are partially responsible for inhibition of binding.

In vitro, incubation of bacterially expressed dCREB2 protein with casein kinase I or II, or Schneider cell extracts, but not PKA, calcium-calmodulin kinase II (CaMKII), or GSK-3, inhibits DNA binding. Subsequent phosphatase treatment partially reverses this inhibition of DNA binding. In vitro phosphorylation with PKA, CaMKII or IV, or GSK-3, does not reverse the inhibition produced by casein kinase I, demonstrating that this inhibition is "dominant" to other phosphorylation events. Bacterially expressed mutant protein (containing alanine substitutions in all four conserved serine sites) is partially immune to Schneider extract-mediated inhibition of binding. Tryptic analysis of bacterially expressed protein treated in vitro with casein kinase I, or Schneider cell extracts, and γ-ATP, have identical phosphopeptide patterns.

Cell-free extracts made from mouse tissue (brain, heart, kidney) show that, unlike flies, there are two approximately equal pools of protein: one is binding-competent and one is inhibited from binding unless treated with phosphatase. Preliminary transfection experiments show that a mutant rat cAMP response element-binding protein (CREB) gene (containing five serine to alanine substitutions) has greater transcriptional activity than the wild-type gene. This suggests that phosphorylation of the conserved casein kinase sites can inhibit DNA binding and transcriptional activity of a CREB activator isoform. Since most, if not all, of the fly dCREB2 protein seems to exist in a phosphorylated form, one of the major regulatory steps in the activation of dCREB2 must be dephosphorylation. It is hypothesized that one of the signaling pathways activated during learning and memory formation must target the relevant phosphatase(s).

Regulation of dCREB2 Subcellular Localization

C. Margulies, W. Jiang, P. Wu, H. Zhou

We have previously reported that much of the dCREB2-b (blocker) protein, whether endogenous or transgenic, is localized to the cytoplasmic compartment. Deletion of the amino-terminal portion of the protein or mutation of all four conserved casein kinase sites results in more protein localization to the nucleus. We are using the yeast two-hybrid system to search for proteins that interact with the *Drosophila* protein.

S162, a Lethal Mutation in the *dCREB2* Gene

M. Tello, E. Drier, H. Zhou

We have identified the DNA alteration in the *S162* mutation. A stop codon is present in exon 7 of the *dCREB2* gene, resulting in premature truncation of the protein. Western blot analysis supports this finding, since dCREB2 protein from escaper flies shows a pair of truncated proteins, about 5–8 kD shorter than the wild-type doublet. The larval/pupal lethality of this mutation can be rescued using a 9-kb genomic transgene that codes for the wild-type proteins. Lethality cannot be rescued using a 9-kb fragment containing a premature stop codon engineered into the dCREB2 open reading frame.

Role of Sleep in Memory Formation

M. Cowan, P. Wu, H. Zhou
[in collaboration with Joan Hendricks,
University of Pennsylvania]

We have previously shown that there is periodic, circadian-regulated, CRE-mediated transcription in the living fly and that one peak occurs during the dark period of a 12-hour light:12-hour dark cycle. In flies, proteins from the *dCREB2* gene show highest binding affinity to CRE sites in vitro and are one of two protein families that bind to these sites. Flies show many of the characteristics of "sleep" during the nighttime period, including long periods of locomotor inactivity and rebound (the tendency to rest more) if part of this period is disrupted. Because there is a lot of speculation about the possible role of sleep in mammalian memory consolidation, we are trying to demonstrate that this nightly peak in CRE-mediated transcription is important for memory formation. Experiments using light as the experimental variable to disrupt the circadian system and cycling transcription yield inconsistent results. We believe the inconsistencies are due to the pleiotropic effects of light itself. Therefore, we are currently using molecular approaches to disrupt the circadian system, and periodic CRE-mediated transcription, to ask if the persistence of this cycling transcription is needed for consolidation or maintenance of previously formed memories.

In a related experiment, flies that are entrained to a 12-hour light:12-hour dark period show a striking "time of day" effect on memory formation. Flies trained at six different time points during the 24-hour cycle show significant differences in their ability to form memories. Flies trained shortly after lights-out perform the best, whereas flies trained late in the dark cycle do the worst. The nighttime peak in CRE-mediated transcription occurs shortly after lights out.

PKMζ-mediated Enhancement of Memory Formation

E. Drier, M. Cowan, P. Wu, H. Zhou
[in collaboration with Todd Sacktor,
State University of New York, Brooklyn]

Experiments in hippocampal slices and honeybees suggest the critical involvement of nonconventional PKC family members in the maintenance of long-term potentiation (LTP) and long-lasting memory. Induction of a mouse *PKMζ* (the truncated version of PKCζ) transgene enhances memory formation after a single training trial, resulting in significant memory retention 24 hours after training. There is no 24-hour memory in wild-type flies, whether or not heat-shocked, or transgenic flies that are not induced. Induction of the *PKMζ* transgene also enhances memory after 10x massed trials, producing robust retention 4 days after training. Wild-type flies, or transgenic flies that were not induced, do not have any memory 4 days after 10x massed training. To produce enhancement, the transgene must be induced after behavioral training, and there is a short "window" when induction is effective. Induction of *PKCζ*, the full-length gene, does not enhance memory after a single training trial.

We are investigating which phases of memory PKMζ enhances as well as its molecular mechanism(s).

Identification of a *Drosophila* Atypical PKC Gene

M. Tello

In mammals, there are 11 members of the PKC family, which are divided into three subgroups: conventional, novel, and atypical. The conventional PKC genes require Ca^{++} and DAG, the novel genes require DAG, and the atypical genes require neither.

Despite these biochemical distinctions, little is known about the physiological functions of each subgroup, nor the relationship between them. In *Drosophila*, one novel and two conventional genes have been described. Scanning the sequence database, we have identified a *Drosophila* atypical family member and are beginning the basic molecular characterization of this gene.

Technologies for Disrupting Gene Function in Animals

M. Stebbins, Z. Lin, W. Jiang, H. Zhou, P. Wu,
K. La Vine, E. Drier, K. Seidenman [in collaboration
with Wolfgang Hillen, University of Erlangen; Gerry Byrne,
Nextran; Matt During, Thomas Jefferson]

Tetracycline-inducible System: We have improved the tetracycline-inducible system for use in *Drosophila*. By using insulator elements, we can achieve about 100x repression of target gene expression, measured in the living fly. This change in gene expression can be spa-

tially and temporally regulated by combining the tetracycline and Gal4-UAS systems. Spatially and temporally regulated repression can be achieved in the adult or embryonic stages. In the "tet on" version of this system, the addition of doxycycline causes DNA binding and transcriptional activation. If insulators and gene expression parameters (removal of a cryptic splicing site, use of mammalian codon usage) are optimized, doxycycline feeding results in about a 10–20x induction (which can be spatially delimited) in adult flies.

We are currently testing a new "tet on" *trans*-activator in flies, which has a whole new set of mutations that changed the binding properties of the protein. In primary neurons, this new *trans*-activator is 3–4x more active than the original one.

When implemented in mice, the old "tet on" *trans*-activator does not perform well. Molecular analysis shows that although the *trans*-activator protein is detectably expressed, it has more degradation than when expressed in flies. In addition, both mouse and fly extracts show endogenous protein(s) that can bind to the expanded tetracycline operator sequences, which are commonly used. However, the affinity or levels of the endogenous protein in flies are lower than that in mice.

Alternative Strategies in Mice: We are investigating the possibility of using adenovirus-associated virus (AAV) infection as a means to introduce genes into cells and tissue. The advantage of this virus is that it is relatively harmless, shows a long period of expression in infected tissue, and shows a strong preference for infecting neurons. In addition, TAT-protein-mediated transduction has been shown to be a viable method for introducing proteins into cells and tissue. We are investigating this technology, envisioning its usage in a combinatorial manner with regular transgenic technology and viral infection.

Single-chain Antibodies: Single-chain antibodies are proteins made from the variable region of hybridoma heavy and light chains fused onto one contiguous polypeptide. This peptide retains the binding specificity and most of the affinity of the original monoclonal antibody. We are creating these molecules and testing their feasibility as inactivating molecules in transgenic flies.

The ultimate goal is to have spatial (anatomically delimited), temporal, and subcellular control over their presence and location. It is anticipated that such tools will facilitate the analysis of proteins that participate in the process of memory formation, especially those who change their intracellular location in response to training-induced signaling events.

Development of Mouse Behaviors

E. Friedman, R. Filipkowski

To develop behavioral assays that are useful for evaluating molecules involved in memory formation, we have focused on two different "spatial" tasks: contextual discrimination and a modified Morris water maze. In contextual discrimination, mice are serially exposed to two similar, but discernibly different, conditioning boxes and are shocked in one of the two. Multiple training trials are required for the formation of stable, consolidated memory of the pairing-specific box. If the multiple trials are separated by short intervals, 4-day memory, but not 7-day memory, is formed. If the trials are separated by longer intervals, 7-day memory forms. Preliminary evidence shows that the memory generated after long-interval training is susceptible to protein synthesis inhibition, whereas the memory produced following shorter-interval training is not. These behavioral properties are reminiscent of the spaced/massed distinction in fly behavior, although some details are not identical.

We have modified the training protocal for the Morris water maze, allowing mice to form 7-day memory with only a single day of training. The multiple training trials occur all in one day and have specific requirements in terms of the total number of trials, the grouping of individual trials into blocks of trials, and the interblock intervals. We are investigating the protein synthesis requirements of the different consolidated memories that form after distinct training protocals.

The Search for Memory Genes

R. Filipkowski, K. Seidenman [in collaboration with Tim Tully, Cold Spring Harbor Laboratory]

We are using array-based methods to search for genes whose expression changes during long-term memory formation. Our strategy is to compare different models of neuronal plasticity, cellular plasticity and behavior for genes that change in more than one situation, or for genes whose pattern of response is similar. We are focusing on hippocampal-dependent behavioral tasks, such as contextual discrimination, fear conditioning, and the Morris water maze.

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NEURAL BASIS OF LEARNING AND MEMORY IN *DROSOPHILA*

Y. Zhong F. Hannan H.-F. Guo Y. Wang J. Pathenveettil J. Tong I. Hakker J. An

We are interested in the neural basis of learning and memory, and our research is currently following two paths. First, we are establishing *Drosophila* models for studying genes involved in human neurodisorders that impair learning and memory. In particular, we are interested in genes known to contribute to neurofibromatosis 1 (*Nf1*) and Alzheimer's disease. *Nf1* patients are identified by neurofibromas and other symptoms including learning defects. We are investigating a hypothesis that the tumor suppressor gene *Nf1* not only acts as a Ras-specific GTPase activating protein (GAP), but also is involved in mediating G-protein-stimulated activation of adenylyl cyclase (AC), and this NF1-dependent AC pathway is required for learning. In the case of Alzheimer's disease, our study has been limited to examining how presenilin and accumulation of Aβ affect the age-dependent decline of learning ability. This approach, on the one hand, serves as a new way to identify biochemical cascades underlying learning and memory in *Drosophila*. On the other hand, it may also provide insights into pathogenesis of the diseases. Second, we 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. The specific projects are described below.

NF1-regulated cAMP Pathway in Learning

H.-F. Guo, J. Tong, F. Hannan

The tumor suppressor gene *Nf1* encodes a large protein containing a fragment homologous to Ras-GAPs, which inhibit Ras activity. *Drosophila* NF1 is highly conserved since 60% of its 2803 amino acids are identical to those of human NF1. Our previous electrophysiological, biochemical, and behavioral analyses of *Drosophila Nf1* mutants have indicated that in addition to functioning as a Ras-GAP, NF1 regulates activation of adenylyl cyclase. This NF1-dependent cAMP pathway is crucial for learning and memory. Our recent effort in biochemical assays of AC activity has revealed that G-protein-stimulated AC activity consists of an NF1-dependent component and an NF1-independent component. The NF1-dependent activity is largely mediated via the *rutabaga* (*rut*)-encoded AC. The NF1-dependent component is largely mediated via the *rutabaga* (*rut*)-encoded AC, which is required for learning in *Drosophila*.

Site-directed Mutagenesis in Human NF1

F. Hannan, I. Hakker

To understand how NF1 regulates AC activity, we have conducted yeast two-hybrid screening in an attempt to determine whether NF1 interacts directly with Rut-AC or to identify intermediates that mediate interactions between NF1 and Rut-AC. The effort has failed to gain positive evidence. We are now taking another approach by utilizing point mutations found in NF1 patients to investigate problems related to how NF1 regulates AC activity. Our collaborator Dr. Nürnberg (Institut für Medizinische Genetik) has compiled a list of 17 missense mutations after analyzing approximately 400 patients for mutations in the whole coding region. Some of these mutations fall into the region critical for Ras binding, and one of them has been shown to disrupt the binding of Ras to NF1 drastically. A large number of mutations fall into domains without defined functions. All of these mutations will allow us to investigate the following issues. First, mutations falling in the Ras-related-domain will

enable us to test whether Ras activity or binding of Ras is required for NF1-regulated AC activity. If expression of human NF1 with such mutations can still rescue *Drosophila Nf1* phenotypes related to the cAMP pathway, Ras activity or binding of Ras is not required for NF1-dependent regulation of AC activity. Second, studies of mutations outside the Ras-related-domain will help to determine regions of NF1 important for regulating AC activity. It is expected that expression of human NF1 with some mutations will be unable to rescue the phenotypes, and the regions in which the mutations are located must be important for regulation of AC activity. We have shown that induced expression of human NF1 was capable of rescuing *Drosophila* NF1 phenotypes, including the small body size, defective neuropeptide response, and reduction in G-protein-stimulated AC activity. We are now conducting site-directed mutagenesis and making constructs that contain mutated human NF1. We will then generate transgenic flies carrying these constructs for testing effects of these mutations as outlined above.

NF1-regulated cAMP Pathway in Vertebrates

J. Tong

All of the above studies have been confined in *Drosophila*. Yet, studies of NF1 function in vertebrates have largely been limited to GAP activity. It remains to be determined whether NF1 also regulates AC activity in vertebrates. The observation that expression of human NF1 rescues fly NF1 phenotypes resulting from impaired NF1-regulated AC activity suggests that a similar NF1-regulated AC pathway is also expected in vertebrates. We want to study this issue by assaying AC activity in *Nf1* knockout mouse. Since these *Nf1* mutant mice are embryonically lethal at stage E13.5, our assay has used brain tissue from embryos at E12.5 to E13.5. Preliminary data indicated that GTPγS-stimulated AC activity was reduced in NF1 homozygous mice as compared to heterozygous or wild type, supporting the idea that NF1 is both structurally and functionally conserved between vertebrates and invertebrates.

Ensemble Coding and Odor Perception: Ca^{++} Imaging of Odor-evoked Neural Activity in the Mushroom Body of the Living *Drosophila*

Y. Wang, J. An, H.-F. Guo

Drosophila has been a powerful genetic model for dissecting the molecular basis of learning and memory. Yet, it has fallen short for studying the physiology of learning and memory because of the small size of its brain and central nervous system neurons. We have now developed an imaging method that will allow us to monitor fly brain activity by neural activity-dependent changes in intracellular Ca^{++} concentrations. Odor-induced neural activity was recorded by Ca^{++} imaging in the cell body region of the *Drosophila* mushroom body, the second relay of the olfactory central nervous system. Its simple organization and superficial position have allowed visualization of odor-induced widely distributed spatial activity patterns. The activity pattern, but not amplitude, of an odor response is specific for the odor chemical identity and concentration. This specific activity pattern can be altered by genetic manipulation of an odor-binding protein; the alteration of the spatial representation of an odor is closely associated with a behavioral defect of odor preference. These results strongly support the idea that olfactory information is internally represented or encoded by spatially distributed activity patterns at the second relay of the olfactory system.

In Press

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COMPUTATION IN THE AUDITORY CORTEX

A. Zador M. DeWeese
E. Verdee

We use a combination of theoretical and experimental approaches to study how the brain computes. The brain is able to solve hard computations that remain far beyond the reach of the fastest computers. Our goal is to understand this computation at the synaptic, cellular, behavior, and algorithmic levels. One example of such a hard computation is the "cocktail party problem": our ability to attend selectively to a single voice, effortlessly filtering it out from the others that make up the banter that surrounds us. The model system in which we study this computation is the rat auditory cortex. Since arriving here at Cold Spring Harbor Laboratory this year, we have begun to pursue two related projects.

SYNAPTIC MECHANISMS UNDERLYING AUDITORY STIMULUS-EVOKED RESPONSES

A first step toward understanding complex processing in the auditory cortex involves understanding how neurons represent auditory stimuli, and how these representations are computed from the cochlear input five synapses away. We hypothesize that the precise relative timing of excitatory and inhibitory input determines whether a cortical neuron responds to a stimulus within its receptive field. To test this hypothesis, we are using whole-cell patch clamp recording in vivo to measure the synaptic currents elicited by simple and complex auditory stimuli. Patch clamp recordings provide a much richer source of information than do conventional single-unit extracellular recordings because they allow us to monitor not just the *output* of the neuron—the spike train—but the *inputs* as well. Preliminary data using pure tone-pip stimuli support our hypothesis. These data suggest how we can design new stimuli to probe the representations with which the cortex solves hard problems in auditory processing.

NEURAL CORRELATES OF SELECTIVE AUDITORY ATTENTION

When we tune in to one voice at a cocktail party, and tune out the others, we are engaging in a form of selective auditory attention. Our ability to attend selectively is not limited to the auditory domain: Analogous tasks demand selective attention in the visual and even somatosensory domains. In monkeys, visual attention selectively enhances neural activity even in the most peripheral areas of the visual cortex. This enhancement is surprising because early sensory areas have traditionally been thought of as representing the sensory world faithfully, in a way that depends only on the properties of the sensory input itself. The discovery of attentional modulation overturns the notion that the early sensory cortex is a passive "TV screen" available for viewing by a "homunculus" buried deep in the cortex, and instead raises the possibility that the "homunculus" is distributed throughout the cortex.

More than a decade has passed since the first reports of a neural correlate of selective attention in monkeys, but the mechanism underlying the enhancement of neural activity remains largely a mystery. We are therefore developing a simpler preparation for studying selective attention. We are training rodents to perform a simple selective auditory attention task. This simple rodent preparation will have many advantages over the existing primate preparation, as it will permit us to bring to bear the full cellular and molecular armamentarium. A better understanding of the mechanism underlying selective attention may eventually allow us to resolve basic questions about brain function.

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NEURAL ORGANIZATION OF OLFACTORY PERCEPTION AND BEHAVIOR

Z.F. Mainen R. Gasperini K. Greenwood
M. Glander S. Macknik

Our laboratory is working toward understanding the general principles of neural organization that allow organisms to perceive, remember, anticipate, and act on complex sensory information. The goal is an integrative understanding that relates information processing at the microscopic level (individual synapses and neurons) to its macroscopic function. Our research is currently centered on the olfactory system. The principles of chemical sensation are still poorly understood, but the olfactory system offers numerous experimental advantages including known receptor genes linked directly to the initial encoding of stimuli, accessibility by optical imaging techniques, a simpler neuronal architecture compared to the neocortex, and a very direct connection between sensory periphery and central processing. My laboratory opened this fall in the Marks Building, and we are now beginning to use a variety of physiological and advanced optical techniques such as two-photon microscopy and intrinsic imaging to explore perception, learning, and behavior in the olfactory system. The three main areas of research are described below. We are also continuing our investigation of the biophysics of synaptic transmission and Ca^{++} dynamics in collaboration with K. Svoboda here at the Laboratory.

Neural Correlates of Olfactory Perception

S. Macknik, R. Gasperini, Z.F. Mainen

Why do flowers smell more similar to one another than to trees? What is the neural basis for the perceived qualities, similarities, and differences between odors? To address these questions, we are combining optical imaging techniques to probe neural representations with human and rat psychophysical techniques to quantify perceptual characteristics of odors. Because each glomerulus in the olfactory bulb is targeted by neurons expressing a single olfactory receptor type, it is thought that the olfactory glomeruli form a map of chemical structure. Any odor, either a single molecule or a mixture of a many different molecules, activates a specific subset of odor receptors and hence a particular pattern in the olfactory bulb. Because the location of particular receptors within the glomerular map is preserved from individual to individual, the receptor map could provide the basis for the invariant perceptual qualities of odors. Our main hypothesis is that perceptual qualities of odors are direct correlates of the spatial patterns of activity produced across the glomerular input layer to the olfactory bulb. Alternative hypotheses include the possibility that such perceptual maps are only formed later in the olfactory pathway, such as in the olfactory cortex, and the possibility that perceptual qualities are not directly related to any kind of spatial map, but instead rely on nonspatial factors such as synchronization of neural activity. We are testing the main hypothesis by comparing the perceptual discriminability of pairs of odors, measured by errors made by mice in a behavioral task, with the similarity of their glomerular activity patterns, measured by intrinsic imaging of odor-evoked responses in anesthetized mice. We will also test the first alternative hypothesis by carrying out similar imaging in the pyriform cortex.

Neural Mechanisms of Mouse Neonatal Olfactory Learning

K. Greenwood, R. Gasperini, Z.F. Mainen

Why do mice get stuck in peanut-butter-baited traps? Most rodent behavior is based on olfactory sensory cues. As a basis for understanding how particular behaviors are guided by specific sensory stimuli in a mammalian nervous system, we are investigating the neural bases of olfactory behavior in the neonatal mouse. In the developing rodent brain, the basic archi-

tecture is in place, but the most complex central processes are still not on line, leaving the animal with a functional but substantially less complicated nervous system and behavioral repertoire. We view this as a particularly advantageous system in which to pursue a neuroethological understanding of the basic organization of information processing in the brain. Our starting point is the use of behavioral paradigms in which neonatal rodents learn long-lasting preferences and aversions to particular odors they encounter in their environment. We are using classical conditioning techniques to isolate specific learning events. Previous work has implicated structures within the olfactory bulb in the formation and access of olfactory memories in this system. We are pursuing two broad questions. First, what are the cellular and circuit mechanisms involved in the formation of a specific odor preference? Second, what are the changes in cellular and circuit properties responsible for changes in behavior that follow learning?

Imaging Olfactory Structure and Function in *Xenopus* Tadpoles

M. Glander, Z.F. Mainen [in collaboration with H. Cline, Cold Spring Harbor Laboratory]

In collaboration with H. Cline, we are examining the structure and function of the olfactory system in the albino frog *Xenopus*. The developing nervous system of the tadpole is particularly amenable to high-resolution imaging. We are using fluorescent dyes to label olfactory receptors neurons and two-photon imaging to resolve the structure of their axonal arbors. Ca^{++}-sensitive versions of these dyes are being used to obtain functional maps of activity in the olfactory bulb and epithelium. We are also examining the effect of odor experience on the behavior of tadpoles, which, there is reason to believe, may mirror the effects seen in the developing mammalian system.

Biophysical Mechanisms of Synaptic Transmission

Z.F. Mainen [in collaboration with R. Malinow and K. Svoboda, Cold Spring Harbor Laboratory]

We have been using two-photon microscopy to image transient increases in Ca^{++} concentration mediated by NMDA (*N*-methyl-D-aspartate) receptors in single dendritic spines of CA1 pyramidal neurons in hippocampal slices. This optical approach has allowed us to study single synapses at a resolution impossible with traditional electrical recordings and to thereby address outstanding problems in the biophysics of synaptic transmission.

At excitatory synapses in the central nervous system, the number of glutamate molecules released from a vesicle is much larger than the number of postsynaptic receptors. But does release of a single vesicle normally saturate these receptors? Answering this question is critical to understanding how the amplitude and variability of synaptic transmission are set and regulated. To test for NMDA receptor saturation, we compared responses to stimulation with single and double pulses. We found that a single release event does not saturate spine NMDA receptors; a second release occurring 10 msec later produces ~80% more NMDA receptor activation. Our results imply that the amplitude of spine NMDA-receptor-mediated [Ca^{++}] transients (and the synaptic plasticity which depends on this) may thus be sensitive to the number of quanta released by a burst of action potentials and to changes in the concentration profile of glutamate in the synaptic cleft.

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STRUCTURE AND FUNCTION OF BRAIN CIRCUITS

D.B. Chklovskii

I am interested in discovering key organizing principles of the brain and applying these principles to understand brain function. One such principle, that of wiring economy, proved to be particularly useful for understanding the organization of the brain. This principle, which goes back to Cajal, asserts that the brain has evolved under pressure to keep its volume to a minimum. Thus, for a given circuit, the layout of brain components, such as neuronal bodies and synapses, should minimize the length of wiring, i.e., that is axons and dendrites.

WIRING CATASTROPHE IN THE BRAIN

This work was carried out in collaboration with C.F. Stevens (Salk Institute). The mammalian neocortex has an astronomical number of connections: A microliter of gray matter contains approximately 10^5 neurons, 10^9 synapses, and 4 km of axons. In a mouse cortex, 60% of the gray matter is taken up with "wire," half of this by axons and the other half by dendrites. Because of such high volume occupied by wire, any layout inefficiencies resulting in additional wiring would be very costly: Wire would encroach upon the space that could be devoted to "non-wire" such as neurons and synapses. In view of this, evolution must have optimized wiring the brain. This suggests solving a wiring optimization problem to understand organizing principles of the cortex.

Suppose we are given the wiring diagram of the brain that lists all of the neurons and their interconnections. Our task is to design a brain that implements this wiring diagram. This task can be broken into two stages. First, we need to find an optimal placement of non-wire components: neurons and synapses. Second, we need to determine the optimal parameters of the wire components: axons and dendrites. Here, we focus on the second stage and derive the optimal volume fraction taken up by axons and dendrites for fixed relative positions of neurons and synapses. Anatomical data suggest that the observed volume fraction is close to the prediction.

On this basis, we argue that settling on a suboptimal layout in the first stage cannot be offset by an increase in volume but degrades performance by reducing processing speed or simplifying wiring diagram. This happens because of the sequence of events which we call the wiring catastrophe. Layout inefficiencies lead to longer wiring. To maintain the same time delays in axons and attenuation in dendrites, they must be thicker, thus occupying greater volume. This pushes neurons and synapses farther apart. Thus, the wiring length must increase, and so on, resulting in a runaway volume growth.

THEORY FOR THE GLOBAL STRUCTURE OF THE OCULAR DOMINANCE PATTERN

Ocular dominance patterns in the primary visual cortex have been actively studied ever since their discovery about 30 years ago. However, several crucial questions about the appearance of the patterns remained unanswered. One of the questions is what determines the global structure of ocular dominance stripes on the cortical surface. Because the structure is reproducible between different animals, there is likely to be a functional reason.

I predict the orientation of ocular dominance stripes on cortical surface by analyzing the role of VI in binocular stereopsis. If one calculates the predominant direction of stereo disparity in different regions of the visual field and then maps this direction back onto the cortex, the ocular dominance stripes should run in the same direction. This relationship minimizes the length of wiring required to make the appropriate binocular connections between neurons representing the disparities. My theory agrees with the observations made in macaque and Cebus monkeys. By relating the appearance of the maps to the cortical circuitry, my results provide a tool for analyzing the function of the brain circuits. These are timely results because fMRI is about to achieve resolution sufficient to image ocular dominance patterns in humans.

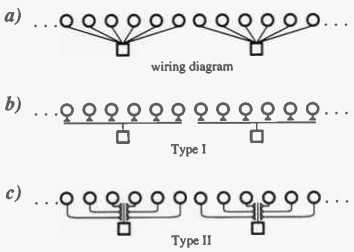

FIGURE 1 The wiring diagram (*a*) for a projection between the layers of input (*circles*) and output (*squares*) neurons can be implemented in two ways; (*b*) wide dendritic arbors and no axonal arbors; (*c*) wide axonal arbors and no dendritic arbors. The neuronal circuit in *b* is preferred because it has shorter wire length than *c*. Convergence of a neuronal circuit favors wide dendrites and no axons.

DERIVATION OF A RULE RELATING DENDRITIC AND AXONAL ARBOR SIZES TO CONVERGENCE AND DIVERGENCE IN NEURONAL CIRCUITS

Understanding brain function requires knowing connections between neurons. However, experimental studies of interneuronal connectivity are difficult, and the connectivity data are scarce. At the same time, neuroanatomists possess much data on cellular morphology and have powerful techniques to image neuronal shapes. In this situation, I propose to use morphological data to infer interneuronal connections. Such inference must rely on rules that relate shapes of neurons to their connectivity.

I use the principle of wiring economy to derive a rule that relates axonal and dendritic arbor sizes to the convergence/divergence of neuronal connections. Qualitatively, the rule states that in a projection between two neuronal layers, neurons in the sparser layer should have wider arbors. I derive a quantitative version of this rule and test it against experimental data. The existing anatomical data from different brain areas such as retina, cerebellum, olfactory bulb, and cerebral cortex support the rule. Because cellular morphology is much better known than neuronal connectivity, this rule provides a useful tool in unraveling brain circuitry.

In Press

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COLD SPRING HARBOR LABORATORY FELLOWS

In 1986, Cold Spring Harbor Laboratory began a Fellows program to encourage independent research by outstanding young scientists who, during their graduate studies, displayed exceptional promise of becoming leading scientists of the future. The purpose of this program is to provide an opportunity for these young scientists to work independently at the Laboratory for a period of 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 Adrian Krainer (1987) and Scott W. Lowe (1995) are currently members of the faculty at the Laboratory. After nine years at the Laboratory, Carol Greider (1988) left to join the Department of Molecular Biology and Genetics at Johns Hopkins University School of Medicine. Eric Richards (1989) is currently in the Department of Biology at Washington University. After finishing his fellowship, David Barford (1991) returned to the Laboratory of Molecular Biophysics at Oxford University. Ueli Grossniklaus (1994) was a member of our faculty before leaving last year to join the Friedrich Miescher Institut in Basel, Switzerland. Marja Timmermans, the current Cold Spring Harbor Laboratory Fellow, joined us from Yale University in 1998.

PLANT DEVELOPMENTAL GENETICS

M. Timmermans P. Vahab
C. Merrick (URP)

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. 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. Early surgical experiments on shoot apices of potato (Sussex, *Nature 167:* 651 [1951]) suggested that the establishment of dorsoventral asymmetry in leaves requires a signal(s) from the shoot apex. Incisions that isolated the incipient leaf primordium from the meristem resulted in the development of radially symmetric organs. However, the genes involved in the initiation and patterning of lateral organs in plants are still largely unknown.

We are using transposable elements to generate mutants that affect the initiation and patterning of lateral organs in maize. In particular, we are analyzing the roles of two genes: *Leafbladeless1* and *Rough sheath2*. A better understanding of the function of these and other genes involved pattern formation will provide new insights into fundamental concepts of plant development. Variations in the expression patterns of such genes are likely to contribute to the diversity of leaf shapes among many plant species. More importantly, however, the isolation and characterization of genes involved in the patterning of leaves may facilitate the manipulation of plant architecture, which has become increasingly more important for aspects of crop yield such as shade tolerance.

ROUGH SHEATH2 IS REQUIRED FOR THE TRANSITION FROM STEM CELL-LIKE GROWTH OF THE MERISTEM TO DETERMINATE GROWTH OF LATERAL ORGANS

A family of homeobox genes, the *Knox* genes, is expressed in the shoot apical meristem and is required for the indeterminate growth of the tissue. Determinate lateral organs such as leaves are initiated by the recruitment of founder cells, which is facilitated by the down-regulation of *Knox* gene expression in a subset of cells within the meristem. The recessive leaf mutant *rough sheath2* (*rs2*) affects 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 number of founder cells recruited into *rs2* mutant leaves is also reduced. We showed that both phenotypes result from the misexpression of

KNOX proteins in *rs2* mutant leaf primordia. *rs2* thus functions as a negative regulator of *Knox* gene expression, and 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, which is the ortholog of the *Antirrhinum Phantastica* gene and encodes a Myb-domain protein. In wild-type apices, *Rs2* is expressed in young leaf primordia in a pattern that is consistent with the *rs2* mutant phenotype. However, the down-regulation of *Knox* gene expression, 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 protein 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. The numbers and sizes of such sectors varied among leaves and did not correlate with normal developmental domains. This variegated accumulation of KNOX proteins in *rs2* mutants suggests that *Rs2* represses *Knox* expression through epigenetic means.

We are currently studying the mechanism by which *Rs2* regulates *Knox* gene expression. We are taking several approaches to identify genes that act together with *Rs2* in this process. As a first step, we have generated *Rs2*-specific monoclonal and polyclonal antibodies. These will also be used to determine the precise expression pattern of *Rs2* throughout development and to analyze potential changes in *Rs2* accumulation in other maize mutants affecting leaf initiation and patterning.

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 previously shown that the recessive *leafbladeless1* (*lbl1*) mutation causes 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 of abaxialized sectors on the adaxial leaf surface. In addition, we have shown that the number of founder cells incorporated into *lbl1* leaf primordia is strongly reduced. These observations suggest (1) that *Lbl1* plays a direct or indirect part in the down-regulation of *Knox* genes during organ initiation, (2) that adaxial/abaxial patterning of the maize leaf primordia occurs within the meristem during initiation, and (3) that lateral growth of the organ occurs at the boundary between adaxial and abaxial domains. This latter point is particular intriguing because lateral growth of insect wings occurs similarly at the dorsal/ventral boundary.

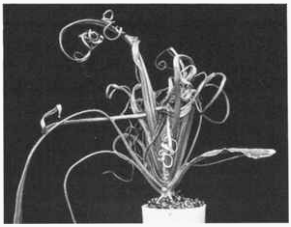

FIGURE 1 A maize plant that carries a mutation in the *leafbladeless* (*lbl1*) gene shows threadlike leaves and stunted growth. These and other defects result from the failure to establish the adaxial domain of the leaf. This dorsoventral patterning defect also affects lateral expansion of the leaf, which normally occurs at the adaxial/abaxial boundary. As a result, *lbl1* mutant leaves develop as radially symmetric rather than as flattened organs.

Several new alleles of *lbl1* were obtained from EMS and transposon-mutagenized populations. The most severe allele of *lbl1* results in embryo lethality. Even though the root system develops normally in the mutant, the shoot arrests early in development after the formation of a variable number of short filamentous organs. In addition, two new mutations with defects in dorsoventral patterning of the leaf have been identified. To characterize these and the different *lbl1* mutants in more detail, we have cloned the maize homologs of several *Arabidopsis* genes that are expressed specifically in either the adaxial or abaxial domains of the leaf. These maize homologs will be used in in-situ hybridization experiments. We have also begun to analyze the double mutant phenotypes of *lbl1* and the other dorsoventral patterning mutants. Preliminary results suggest that all mutations affecting dorsoventral patterning that have been identified so far, act in the same pathway.

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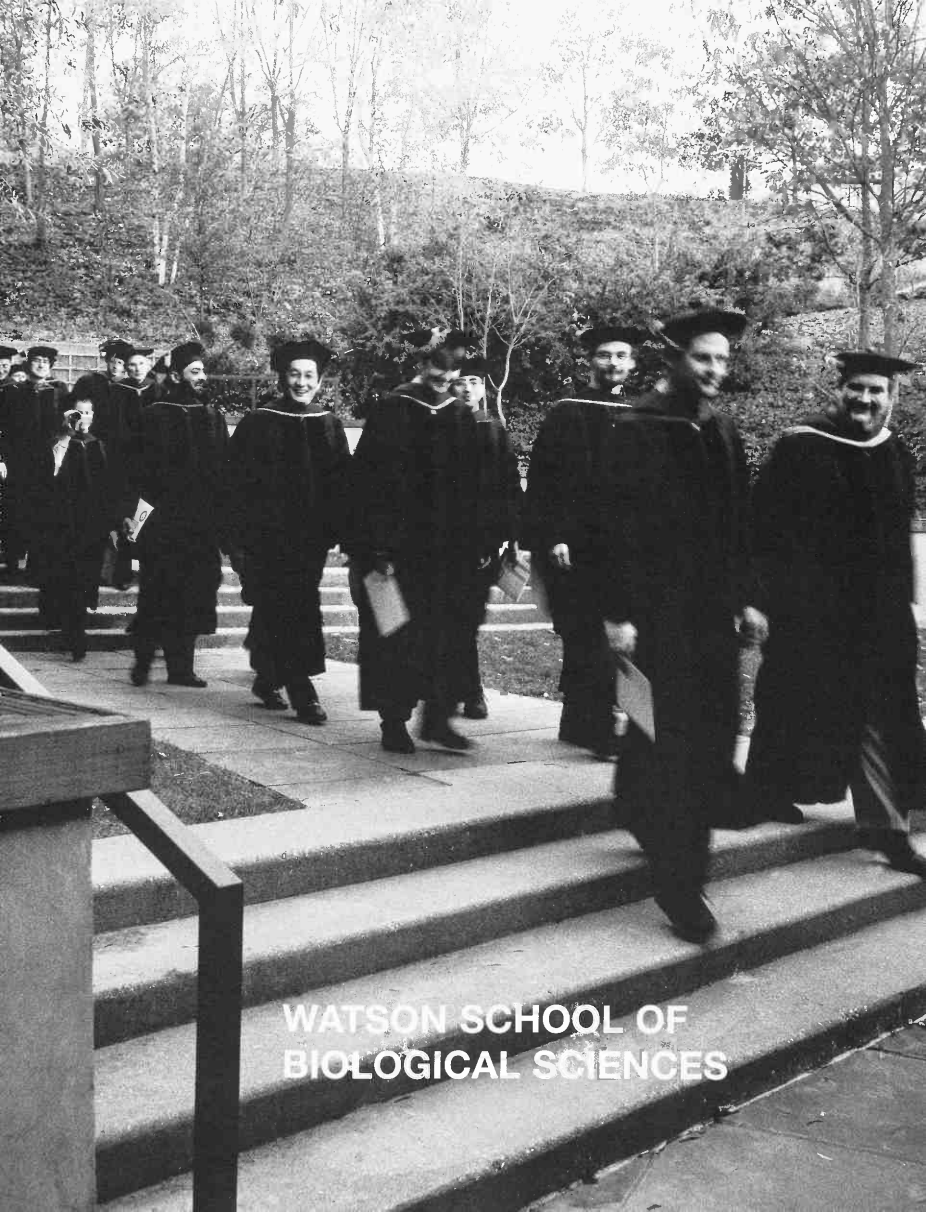

WATSON SCHOOL OF BIOLOGICAL SCIENCES

EXECUTIVE COMMITTEE MEMBERSHIP

Chair:	Winship Herr	
Faculty Members:	Hollis Cline	Robert Martienssen
	David Helfman	William Tansey
	Michael Hengartner	Jan Witkowski
	Scott Lowe	
Student Representatives:	John Mignone, SUNY Stony Brook	
	Michelle Cilia, Watson School	
Secretary:	Lilian Gann	

ADMISSIONS COMMITTEE MEMBERSHIP

Chair:	Winship Herr	
Faculty Members:	Shiv Grewal	David Jackson
	Gregory Hannon	Leemor Joshua-Tor
	Michael Hengartner	Lincoln Stein
	Nouria Hernandez	Jerry Yin
Secretary:	Lilian Gann	

EXTERNAL ADVISORY COMMITTEE MEMBERSHIP

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Chairman, Department of Cell and Molecular Pharmacology
University of California, San Francisco

Gail Mandel
Professor, Department of Neurobiology and Behavior, SUNY Stony Brook
Investigator, Howard Hughes Medical Institute

Marguerite Mangin
Associate Dean of Graduate Studies, The Rockefeller University

Barbara Meyer
Professor, Department of Biochemistry
University of California, Berkeley

Frank Solomon
Professor, Department of Biology and Center for Cancer Research
Massachusetts Institute of Technology

Shirley Tilghman
Professor, Department of Molecular Biology, Princeton University

WATSON SCHOOL OF BIOLOGICAL SCIENCES DEAN'S REPORT

On September 7, 1999, Cold Spring Harbor Laboratory opened its doors to the first class of the Watson School of Biological Sciences. What had been a vision for the new millennium only four years before had become a reality—a reality that was certain to have a significant change on the life of the Laboratory. The immediate reality was a major, year-long effort, involving all the elements of the Laboratory, to prepare for the students' arrival and the Laboratory's first academic term.

Recruitment of the Founding Class

The most important task at the beginning of the year was to recruit an outstanding founding class of five to six students. The school was aided by extensive news media coverage in the fall of 1998 engineered by the Public Affairs Department and its director, Deborah Barnes. Articles or news pieces appeared in *Science, Nature*, and the *Chronicle of Higher Education*, as well as local news media. Furthermore, we placed advertisements in the major scientific journals, the Watson School Web site was launched, and posters, brochures, and letters were mailed to biology departments and colleagues at academic institutions around the world. In return, the Laboratory received approximately 1000 requests for application forms. By the deadline of February 1, 1999, 130 students had submitted completed applications.

The Watson School's Admissions Committee had the daunting, albeit exciting, task of reviewing all the applications and narrowing them down to a group of candidates that it felt had the ability to excel in our new and intensive doctoral program. Twenty applicants were thus selected and invited to the Laboratory for interviews. Nineteen students accepted our invitation, and each enjoyed a two-to-three-day visit to the campus and discussions with 10 to 15 members of the research and nonresearch faculty. The candidates were all excellent. We found ourselves in the enviable position, as had been hoped, of choosing from among the world's most promising students. Nine students were offered places in the Watson School—six of them accepted. Each student has an outstanding academic record complemented by many enriching experiences, and two have been awarded the prestigious Howard Hughes Medical Institute predoctoral fellowship.

Cold Spring Harbor Laboratory has a long tradition of shared graduate programs with the State University of New York (SUNY) at Stony Brook. Before the Watson School was established, there was the concern that the new school might inadvertently discourage students from applying to the shared SUNY Stony Brook programs and, thus, possibly, lose them from the pool of students who come to Long Island for their doctoral studies. As a result of this concern, the Watson School and SUNY Stony Brook graduate programs in the life sciences established a co-application mechanism, whereby applicants to the Watson School could also automatically apply to the SUNY Stony Brook programs in Genetics, Molecular and Cellular Biology, Molecular Genetics and Microbiology, and Neurobiology and Behavior. We are pleased that as a result of this shared application mechanism, five students joined either the Genetics or the Molecular and Cellular Biology programs at SUNY Stony Brook this fall. This success was due in large part to the extraordinary efforts of Peter Gergen, chair of the joint Genetics and Molecular and Cellular Biology admissions committee. We have Lawrence Martin, dean of the Graduate School at SUNY Stony Brook, to thank for suggesting the successful co-application mechanism.

In establishing the Watson School, the Laboratory was, from the outset, hopeful that it would be able to recruit students who had participated in the Laboratory's summer Undergraduate Research Program. This program, which celebrated its 40th anniversary this year, has year after year recruited some of the best undergraduate talent in the world to spend 10 weeks in the summer at the Laboratory

to get a flavor for scientific research. Our hopes of attracting such students were not dashed. One of the entering Watson School students, Elizabeth Thomas, heralds from the undergraduate class of 1997, when she spent the summer studying pre-mRNA processing with Adrian Krainer—and established our Undergraduate Research Program Web site.

The successful recruitment of our founding class depended heavily on the efforts of all the Watson School faculty, including the Laboratory's president and director. Jim Watson and Bruce Stillman met extensively with nearly all the candidates. And Jim Watson was even known to follow up with some candidates by finding them at the Blackford dining hall during breakfast for a few additional words!

Preparing for the First Class

Our success in recruiting outstanding students invigorated us to prepare for the fall, which was fortunate because there was no time for pause. Over the spring and summer months, much effort was spent preparing for the Fall Course curriculum. A Core Course Coordination Working Group consisting of Lilian Gann, David Helfman, Michael Hengartner, Winship Herr, and William Tansey met regularly over the summer months to discuss the development of the Scientific Reasoning and Logic (SRL) and Scientific Exposition and Ethics (SEE) core courses and coordinate them with each other and the Specialized Disciplines courses. They met with instructors of the Specialized Disciplines courses to discuss the development of those courses (in bioinformatics, transcription, and neurobiology) and how they might best be integrated into a coordinated Fall Course curriculum. During this period, the SEE course instructors, Deborah Barnes, Adrian Krainer, and William Tansey, met regularly to discuss how to teach communication and ethics as one coherent course. The SRL instructors, Grigori Enikolopov, David Helfman, Michael Hengartner, Winship Herr, David Jackson, Scott Lowe, and W. Richard McCombie, divided the weekly segments among themselves and, together with other Watson School faculty, developed the individual problem sets and selected readings to be assigned for discussion with the students. To integrate and refine the course segments, the SRL course instructors took advantage of the Banbury Conference Center for a two-day retreat in August. Lastly, our assistant dean, Lilian Gann, put her organizational skills to the test by establishing a School calendar, so that all of the students and faculty would end up in an available classroom at the right time. All of these efforts proved essential to the success of the first Fall Course curriculum.

But not only courses had to be prepared. We also had to arrange to offer foreign students F1 visas, get facilities ready, and obtain equipment.

The task of obtaining F1 visa status for the School fell to Merilyn Simkins and Cheryl Sinclair from our Human Resources department. As spring became summer, so the possibility of obtaining our visa status in time appeared to diminish. In mid-June, it came to light that there was some confusion over our accreditation status. Barbara Meinert and Madeleine Ries of the State Education Department, who had helped us so much with getting our accreditation, came to our rescue, providing us with the necessary letters for the Immigration and Naturalization Service. Our F1 visa status came through at the end of June.

The school's permanent home, Urey Cottage, would not be ready for occupancy during the 1999–2000 academic year, so temporary accommodation was required. Art Brings, director of Facilities, and his team worked fast and furious to identify and prepare a study area for the students in the lower floor of the Wawepex building. They also had to prepare a suitable classroom for the Scientific Reasoning and Logic core course. The new Marks Imaging Building was in the final stages of completion and, again, Art and his team came through and were able to accelerate the work on the Gerry seminar room in Marks so that it would be available for our first class. We are extremely grateful for all their efforts.

The final hurdle in getting ready to deliver courses was getting the appropriate computer facilities in place. We had worked extensively with the director of Information Services, Gerald Latter, over the summer months, and things seemed to be going smoothly, or so we thought. We had received notification on August 27 that the students' laptop computers had been shipped and were all confident that

ENTERING CLASS OF 1999

Amy Anne Caudy, Washington University, St. Louis, Missouri
George A. and Marjorie H. Anderson Fellow
Howard Hughes Medical Institute Predoctoral Fellow

Michelle Lynn Cilia, Boston University, Boston Massachusetts
William R. Miller Fellow

Ahmet M. Denli, Bilkent University, Ankara, Turkey
David Koch Fellow

Emiliano M. Rial Verde, University of Buenos Aires, Argentina
David and Fanny Luke Fellow

Elizabeth Ellen Thomas, Evergreen State University, Olympia, Washington
Farish-Gerry Fellow
Howard Hughes Medical Institute Predoctoral Fellow

Niraj H. Tolia, Imperial College of Science, Technology, and Medicine, London, United Kingdom
Leslie C. Quick, Jr. Fellow

Seated left to right: Niraj Tolia, Michelle Cilia, Elizabeth Thomas
Standing left to right: Emiliano Rial Verde, Amy Caudy, Ahmet Denli.

they would be up and running by matriculation on September 7. On September 7, however, we received the following e-mail message from Jerry: *"I just got off the phone with the shipper of the laptops. The bad news is they got screwed up in shipment and ended up in Baltimore. The other bad news is that they do not at this moment know where they are."* The only thing left to do was laugh! Thankfully, Jerry and his team received the computers the following day and had them operational for our first week of the SRL course on macromolecular structure.

In addition to readying the facilities and equipment, we had to establish a handbook of School regulations! To help prepare this and learn about how graduate schools are run, Lilian Gann and I drove to Portland, Maine, in July to attend the Council of Graduate Schools' Deans' Workshop. There we learned from people who have been doing this much longer than we have. In addition to hearing how things can go awry—and be righted—we met many graduate school administrators, who gave us invaluable insights into what it is to be a graduate school, albeit the smallest one around!

With the help of what we learned in Maine, much of what we had already done to become accredited by the State Education Department, and a Rockefeller University handbook given to me 2 years earlier, Lilian Gann, with modest help from me, prepared a handbook for the entering Watson School students. And in the nick of time! It was well past midnight on September 7 when we finished it—matriculation took place just seven hours later.

The Founding Class Arrives

The students arrived during August and the beginning of September, and on September 7, 1999, the first class of the Watson School of Biological Sciences matriculated. It was truly a historic occasion for the Laboratory. The first class consists of six students (see previous page), each sponsored by a Watson School fellowship. They came from across the United States—Massachusetts, Missouri, and Washington—and abroad—London, Ankara, and Buenos Aires—and are interested in a wide range of fields, including bioinformatics, cancer, gene expression, neurobiology, plant biology, and structural biology. The students have adapted well to being pioneers in the Watson School. The permanent residence for the students—the Knight House—was not ready until the end of December; those students requiring Laboratory housing were placed in temporary housing on the grounds—some in a cabin in the woods in keeping with the pioneering spirit!

An Intensive Fall Course Curriculum

The goal of the Fall Course curriculum is to teach students how to think independently and critically so that in the future they can acquire, on their own, new knowledge in areas they have chosen to explore. The Fall Course curriculum provided intense instruction in a series of integrated interdependent courses. We were able to do this, in part, because we had maintained a small class size. Students could receive individual attention and participate actively in class activities.

One of the great unknowns before this fall was how faculty of an institution with a rich tradition of research, but not teaching, would respond to the demands of teaching. I am pleased to report that the response of the faculty was nothing less than exceptional. Essentially without exception, faculty devoted much effort to lectures and came out of the experience excited. The commitment of faculty was well beyond expectations—over 70% of faculty participated in teaching either as full course instructors or as guest lecturers. This success grew in large part out of the excellence and commitment of the six students. I often heard remarks from faculty about how they had enjoyed the interactions with the students during the lectures.

The three core courses in which the students participated during the fall were SRL, SEE, and Research Topics (a research seminar series by all research faculty), which spanned the length of the fall term. In parallel, students participated in three tandem, four-week lecture courses in the specialized disciplines. Students were also introduced to the Laboratory through the annual Laboratory-wide

Symposium, and graduate student and Laboratory-wide seminars. The schedule was demanding, to say the least—but we all made it! Because the courses had been so carefully integrated, we were able to gauge when we had overloaded the students and take corrective action. The students were also quick to bring concerns to our attention at the weekly "Dean's Tea," where each Monday afternoon Lilian Gann and I met for discussions with the students over tea and cookies. In addition to feedback via the teas, the SRL and SEE core course instructors met separately each week to review not only student progress, but also their own progress. These and other experiences have taught a new teaching faculty how to improve the course didactics.

The Fall Course term also saw the beginning of the academic mentoring program headed by William Tansey. To achieve a high level of mentoring and guidance, the Watson School instituted a "two-tier mentoring" program, through which each student has an academic mentor and a research mentor.

Shortly after matriculation, each student was assigned a faculty member as an academic mentor. To make the mentor assignments, self-selected faculty put their names forward as eligible mentors and students interviewed the ones they were most interested in. After the interviews, knowing the preferences of students and faculty, I matched them up for the mentoring program. Through regular meetings, the academic mentors are able to follow student progress and guide them through their academic development.

Special Events

The establishment of the Watson School of Biological Sciences has also generated great excitement within the institution generally. Last fall, there were two events that were centered around the School: a gala concert with Emanuel Ax, Yo-Yo Ma, and Midori, which helped raise valuable funds for the School, and an inaugural convocation of the Watson School.

Watson School Convocation

On November 5, the Watson School held its inaugural convocation. The purpose of the convocation was to celebrate the opening of the Watson School and to recognize the contributions of Cold Spring Harbor Laboratory to education in the biological sciences in its 109-year history. The Watson School awarded three scientists who best illustrate the Laboratory's contributions to education in the biological sciences—David Baltimore, Seymour Benzer, and Gerald Fink—the Honorary Degree of Doctor of Science. David Baltimore illustrates the importance of the Laboratory's Undergraduate Research Program, Seymour Benzer exemplifies how the Laboratory's postgraduate courses have influenced the careers of scientists, and Gerald Fink is a model of the influence and dedication of the Laboratory's postgraduate course instructors. Their contributions are described in greater detail below.

At the convocation, we were also honored to have Susan Hockfield, dean of the Graduate School of Arts and Sciences at Yale University and an alum of Cold Spring Harbor Laboratory, present the keynote address. In her address, she spoke of graduate education and the role Cold Spring Harbor Laboratory may play in this enterprise. She paid particular attention to the Watson School's goal of training doctoral students in a four-year program and pointed out how, through our programs in extensive mentoring, teaching students to think critically and logically, and emphasizing that learning is a lifelong endeavor, the School may succeed. For me, it was a very special speech because it laid out so clearly the goals of the Watson School.

The convocation also gave the Watson School an opportunity to put the achievements of one of its first-year students on display. Michelle Cilia, who hails from New Hyde Park on Long Island and is a former middle and high school student of the DNA Learning Center, is an accomplished singer. She gave a virtuoso performance of excerpts from "House of Comedy", an opera composed by Christian H. McLeer. Michelle and Christian have been friends since childhood, and he accompanied her singing on the piano. All in all, it was a wonderful occasion.

James D. Watson, "How Science Happens"

In recognition of Jim Watson's contribution to Cold Spring Harbor Laboratory and to research and education in the biological sciences, the Board of Regents of the University of the State of New York, on behalf of the State Education Department, on February 3, 1999, granted the Laboratory permission to name the School in Jim Watson's honor—we became the Watson School of Biological Sciences. In the fall, the School was further honored when Jim gave a series of scientific lectures to the Cold Spring Harbor Laboratory community to commemorate the opening of the School. These lectures, entitled "How Science Happens" and open to the public, gave the audience insights into scientific processes and covered four stages in Jim's career:

- discovering the double helix,
- George Gamow and his combinatorial approach to the genetic code,
- finding the genes of DNA tumor viruses that unlock cellular DNA synthesis, and
- recombinant DNA and the beginnings of the Human Genome Project.

Gavin Borden Lecture

The fifth Gavin Borden Visiting Fellow Lecture (so named after the energetic and charismatic publisher of *The Molecular Biology of the Cell*, who died in 1991 of cancer) was given by Joseph L. Goldstein, M.D., of the University of Texas Southwestern Medical Center. The lecture, entitled "A Proteolytic Pathway That Controls Cholesterol Content of Membranes, Cells, and Blood," was followed by a reception and dinner for the speaker with the graduate students. The next day, Dr. Goldstein met with Laboratory faculty and, after lunch with the faculty, with all the graduate students again for an informal discussion.

Career Pathways for the Biology Ph.D.

In the Career Pathways for the Biology Ph.D. seminar series, students and postdoctoral fellows are exposed to the many different career opportunities for Ph.D. graduates in biology. Biology Ph.D.s who have taken a variety of different career paths are invited to discuss their career choices and to describe how they use their Ph.D. training in their jobs. The guest speakers for this year's seminars were two former SUNY Stony Brook graduate students who studied at the Laboratory. George Tokiwa was a graduate student with Bruce Futcher and studied regulation of yeast cell growth. Carmella Stephens was a graduate student with Ed Harlow and studied the adenovirus E1A transforming protein.

- George Tokiwa, Ph.D., Orchid Biocomputer Inc.
  "From CSHL to Life in a Start-up Biotech Company ... a Personal Experience"
- Carmella Stephens, Ph.D., Baker and Botts, L.L.P.
  "Patenting in Biotechnology: Challenges for the Patent Attorney"

Shared Programs with SUNY Stony Brook

The six Watson School students were not the only students to arrive at the Laboratory in 1999. Although the Laboratory only became a degree-granting institution in 1998, it has been involved in graduate education leading to the Ph.D. degree in the biological sciences for over 25 years. Many graduate students from institutions with Ph.D.-degree–granting authority, most notably SUNY Stony Brook, have performed their doctoral research at Cold Spring Harbor Laboratory. The Laboratory remains committed to these shared programs with SUNY Stony Brook, which continue to be a source of excellent young scientists. Indeed, many of the Watson School faculty members have enjoyed the opportunity to design and teach graduate courses at SUNY Stony Brook and have found this invaluable in the

development of their own courses in the Watson School. This year, we welcomed the following students, all from SUNY Stony Brook, to Cold Spring Harbor Laboratory:

Student	CSHL Research Mentor	SUNY Stony Brook Affiliation
Anitra Auster	Leemor Joshua-Tor	Genetics Program
Emily Berstein	Gregory Hannon	Genetics Program
Hsu Hsin (May) Chen	Nicholas Tonks	Molecular and Cellular Biology Program
Laureen Connell	David Helfman	Molecular and Cellular Biology Program
Sujit Dike	Bruce Stillman	Molecular and Cellular Biology Program
Farida Emran	Nouria Hernandez	Department of Pharmacology
Ping Hu	Nouria Hernandez	Molecular and Cellular Biology Program
Ajit Janardhan	Jacek Skowronski	Genetics Program
Kendall Jensen	Hollis Cline	Department of Neurobiology and Behavior
Manpreet Katari	W. Richard McCombie	Genetics Program
Edward Kim	David Helfman	Genetics Program
Ingrid Koh	Karel Svoboda	Department of Applied Mathematics and Statistics
Sungwoo Lee	David Helfman	Department of Molecular Genetics and Microbiology
Shih-Chieh (Jack) Lin	Yuri Lazebnik	Molecular and Cellular Biology Program
Björn Schumacher	Michael Hengartner	Department of Molecular Genetics and Microbiology
Yvette Seger	Gregory Hannon	Genetics Program

The Watson School Administration

When I was named dean of the Watson School of Biological Sciences in the fall of 1998, I knew that one of my most important challenges was to establish an administration that would have the desire and vision to create a new school. In meeting this challenge, I have been most fortunate.

First, I was able to depend on the energy and experience of Jane Reader to help in the first critical phase, that of recruiting the founding class. Jane has worked for many years as administrator of the Undergraduate Research Program, first with me and then with Michael Hengartner since he took over the program in 1995. She had also helped me in earlier efforts to recruit students to our shared graduate programs with SUNY Stony Brook. In those roles, she gained much experience in how to manage a recruitment effort, a complex and intense enterprise, and she brought this expertise to the very successful recruitment of the founding class of the Watson School. The School is deeply indebted to Jane for that effort at such a critical time.

Just as the students began coming for interviews in early March, Lilian Gann arrived from England to assume the position of assistant dean. Good fortune played a role in this development, as the Laboratory simultaneously recruited her husband, Alexander Gann, to join the Laboratory Press as senior editor, textbooks. In Lilian Gann, the Watson School found the perfect candidate for assistant dean. Lilian received her Ph.D. in the biological sciences from the University of St. Andrews in Fife, Scotland, for her studies of 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 ICRF and also earned a master's degree in business administration (MBA) from the University of Westminster in London. In her role as administration manager, she was involved in both the graduate student and postdoctoral programs. Most recently, Lilian was director of cancer support services at CancerBACUP, a U.K. cancer charity focused on helping people live with cancer. Lilian's contributions to the Watson School so soon after her arrival have been striking. I regard recruiting Lilian to the Watson School as my single most important accomplishment in establishing the School.

In October 1999, the School's administration was further enhanced when Janet Duffy joined us as admissions and academic records administrator. Janet is a graduate of Hofstra University, where she majored in English literature, with a minor in secondary education. After a brief spell as a high school teacher, she switched to real estate administration and now, to our benefit, academic administration.

The Watson School may not have a large administration, but it is blessed with a very effective one!

Graduate Program Development and Oversight

Throughout the development of the graduate program, the School has relied on the support and commitment of the Laboratory's scientific and support staff, especially the School's Executive Committee. The Executive Committee meets monthly to address the wide range of issues involved in creating a new program and to oversee the program in general. Issues the committee addresses are numerous and include

- determining the roles of faculty in the program,
- overseeing the committees for admissions and qualifying examinations,
- monitoring graduate student progress, and
- developing and monitoring the curriculum.

The faculty and students on the committee have been instrumental to the process and have devoted considerable time and intellectual resources to the program's development.

External Advisory Committee

Evaluating and implementing changes for improvement are critical to establishing and maintaining an exceptional graduate program. In this, we are assisted by an External Advisory Committee comprising senior figures in science and graduate training. The External Advisory Committee evaluates the quality and effectiveness of our academic programs and provides advice and guidance. The committee visited the Laboratory in April 1999, primarily to review the planned Fall Course curriculum. At that time, they also interviewed the Laboratory and School administration, faculty, students, and postdoctoral fellows. After this visit, the External Advisory Committee prepared a report of its findings, together with recommendations for improving the graduate program. These findings and recommendations were instrumental in highlighting areas of change to improve the Fall Course curriculum that could be implemented over the spring and summer months. We were exceptionally pleased with the enthusiasm the External Advisory Committee expressed for the Watson School graduate program.

A Dedicated Endowment

One of the very special features of the Watson School is the commitment the Laboratory has made to ensuring its success by raising an endowment dedicated to the support of the School. By covering the costs of the four-year training of the graduate students, including tuition, stipend, and research expenses, the School can guarantee that the education of each student remains focused on his or her academic development. The Watson School's capital campaign has been immensely successful in large part because of the dedicated efforts of its chairman, David Luke, and the generous contributions of our many benefactors. I am especially thankful to Marjorie and William Matheson for endowing the George A. and Marjorie H. Anderson Founder's Endowment. Without that early and generous support the Watson School could not possibly have begun in such a splendid manner.

In Conclusion

And so it has been an exciting year indeed! This adventure of establishing a new school has been a resounding success because of the support and contributions of the entire Laboratory community and its friends. I have had a most marvelous experience working with so many outstanding and generous colleagues. If such dedication continues, the School is guaranteed success as it grows and flourishes.

April 2000

Winship Herr

FALL COURSE CURRICULUM

CORE COURSES

Scientific Reasoning and Logic

FUNDED IN PART BY **George A. and Marjorie H. Anderson Founder's Endowment**

INSTRUCTORS **Grigori Enikolopov**, **David Helfman**, **Michael Hengartner**, **Winship Herr**, **David Jackson**, **Scott Lowe**, **W. Richard McCombie**

GUEST LECTURERS **Alexander A.F. Gann**, **Gregory Hannon**, **Leemor Joshua-Tor**, **Adrian R. Krainer**, **Yuri Lazebnik**, **Robert Martienssen**, **Jacek Skowronski**, **David L. Spector**, **Lincoln Stein**, **Arne Stenlund**, **Bruce Stillman**, **Linda Van Aelst**, **Michael Wigler**, **Jan Witkowski**, **Rui-Ming Xu**

VISITING LECTURER **James Konopka,** SUNY Stony Brook

A fundamental aspect of earning the Ph.D. is training in the pursuit of knowledge. In this core course, which forms the heart of the Fall Course curriculum, faculty covered (1) a broad base of knowledge in the biological sciences, (2) the scientific method, and (3) how to think critically. This course consisted of 13 weekly segments, each of which had a different theme. Each week, students read an assigned set of research articles (generally five articles) and provided written answers to a problem set that guided them through two (or, on occasion, one) of the articles. Twice weekly, students attended lectures related to the week's topic, which included concepts and experimental methods. During the week, the students met to discuss among themselves the assigned papers not covered by the problem set. At the end of each weekly segment, the students submitted their problem sets and spent the evening discussing with faculty the assigned articles not covered by the problem set. The weekly topics were

Week 1 Macromolecular Structure
Week 2 RNA Processing
Week 3 DNA Replication and Chromosome Dynamics
Week 4 Protein Synthesis, Protein Kinesis, and the Cytoskeleton
Week 5 Signal Transduction
Week 6 Cell-cycle Regulation
Week 7 Cancer Genes
Week 8 Apoptosis
Week 9 Virus-Host Cell Interactions
Week 10 Mobile Genetic Elements
Week 11 Genome Structure and Evolution
Week 12 Cell-Cell Communication
Week 13 Development

Research Topics

ARRANGED BY **Lilian Gann**
David Helfman

This course spanned the fall term. It provided students with an in-depth introduction to the fields of research that Laboratory scientists investigate. Students and faculty attended a weekly evening Research Topics seminar, at which faculty members presented seminars on their current research topics and methods of investigation. The students learned how to approach important problems in biology. These seminars, together with the annual fall in-house symposium, provide students with a basis for selecting laboratories in which to do rotations. The weekly speakers were as follows.

Week 1	Andrew Neuwald, Leemor Joshua-Tor, Rui-Ming Xu
Week 2	David Helfman, Adrian Krainer, Michael Wigler
Week 3	Nouria Hernandez, Tatsuya Hirano
Week 4	David Jackson, David Spector
Week 5	Grigori Enikolopov, Linda Van Aelst
Week 6	Shiv Grewal, Ryuji Kobayashi, Bruce Stillman
Week 7	Masaaki Hamaguchi, Arne Stenlund, William Tansey
Week 8	Gregory Hannon, Michael Hengartner, Yuri Lazebnik
Week 9	Winship Herr, Scott Lowe, Jacek Skowronski
Week 10	Robert Martienssen, Tim Tully, Jerry Yin
Week 11	W. Richard McCombie, Lincoln Stein, Michael Zhang
Week 12	Dmitri Chklovskii, Roberto Malinow, Karel Svoboda, Yi Zhong
Week 13	Hollis Cline, Zachary Mainen, Anthony Zador

Scientific Exposition and Ethics

FUNDED IN PART BY **Nicholas C. Forstmann and the Edward H. Gerry Visiting Lectureship**

INSTRUCTORS **Deborah M. Barnes**
Adrian Krainer
William Tansey

GUEST LECTURERS **Alexander A.F. Gann**
Terri Grodzicker
Nouria Hernandez

VISITING LECTURERS **Robert P. Charrow, Esq.,** Crowell & Moring LLP
Robert Day, University of Delaware
Ellie Ehrenfeld, National Institutes of Health
Boyce Rensberger, Massachusetts Institute of Technology

This core course offered instruction about the fundamental elements of scientific exposition—writing skills and public speaking—and ethics. The abilities to communicate effectively and to appreciate the intricacies of ethical issues are essential skills for biologists; communications and ethics were taught in a series of example-based lectures and discussion groups. Writing skills included the fundamentals of modern scientific English and the organization and preparation of papers, research abstracts, and grant

applications. Oral presentation skills were taught by scientists with excellent, albeit different, modes of presentation. Together with instructors, students also critiqued formal seminar presentations at the Laboratory. Instruction and discussions about ethics included the ethical implications of biological discovery for society, as well as the nature and boundaries of ethical behavior of individual scientists and their rights and responsibilities. A primary objective of the course was that students understand that exposition and ethics should be an integral part of scientific research. An added benefit of this course was that two of the guest lecturers, Robert Day and Ellie Ehrenfeld, were also able to give additional lectures to the Cold Spring Harbor Laboratory community.

SPECIALIZED DISCIPLINES COURSES

Fundamentals of Bioinformatics

FUNDED IN PART BY **Edward H. and Martha F. Gerry Lectureship**

INSTRUCTORS **Andrew Neuwald**
Lincoln Stein
Michael Zhang

In biology today, the computer is as essential a tool for research as the microcentrifuge and electrophoresis unit. With computer software, scientists can digest the enormous amount of information produced by the human genome project, answer questions about evolution, model complex processes such as signal transduction and gene regulation, and manage and organize experiments. This course provided a practical introduction to bioinformatics, including computational biology, biological data modeling, and laboratory workflow management. Topics covered included database basics, Web-based resources for genome maps and sequence data, elementary probability and statistics, protein- and DNA-sequence analysis, phylogenetic analysis, and molecular evolution.

Mechanisms of Transcriptional Regulation: From *E. coli* to Elephants

FUNDED IN PART BY **Martha Farrish Gerry Visiting Lectureship**

INSTRUCTORS **Winship Herr**
William Tansey

GUEST LECTURERS **Alexander A.F. Gann**
Nouria Hernandez

VISITING LECTURER **Walter Gilbert,** Harvard University

This course covered both prokaryotic and eukaryotic transcriptional regulation. The discussion of prokaryotic transcription was centered on a historical perspective of the discovery of key mechanisms of transcriptional regulation in the *Escherichia coli lac* operon. Discussions about eukaryotic transcription built on

the information shared from studies of prokaryotes and emphasized fundamental similarities—and important differences—between eukaryotic and prokaryotic transcriptional regulatory mechanisms. Two recurring themes in this course were the complementary nature of studies of diverse organisms and the importance of diverse approaches to scientific discovery.

Mechanisms of Synaptic Plasticity and Learning

FUNDED IN PART BY **John Klingenstein Lectureship**

INSTRUCTORS **Hollis Cline**
Roberto Malinow
Karel Svoboda

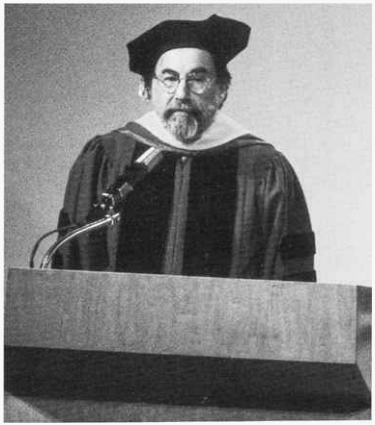
This course explored the cellular plasticity that underlies changes in brain function associated with development and with learning and memory. The study of the development of the visual system shows how organized topographic sensory projections develop in the central nervous system. Discussions of research on the mammalian hippocampus using behavioral, cellular, and molecular approaches were analyzed to describe the cellular basis of learning and memory in the adult brain. Ultimately, students gained an understanding of neural plasticity in development and in learning and memory.

WATSON SCHOOL OF BIOLOGICAL SCIENCES INAUGURAL CONVOCATION

On November 5, 1999, Cold Spring Harbor Laboratory celebrated the opening of the Watson School of Biological Sciences with an inaugural convocation. The event included a celebration of the 109-year history of science education at the Laboratory and the awarding of the Honorary Degree of Doctor of Science to three outstanding scientists—David Baltimore, Seymour Benzer, and Gerald Fink. Following are the citations that describe each recipient's association with educational activities at the Laboratory, and the respective acceptance speeches.

David Baltimore

Citation



David Baltimore is president of the California Institute of Technology. Born in New York City on March 7, 1938, Dr. Baltimore received his B.A. degree from Swarthmore College in 1960 and his Ph.D. degree in biology from Rockefeller University in 1964 for studies on RNA viruses, including poliovirus. After postdoctoral studies at the Massachusetts Institute of Technology and Albert Einstein College of Medicine with James Darnell and three years at the Salk Institute as a research associate, Dr. Baltimore joined the MIT faculty in 1968. There, in 1970, Dr. Baltimore isolated, contemporaneously with Howard Temin, the reverse transcriptase enzyme, which copies RNA into DNA. In 1975, he shared the Nobel prize in Physiology or Medicine with Howard Temin for this extraordinary discovery. He has since been the founding director of the Whitehead Institute, president of Rockefeller University, and chairman of the National Institutes of Health AIDS Vaccine Research Committee.

In 1959, while an undergraduate at Swarthmore College, Dr. Baltimore spent the summer at Cold Spring Harbor Laboratory as a member of the first class of the Undergraduate Research Program. He studied the genetics of bacteriophage viruses with George Streisinger. Dr. Baltimore best illustrates the influence the Laboratory's Undergraduate Research Program has had in preparing outstanding scientists in the biological sciences.

Acceptance Speech

"The Cold Spring Harbor Laboratory has always embodied for me the deepest values of science. The emphasis on basic advances, the commitment of the staff to a full-time life in science, makes the Lab an ideal to emulate. So, I can't think of any higher honor than to receive this honorary degree from the

Watson School, especially because it comes for the work I have done in the field that I chose here so many years ago.

"In 1959, I was in the first URP class, and I had no idea how lucky I was. I came here because I had heard about bacteriophages and I wanted to see what the plaques they made looked like. That odd impetus—and my own knowledge that Cold Spring Harbor was just down Route 25A from my hometown of Great Neck—changed my life. It is a remarkable symmetry of my career that I was in the first URP class here and am now the recipient of the first honorary degree from the Watson School.

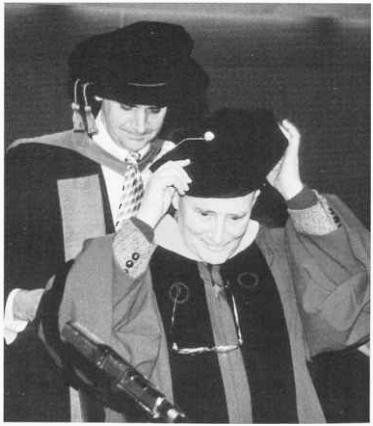
"The Watson School is sure to do great things. Many people say that the world doesn't need—that America doesn't need—more Ph.D.s. But I think there are never too many people of excellence who have been trained by the kind of faculty that is here. This faculty is able to impart to young students an understanding of what they have gotten into and where they can go with it. I'm not positive that four years is enough for a Ph.D. today. It is a complicated world that the students are entering. But it is an experiment worth trying—in a sense, going back to. This school is new, but there is no question that the faculty has the right stuff to make it a school of great excellence.

"I am relatively new to Caltech. I've only been there for two years, and I still see Caltech from the outside. Caltech, to me, has redefined excellence because what it means there—and what it should mean anywhere—is only doing things when you can do them exactly the way you feel is right. It means having the best people and very deep resources—then you can insist that only the best students are good enough to come and work with the people at your institution.

"As I walk around here, I've been comparing Cold Spring Harbor in 1959 and Cold Spring Harbor now. I think of it then as a few, sort of quaint shacks on the harbor—and now they have metamorphosed into palaces. It's like a sort of skinny, ugly caterpillar that emerges as an elegant butterfly. There is no secret who is the magician: Jim Watson, a great builder of institutions. And now his institution is to metamorphose again, this time into a school. Congratulations Jim, Bruce, and Dean Winship. Thank you."

Seymour Benzer

Citation



Seymour Benzer is James Griffin Boswell professor of neuroscience at the California Institute of Technology. Born in New York City on October 15, 1921, Dr. Benzer received his B.A. degree from Brooklyn College in 1942 and his Ph.D. degree in physics from Purdue University in 1947 for studies on germanium crystal rectifiers, a predecessor to the transistor. After reading *What Is Life?* by Erwin Schrödinger and learning of Max Delbrück's studies of mutations in phage, Dr. Benzer was convinced by Salvador Luria to attend the Cold Spring Harbor Laboratory Phage Course in the summer of 1948—the same summer that James Watson first came to the Laboratory as Luria's graduate student. While attending the Phage Course, Dr. Benzer decided to change fields and subsequently became a preeminent biologist. His early studies

focused on the structure of the gene through an exquisitely detailed genetic analysis of the *rII* region of phage T4. During the 1960s, Dr. Benzer switched his interests to the study of animal behavior, using *Drosophila* genetics to uncover genes that control behavior. Dr. Benzer has been widely recognized for his outstanding contributions to the biological sciences. He was awarded the Lasker award in 1971 and the National Medal of Science in 1983. As a preeminent biologist who was in part directed to the biological sciences by his attendance at a Cold Spring Harbor Laboratory course, he exemplifies the impact Laboratory courses have had on scientists in the field of biology.

Acceptance Speech

"This morning, I gave a lecture on the current work of my group at Caltech in seeking the genetic basis of longevity. Afterward, Gerald Fink brought up a quotation from Mark Twain saying that a better design would be to be born at 80 and age backwards, ending up around 16. That kind of time travel has not yet been achieved. We are working on it.

"The best means I can offer for going backwards is to show some photographs of Cold Spring Harbor, taken when I was a student in the Phage Course in the summer of 1948. There, I underwent a quantum change from physics to biology, simply as a result of 3 weeks of exposure to bacteriophage. The first slide shows a picture of myself in the course, when I had more hair. In the background, you can see Gunther Stent, another later-to-become distinguished member of that class. The third person is Bernie Davis, to whom I have always been grateful because he was the first person to explain to me what an antibody was.

"At that time, little enough was known about bacteriophages that it was possible to know almost everything that had been done. Once you mastered the one-step growth experiment, you could begin. The biggest hurdle in getting started was learning how, while holding a test tube in one hand, to hold both a cotton plug and a pipette in the other. The rest was easy. I am forever indebted to my lab partner, Peggy Lieb, for having taught me that.

"One of the traditions was, at the end of the Phage Course, to conduct a costume party graduation. The main activity was worshipping a portrait of Max Delbrück, who had initiated the course, although, by then, it was given by Mark Adams. In the picture, you can also see Salvador Luria, just below the string at the left, and others who have since had distinguished careers.

"I must say that I am taken aback by the austerity of today's occasion, which is in utter contrast to all the earlier experiences I have had at Cold Spring Harbor. Informality then extended to everything. As proof, I offer this picture of Andre Lwoff. I know how he behaved in Paris because I spent a year at the Pasteur Institute in his laboratory, where he was very much the *patron*—distinguished, polite, and formal. And here he was at Cold Spring Harbor shooting a water pistol. I believe he was aiming at me, because I was holding the camera, in an attempt to destroy the evidence.

"Jim Watson, at that time, was making his living by waiting tables in Blackford. That is Jim on the right side of the picture, unfortunately in a fringe area. Although he has risen from that lowly status to where he is now, it is ironic that he is still very much preoccupied with getting food onto the table. In the next picture, we see how he was attired in those days. Jim made a practice of keeping his sneaker strings untied. If some kind person, out of pity, tied them up, Jim would immediately untie them again. You can see some of the changes that occur with



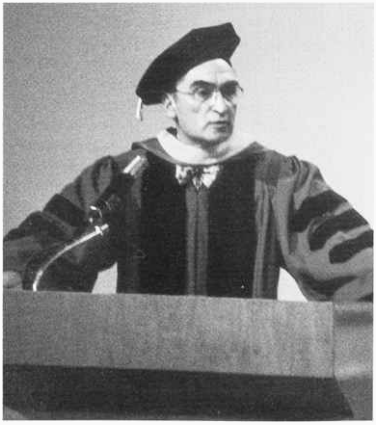
age in morphology, behavior, and attire, by comparing with the contemporary version sitting here on the stage.

"Finally, on one occasion, when Jim sampled the food at Cold Spring Harbor, he decided that something simply had to be done about it, as you can see in the picture. I recall a group reaction, on one occasion, to dehydrated eggs, which may have helped to turn the tide.

"So, I must say thank you to Cold Spring Harbor, Jim Watson, Salvador Luria, Max Delbrück, and the unusually competent and dedicated people who keep this great place running, and who will surely make this already world-class school into one having the name as well as the game."

Gerald R. Fink

Citation



Gerald R. Fink is director of the Whitehead Institute for Biomedical Research and American Cancer Society Professor of Genetics at the Massachusetts Institute of Technology. Born in Brooklyn, New York, on July 1, 1940, Dr. Fink received his B.A. degree from Amherst College in 1962 and his Ph.D. degree in genetics from Yale University in 1965. After performing his postdoctoral studies with Bruce Ames at the National Institutes of Health, Dr. Fink joined the faculty at Cornell University in 1967, where he became professor of biochemistry in 1979. In 1982, he moved to the Massachusetts Institute of Technology as professor of molecular genetics. Dr. Fink is known for his pioneering work in genetics. He has developed yeast as a model system for studying the basic molecular biology of eukaryotic organisms. He was the first to show that DNA could be taken up by yeast, leading to its stable transformation, and he has elucidated mechanisms for the control of amino acid synthesis and sexual reproduction in yeast. More recently, he has been a leader in the development of the small flowering plant *Arabidopsis thaliana* as a model for the study of plant development. He was elected to the National Academy of Sciences in 1981 and received the Genetics Society of America

Medal in 1982. In 1970, encouraged by James Watson who had recently assumed the Laboratory directorship, Dr. Fink initiated the well-known Cold Spring Harbor Laboratory Yeast Course with Fred Sherman. Four years later, Dr. Fink additionally joined David Botstein and John Roth on sabbatical leave at the Laboratory. Drs. Fink and Sherman continued to teach the Yeast Course for 17 years —It played, and continues to play, a seminal role in the development of yeast as a model organism for the study of eukaryotes. Many of the leaders in the field of yeast biology were introduced to yeast through this course. Dr. Fink best illustrates the extraordinary intellectual leadership, teaching ability, and dedication of the Cold Spring Harbor Laboratory course instructors who are responsible for the Laboratory's pre-eminent role in postgraduate education in the biological sciences.

Acceptance Speech

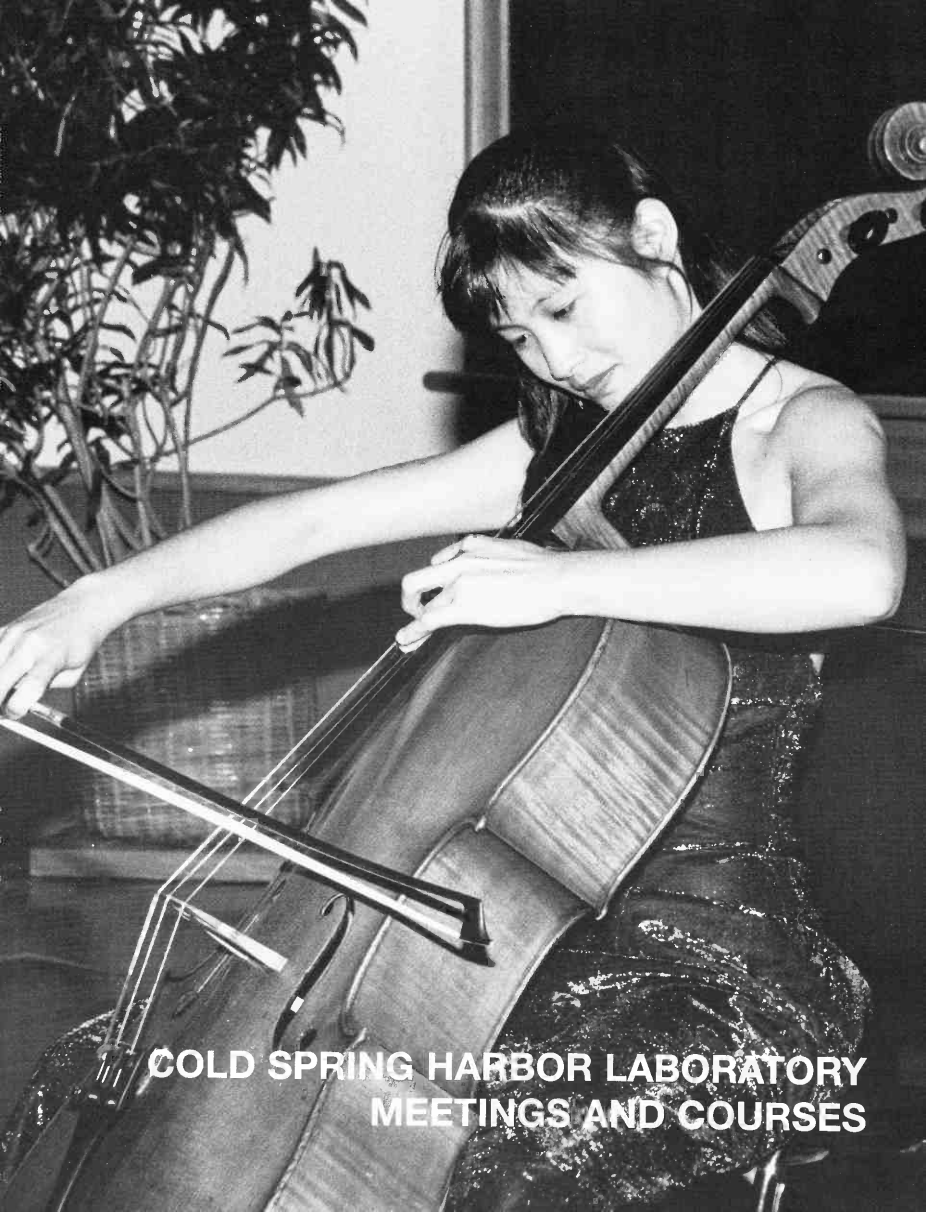
"You have heard that I taught here for 17 years, and as I reflect on my years of teaching here at Cold Spring Harbor, it's very easy for me to understand why every summer I was magnetically drawn back here. Personally, the Lab was enormously hospitable to my family and me. For a month in the summer, my wife, Rosalie, and my two daughters, Julie and Jennifer, were guests of the Cold Spring Harbor community and shared this lovely scenery, and perhaps as interesting, the spicy gossip that had been stored up over the winter.

"Scientifically, a Cold Spring Harbor course offered me the opportunity to seed a new field, because a course at Cold Spring Harbor was really a different challenge, different from anything that a university course offered. I think university courses are often captured by Peter Druckers' comment, "When a subject becomes totally obsolete, we make it a required course."

"A Cold Spring Harbor course is built on the presumption of novelty, and for me, teaching here had the allure of starting something different with the prospect of making remarkable discoveries. The techniques and concepts that we taught were really not in a single book, and you couldn't learn them at any university. This pioneering spirit gave us, both teachers and students, the shared sense that we were standing at the threshold of a golden lode. We knew there was great potential and we were eager to set about mining it.

"I must say that the isolated environment of Cold Spring Harbor contributed a kind of mystical quality that seems simultaneously to expect and portend great discoveries, and as a consequence, I as a teacher had the best of all possible situations: Curious students, desperate to learn, and smart enough to force me to learn. And furthermore, we had nothing else to do for three weeks. Actually, the longevity of the course required considerable political skill on my part because every year Jim Watson would call me into his office and declare, "This is the last year of the course!" and, "There can't be anymore interest in this subject!" This pronouncement was Jim's method of quality control. In the ritual psychodrama that followed, I would plead the case for just one more year to an ever more skeptical grand inquisitor. Inevitably, these sessions ended with Jim's comment, "Well, I guess we'll try it for one more year."

"Jim would not allow for obsolescence. And I detect this both in Winship and all of the staff, and I can see that Bruce is looking for the same kind of novelty. By 1989, Jim and I agreed that it was time for me to stop teaching the course. Our former students were professors that now populated every major university in this country and in Europe. Cold Spring Harbor has created, I think, the most unique postgraduate program in the world and it has built on the presumption of novelty. Today, we are celebrating the nativity of this Graduate Program. I am delighted to be honored at the birth of the Watson Graduate School, which I think is the child of a remarkable postgraduate program. Science will not be the same."



COLD SPRING HARBOR LABORATORY
MEETINGS AND COURSES

ACADEMIC AFFAIRS

With courses ranging from bioinformatics to the cloning of genes expressed in single neurons and with our meetings schedule highlighted by the Laboratory's world famous Symposium, the 1999 academic program was designed to serve the broader scientific community. This year, more than 6000 scientists, ranging from graduate students to senior faculty, participated in laboratory and lecture courses, workshops, and large conferences. These scientists represent an international group who come from universities, medical schools, research institutes, and industry.

The academic program includes a series of 3-week laboratory courses that are held in the summer, as well as 1- and 2-week laboratory courses that are given in the spring and fall. They are held in the Howard Hughes Medical Institute (HHMI) teaching laboratories in the Beckman Neuroscience Center and in Delbrück Laboratory. The Informatics courses take place in the Bush Auditorium, which is adapted for them, and the Neurobiology lecture courses are held at the Laboratory's Banbury Center. The new Marks Imaging Laboratory, which opened in the fall, contains a teaching laboratory and was inaugurated with a course on DNA Microarrays.

Courses cover areas including molecular biology, structural biology, neurobiology, genomics, and informatics. A new course on Genome Informatics was added this year, joining the course on Computational Genomics. Both were heavily oversubscribed, highlighting both the quality of the courses and the need for training in this area. In fact, all of the courses had more applicants than could be accommodated, which is a reflection of their timeliness as well as the expertise of the instructors. The instructors include a few Cold Spring Harbor faculty, but most come from universities and research institutes from around the world. Their energy, enthusiasm, and very hard work are the key to the success of the program. Many of them return for several years to teach at the Laboratory for which we continue to be very grateful. Course instructors, assistants, lecturers, and students are listed in the following pages.

The courses were supported this year by grants from NIH, NSF, HHMI, the Esther and Joseph A. Klingenstein Fund, the Grass Foundation, the Merck Genome Research Institute, and the Burroughs Wellcome Fund. We were extremely pleased that our 4-year grant from HHMI was renewed this year and will continue to support neurobiology courses as well as a range of new courses. We also receive valuable loaned equipment and donated supplies from a variety of companies, which are listed below.

Nineteen meetings were held at the Laboratory this year, including our annual Symposium. Signaling and Expression in the Immune System was the title of the 1999 Symposium. The meeting brought together 445 scientists to discuss their latest research and was organized by Bruce Stillman. The other meetings covered a broad range of topics, extending from Telomeres and Telomerase to Microbial Pathogenesis and Host Response. Many of the meetings filled the Grace Auditorium to capacity, including Programmed Cell Death, Genome Sequencing and Biology, Retroviruses, and Mechanisms of Eukaryotic Transcription. An important feature of these meetings is that most talks are selected by the organizers from submitted abstracts. This not only results in presentation of the most recent and innovative work, but also allows more junior scientists to speak about their research. In fact, at many meetings and lectures, senior scientists announce that they had their first opportunity to present a talk at a Cold Spring Harbor meeting. The organizers, session topics, and session chairs for the 1999 meetings are listed below.

The success of the very large program of courses and meetings is also due to the skilled efforts of many people at the Laboratory. Two of these, who are very well known to the scientific community, are retiring this year. They are Micki McBride, the Course Registrar, and Herb Parsons, the Director of Audiovisual Services. Both Micki and Herb have worked with enormous professionalism, tact, and good humor to contribute in a very significant way to the excellence of the program. While Micki is

moving and will no longer be a neighbor, we are pleased that Herb will continue to do special projects for the laboratory. They will be replaced by their very able colleagues, Andrea Stephenson as the new Course Registrar, and Ed Campodonico as the new Manager of Audiovisual Services. The staff of the Meetings and Courses office handle all aspects of the day-to-day management of the programs of meetings and courses at the Laboratory. This ever-growing job is carried out with skill and efficiency by a group that includes Michael Glaessgen, Drew Mendelson, Kathy Ruffolo, Mary Smith, Marge Stellabotte, Andrea Stephenson, Diane Tighe, Jenna Williams, and Barbara Zane. Housing and front desk operations are skillfully handled by Nancy Weeks, Donna Dykeman, and Andrea Newell, and audiovisual services are ably provided by Ed Campodonico, William Dickerson, John Parsons, and the part-time audiovisual staff. Staff from several other departments are crucial to the success of the courses: Wendy Crowley manages the educational grants; Cliff Sutkevich and his staff set up and maintain the courses equipment; the Information services group under Gerry Latter provide computer support for all courses; and Leigh Johnson in the Library handles the book and journal needs of the course program.

Terri Grodzicker
Assistant Director for Academic Affairs

David Stewart
Director of Meetings and Courses

Symposium 64 barbecue.

64th COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

Signaling and Gene Expression in the Immune System

June 2–7 445 participants

ARRANGED BY **Bruce Stillman,** Cold Spring Harbor Laboratory

The concept of this Symposium emerged from two separate sources. During the past decade, our understanding of the signal transduction pathways that lie downstream from cytokine action at the cell surface has advanced rapidly. As a consequence, I had imagined that a symposium on cytokines would be of considerable interest on its own, especially given the medical importance of the topic. At the same time, discussions with Stephen Smale and Rudi Grosschedl one summer during the Cold Spring Harbor Laboratory Gene Expression Course led to the suggestion of a symposium dealing with gene expression in the immune system, events that lie at the other end of immune cell signaling pathways. The obvious solution was to merge these two interesting topics.

Although not meant to be a Symposium covering all aspects of modern immunology, the time was certainly right to hold another meeting on some aspect of immunology in this series, now in its 64th year. The Antibodies Symposium in 1967 followed immediately after the cracking of the genetic code, itself celebrated at the 1966 Symposium, and naturally focused on immunoglobulin production and amino acid sequence diversity. The Origins of Lymphocyte Diversity Symposium in 1976 saw the beginnings of a molecular understanding of this diversity when Susumu Tonegawa presented his exciting discovery of immunoglobulin gene rearrangements that explained, in large measure, the mechanism of generating B-cell diversity. This meeting, held in the aftermath of the discovery by Doherty and Zinkernagel of MHC restriction, was also in the heyday of T-cell immunology.

It was nearly a decade and a half before the 1989 Symposium on Immunological Recognition heralded the understanding at the structural level of antigen presentation and host cell restriction. It was about this time that cytokine research was starting to take off and the concept of Th1 and Th2 T cells appeared. What has emerged since is a complicated, yet fascinating, story about how the immune cell network is controlled, both during development and in the adult. Cytokine signaling and differential gene expression underlie much of modern immunology research. The bringing together of these two apparently separate areas was an experiment that was worth trying. This field has been greatly aided by technologies such as gene deletions and gene replacements in mice, as well as by rapid developments in our general understanding about signal transduction and gene expression. If we really grasp how these complex signaling pathways interact and how they can be manipulated, by both extracellular and intracellular means, then medical problems such as inflammation, autoimmunity, and immune system disorders may lead to rational therapy. Manipulating the immune system by such means has long been a goal, but at the moment, unexpected results are more common than not.

The final program contained 72 oral presentations and 210 poster presentations, making it a busy five days for the 445 participants who packed Grace Auditorium.

The Symposium started with a fascinating first night of introductory talks from David Goeddel, Tak Mak, James Darnell, and Martin Gellert. I thank Richard Flavell for agreeing to summarize the meeting and for writing such a marvelous and thoughtful summary, matching the great summaries of previous symposia.

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, affiliates, and contributors of our meetings program is essential for these symposia to remain a success, and we are most grateful for their continued support. *Corporate Sponsors* include 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; Du Pont Pharmaceuticals Company; Eli Lilly and Company; Forest Laboratories; Genentech, Inc.; Genetics Institute; Glaxo Wellcome Inc.; Hoechst Marion Roussel; Hoffmann-La Roche Inc.; Johnson & Johnson; Merck Research Laboratories; New England BioLabs, Inc.; Novartis Pharma Research; 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; and Zeneca Group plc. *Plant Corporate Associates* include Monsanto Company; Novartis Agricultural Discovery; Kyowa Hakko Kogyo Co., Ltd ; Pioneer Hi-Bred International, Inc ; and Westvaco Corporation. We also thank our *Foundation Associate*, Albert B. Sabin Vaccine Institute, Inc , at Georgetown University; our *Corporate Affiliates*, Affymetrix, ICOS Corporation, and Sugen; and our *Corporate Contributors*, Alexis Corporation, Biogen, Inc., Genome Systems, CuraGen, Pharmingen, Qiagen, StressGen Biotechnologies Corp , Trevigen, Inc , and Zymogenetics, Inc

T. Honjo, M. Davis, C. Goodnow

Y. Linderson

M. Nussenzweig, U. Weiss

B. Stillman, S. Smale

PROGRAM

Introduction
Chairperson: B. Stillman, Cold Spring Harbor Laboratory

Gene Regulation and Chromatin
Chairperson: R. Grosschedl, University of California, San Francisco

T-cell Differentiation and Receptor Signaling
Chairperson: A. Weiss, University of California, San Francisco

Transcription Factors in the Immune System
Chairperson: T. Maniatis, *Harvard University, Cambridge, Massachusetts*

Cytokine Signaling
Chairperson: L. Glimcher, *Harvard School of Public Health, Boston, Massachusetts*

Recombination, Mutation, and Allelic Exclusion
Chairperson: K. Rajewsky, *University of Cologne, Germany*

Development
Chairperson: U. Storb, *University of Chicago, Illinois*

Cell Death and Survival
Chairperson: J . Allison, *University of California, Berkeley*

B Cells
Chairperson: F. Alt, *HHMI, Children's Hospital, Boston, Massachusetts*

Activation, Selection, and Commitment
Chairperson: M. Davis, *Stanford University School of Medicine, California*

Dorcas Cummings Lecture: Repairing the Body: The Promise of Blood and Tissue Stem Cells
Speaker: Irving Weissman, *Stanford University School of Medicine*

T-cell Receptor Signaling
Chairperson: K. Jones, *The Salk Institute, La Jolla, California*

Nf-κB and NFAT
Chairperson: S. Smale, *University of California, Los Angeles*

Summary: R. Flavell, *Yale University*

C. Janeway

R. Flavell, J.D. Watson

J. Biro, M. Reth

BIOTECHNOLOGY CONFERENCE

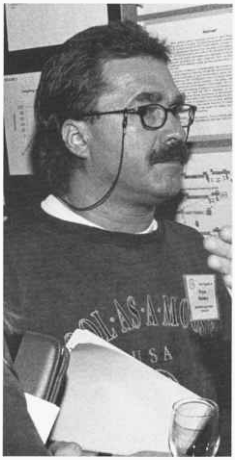
Vector Targeting Strategies for Therapeutic Gene Delivery

March 11–14 151 participants

ARRANGED BY **David Curiel,** University of Alabama, Birmingham
Wayne Marasco, Dana Farber Cancer Institute

The paramount requirement for advancement of gene therapy is the development of vector systems with the capacity for efficient and cell-specific gene delivery. The achievement of this goal requires that the vector system recognizes specific cell signatures. For efficiency, both nonviral and viral vectors have been engineered. For identification of cell-specific markers, a variety of high-throughput methods have been advanced. This special conference provided an update of key technologies relevant to developing vectors with efficient, cell-specific gene delivery. Since the last biotechnology conference on this topic, held in 1997, significant progress had been made in this field. Especially noteworthy was the progress in viral vector tropism modifications. This progress has predicted the translation of novel, advanced secretion vectors into human clinical applications. Future work will be required to define the range of cell-specific signatures of relevance in the clinical context. Nevertheless, the linkage of the two technologies—target definition and vector targeting—is already yielding important advancements in the outcomes achievable via gene therapy methods.

This meeting was funded in part by Transgene, S.A.; Selective Genetics, Inc.; Lilly Research Laboratories, A Division of Eli Lilly and Company; Gene Medicine, Inc.; Glaxo Wellcome, Inc.; Zeneca Pharmaceuticals; Genzyme Corporation; Berlex Laboratories, Inc.; Gen Vec, Inc.; SyStemix, Inc., A Novartis Company; Johnson & Johnson; and Megabios Corporation.



W. Marasco

D. Curiel

PROGRAM

Introductory Comments
Chairpersons: D. Curiel, *University of Alabama, Birmingham;* W. Marasco, *Dana-Farber Cancer Institute, Boston, Massachusetts*

Target Definition
Chairperson: W. Stemmer, *Maxygen, Inc., Santa Clara, California*

Retargeted Adenovirus
Chairperson: R. Hawkins, *Christie CRC Research Center, Manchester, United Kingdom*

Genetically Retargeted Adenovirus
Chairperson: D. Curiel, *University of Alabama, Birmingham*

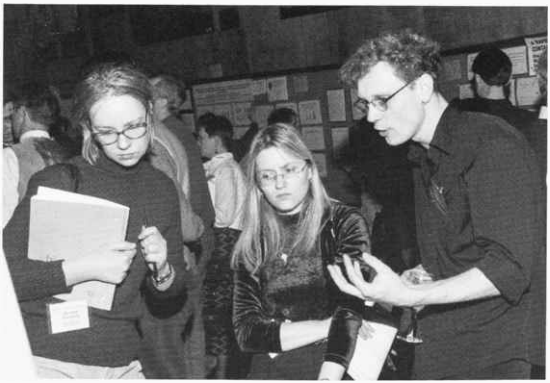
Retargeted Retroviruses
Chairperson: W. Marasco, *Dana-Farber Cancer Institute, Boston, Massachusetts*

Nonviral Vectors
Chairperson: P. Felgner, *Gene Therapy Systems Inc., San Diego, California*

Transcriptional Targeting
Chairperson: N. Lemoine, *Imperial Cancer Research Fund, London, United Kingdom*

Targeted AAV
Chairperson: J. Robbins, *Immusol, Inc., San Diego, California*

Emerging Technology
Chairperson: P. O'Hare, *Marie Curie Research Institute, Surrey, United Kingdom*



Poster session

SPRING MEETINGS

Telomeres and Telomerase

March 25–28 266 participants

ARRANGED BY **Elizabeth Blackburn,** University of California, San Francisco
Titia de Lange, Rockefeller University
Carol Greider, Johns Hopkins University School of Medicine

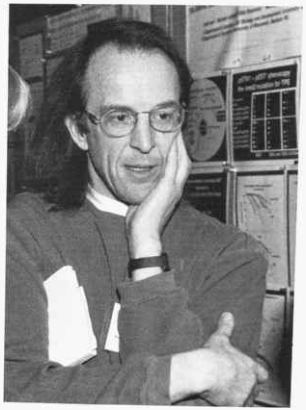
The conference consisted of six sessions of talks and two poster sessions. Each session consisted of two chairs; the chairpersons were established scientists in the field who not only chaired the session (one chair for each half session), but also gave a scientific presentation. This format was chosen to provide a high profile for this new Cold Spring Harbor conference. The rest of the presentations were chosen to represent as many younger scientists as possible, including graduate students and postdoctoral fellows. Attendance exceeded 260 participants, a large fraction of whom presented a total of about 100 posters and 55 talks.

The topics covered all aspects of telomere and telomerase biology, grouped by talk session as follows: telomere structure and function; telomere length regulation; telomeric chromatin and silencing; telomeres and recombination in mitosis and meiosis; regulation of telomerase and telomeres, and telomerase in proliferation, senescence, and cancer.

The scientific content was very high throughout the conference in both the talks and the posters, with significant amounts and quality of new, unpublished data being presented and extensively discussed. Discussion after each talk during the sessions was lively and informative. The conference was verbally judged to be highly successful by all who attended. Participants were very enthusiastic about having another meeting on the same topic in 2001.

This meeting was funded in part by the National Institute on Aging and the National Cancer Institute, branches of the National Institutes of Health.

C. Greider, A. Kass-Eisler



G. Gottschling

Telomerase Structure and Function
Chairpersons: E. Blackburn, *University of California, San Francisco*; C. Price, *University of Nebraska, Lincoln*

Telomere Length Regulation
Chairpersons: T. de Lange, *Rockefeller University, New York, New York*; V. Zakian, *Princeton University, New Jersey*

Telomere Chromatin and Silencing
Chairpersons: D. Gottschling, *Fred Hutchinson Cancer Research Center, Seattle, Washington*; D. Shore, *University of Geneva, Switzerland*

Telomeres and Recombination in Meiosis and Mitosis
Chairpersons: R. Allshire, *MRC Human Genetics Unit, Edinburgh, United Kingdom*; S. Roeder, *Howard Hughes Medical Institute, Yale University, New Haven, Connecticut*

Regulation of Telomerase
Chairperson: S. Bacchetti, *Mcmaster University, Hamilton, Ontario*

Telomeres and Telomerase in Proliferation, Senescence, and Cancer
Chairpersons: C. Greider, *Johns Hopkins University School of Medicine*; H. Cooke, *MRC Human Genetics Unit, Edinburgh, United Kingdom*

C. Harley, S. Primmer

V. Zakian, S. Roeder

Human Evolution

April 21–25 101 participants

ARRANGED BY **Luigi L. Cavalli-Sforza,** Stanford University
Douglas C. Wallace, Emory University School of Medicine

The Human Evolution conference brought together fossil, linguistic, and substantial amounts of molecular genetic information in an attempt to achieve a new synthesis in our understanding of human origins. New paleontological evidence was presented that supports a recent "out-of-Africa" origin for modern *Homo sapiens*, implying that modern humans arose once in Africa between 100,000 and 150,000 years before present (YBP), migrated out of Africa in perhaps two migrations, and replaced contemporaneous *Homo erectus* and Neanderthal individuals. This view is further supported by the recent comparison of Neanderthal and modern mitochondrial (mt) DNA sequences that reveals a striking molecular discontinuity between archaic and modern humans.

Molecular anthropological studies of maternally inherited mtDNA and paternally inherited Y chromosomes were presented which indicate that Africa harbors both the greatest genetic diversity and the oldest lineages, with the two complementary approaches yielding a remarkable consensus that modern humans arose in Africa about 143,000–144,000 YBP. Molecular analysis of variation in autosomal and X-chromosome loci has proved more cumbersome, but work presented at the meeting clearly indicates that the African populations are the oldest and the Native American populations are the youngest. This suggestion is remarkably consistent with the data from mtDNA and Y chromosomes. In contrast, early studies of primate automosomal and X-linked gene trees have given much older coalescence times. Additional analytical tools will be necessary to resolve these discrepancies. One promising approach discussed at this meeting is the analysis of recently arising transposable elements: SINES and LINES. Analysis of recent *Alu* repeats in Africans and Europeans indicates that these two populations diverged about 135,000 YBP.

Besides being used to address global questions of human origins, molecular anthropology has permitted novel approaches to the study of local population issues. Among a number of interesting

D. Nelson, K. Kidd

L. Cavalli-Sforza, S. Starostin

reports, particularly noteworthy was a Y-chromosome study of Jewish individuals with the family name of Cohen, purportedly direct descendants of the Jewish priesthood, which showed that a specific Y-chromosome haplotype is indeed markedly enriched in this population. Intriguingly, this same haplotype is also enriched in the South African Lemba, a black Bantu-speaking population whose members practice Jewish religious rituals and have an oral tradition of Jewish ancestry.

This type of study confirms that considerable historical information can be deduced from our genes. New sources of data and new analytical tools are needed now. A great deal of data remains to be extracted from the autosomal DNA. Moreover, more reliable mathematical methods will be required to accurately extract the information. Not only will such studies tell us much about human history, they will provide vital data on continent- and population-specific variation that will be important for localizing and cloning common disease genes.

This meeting was funded in part by the Oliver Grace Professorship Fund.

PROGRAM

Paleoanthropology and Paleodemography
Chairperson: C. Stringer, *Natural History Museum, London, United Kingdom*

Y Chromosome
Chairperson: P. Underhill, *Stanford University, California*

Roundtable: Language Evolution in Africa, Eurasia, America
Moderator: M. Ruhlen, *Stanford University, California*

mtDNA
Chairperson: D. Wallace, *Emory University School of Medicine, Atlanta, Georgia*

mtDNA, Y Chromosome, Autosomes Compared
Chairperson: L. Cavalli-Sforza, *Stanford University, California*

Primates
Chairperson: M. Weiss, *Wayne University, Detroit, Michigan*

Roundtable: Ethical Issues in Research in Human Evolution
Moderator: H. Greely, *Stanford University, California*

Linkage Disequilibrium
Chairperson: K. Kidd, *Yale University School of Medicine, New Haven, Connecticut*

Technology
Chairperson: P. Oefner, *Stanford University, California*

X Chromosomes
Chairperson: L. Jorde, *University of Utah, Salt Lake City*

Autosomes I
Chairperson: M. Stoneking, *Max-Planck Institute for Evolutionary Anthropology, Leipzig, Germany*

Roundtable: HGDP Organization
Chairperson: L. Cavalli-Sforza, *Stanford University, California*

Autosomes II
Chairperson: J. Bertranpetit, *Universitat Pompeu Fabra, Barcelona, Spain*

M. Stoneking, M. Kayser, S. White

O. Smino, P. Underhill

Learning and Memory

April 28–May 2 161 participants

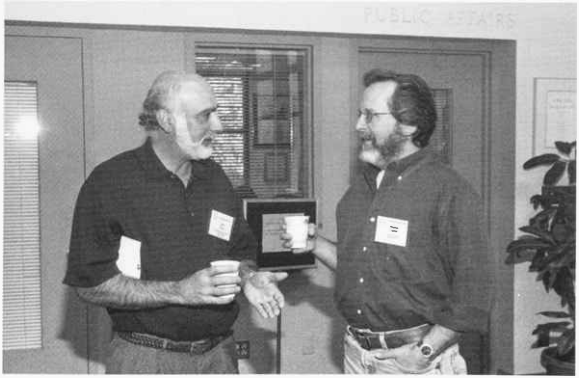
ARRANGED BY **Thomas Carew,** University of California, Irvine
Joseph LeDoux, New York University
Leslie Ungerleider, National Institute of Mental Health

The fourth biennial Learning and Memory meeting consisted of platform and poster presentations and discussions of current research and concepts in the biological basis of memory. Studing the biological bases of learning and memory is a most exciting field in neuroscience. Progress is currently very rapid, particularly at molecular, cellular, neural systems, and computational levels. Commonalties of mechanisms appear to be emerging from studies of gene expression, synaptic plasticity (LTP and LTD), and processes of memory storage in invertebrate systems. In particular, similar changes in the properties of membrane channels and intracellular messenger systems were identified in a number of different neural systems in both vertebrate and invertebrate preparations. Discussion topics ranged from brain systems of memory in humans through brain circuits and systems of memory, to brain plasticity and development, basic processes of learning and memory, mechanisms of synaptic plasticity—for example, long-term potentiation (LTP) and long-erm depression (LTD)—and biophysical and molecular substrates of synaptic plasticity, to genetic approaches to mechanisms of memory.

There were two special foci of the meeting. The first was a strong representation of studies of human memory. World leaders reported on their recent findings which show that rapid advances have been made in using imaging techniques such as PET and fMRI to probe the formation and retrieval of human memories in real time.

The second focus of the meeting was on the use of mutant and transgenic mice to identify the roles of gene expression of particular enzyme systems in synaptic plasticity and memory. The "gene knockout" approach is new and very promising. There was much discussion about the possibility of developing localized, inducible, reverse knockout preparations.

This meeting was funded in part by the National Institute of Neurological Disorders and Stroke and the National Institute on Aging, branches of the National Institutes of Health, and the National Science Foundation.



J. Byrne, T. Carew

PROGRAM

Cellular Analysis of Learning and Memory I
Chairperson: T. Carew, *Yale University, New Haven, Connecticut*

Cellular Analysis of Learning and Memory II
Chairperson: J. Ledoux, *New York University, New York*

Cellular Analysis of Learning and Memory III
Chairperson: H. Eichenbaum, *Boston University, Massachusetts*

Cognitive Analysis of Learning and Memory: Human Studies
Chairperson: L. Ungerleider, *NIMH, National Institutes of Health, Bethesda, Maryland*

Cognitive Analysis of Learning and Memory: Animal Studies
Chairperson: C. Barnes, *University of Arizona, Tucson*

Molecular Analysis of Learning and Memory I
Chairperson: T. Tully, *Cold Spring Harbor Laboratory*

Molecular Analysis of Learning and Memory II
Chairperson: E. Schuman, *California Institute of Technology, Pasadena*

Quantitative Analysis of Plasticity in Neurons and Networks
Chairperson: E. Marder, *Brandeis University, Waltham, Massachusetts*

K. Maubach, M. Bear

R. Malinow, C. Jennings

K. Rankin, S. Vollman

Biology of Proteolysis

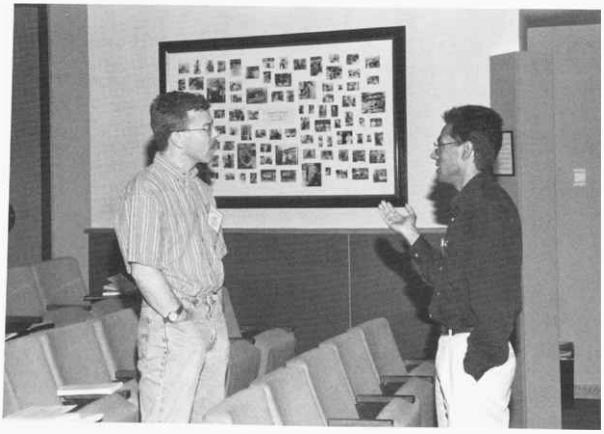
May 5–9 203 participants

ARRANGED BY **Charles S. Craik,** University of California, San Francisco
Mark Hochstrasser, University of Chicago
Susan Gottesman, National Cancer Institute
Yuri Lazebnik, Cold Spring Harbor Laboratory

Proteases are key regulators in a wide range of biological processes, from cell cycle and development to cell death. This biennial meeting provides a forum that fertilizes cross-talk between experts on these processes and experts on the proteases as enzymes.

This second meeting attracted more than 200 scientists, who discussed the role of proteolysis in signal transduction, gene regulation, development, cell cycle, control of protein expression, neoplasia and cell transformation, immune control, and cell death. The reported experimental systems ranged from prokaryotes to animals and plants, whereas the interests of speakers varied from the dissection of protease structure to regulation of cell cycle. Despite such a diversity, the participants commented that the meeting was focused, informative, and very exciting. The cross-talk was made possible in large part by the chairs, who presented extensive overviews at the beginning of each session. In summary, the Biology of Proteolysis meeting was a unifying forum that will increase the understanding of how proteases control life and death.

This meeting was funded in part by the National Cancer Institute; the National Institute of Child Health and Human Development; and the National Institute on Aging, branches of the National Institutes of Health; and the National Science Foundation.



M. Hochstrasser, K. Madura

PROGRAM

Signal Transduction
Chairpersons: J. Goldstein, *University of Texas Southwestern Medical Center, Dallas*; C. Gross, *University of California, San Francisco*

Complexes and Cascades
Chairpersons: W. Baumeister, *Max-Planck Institute for Biochemistry, Martinsried, Germany*; J. Degen, *Children's Hospital Research Foundation, Cincinnati, Ohio*

Chaperones, Quality Control
Chairpersons: A. Horwich, *Yale University School of Medicine, New Haven, Connecticut*; S. Wickner, *National Cancer Institute, Bethesda, Maryland*

Development
Chairpersons: L. Shapiro, *Stanford University, California*; T. Kornberg, *University of California, San Francisco*

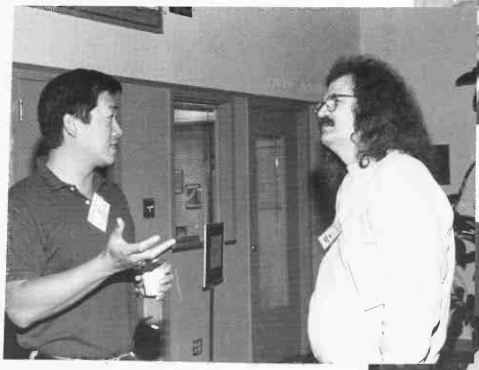
Cell Cycle
Chairpersons: M. Kirschner, *Harvard Medical School, Boston, Massachusetts*; J. Diffley, *Imperial Cancer Research Fund, South Mimms, United Kingdom*

Infectious Disease/Host-Pathogen Interaction
Chairpersons: H. Ploegh, *Harvard Medical School, Boston, Massachusetts*; J. McKerrow, *University of California, San Francisco*

Neoplasia
Chairpersons: Z. Werb, *University of California, San Francisco*; P. Howley, *National Cancer Institute, Bethesda, Maryland*

Cell Death
Chairpersons: N. Thornberry, *Merck Research Laboratories, Rahway, New Jersey*; S. Nagata, *Osaka Bioscience Institute, Japan*

C. Chu, D. Keopp



E. Lam, Y. Lazebnik

M. Holder, S. Gottesman

Tyrosine Phosphorylation and Cell Signaling

May 12–16 193 participants

ARRANGED BY **Nick Tonks,** Cold Spring Harbor Laboratory
Sara Courtneidge, SUGEN, Inc.
Ben Neel, Beth Israel Hospital, Harvard Medical School

This third meeting on Tyrosine Phosphorylation and Cell Signaling at Cold Spring Harbor opened with keynote addresses by Lew Cantley and Ernst Hafen. Dr. Cantley discussed various biochemical approaches to the study of signal transduction and illustrated how tyrosine phosphorylation and phospholipid-dependent signaling mechanisms may be integrated. Dr. Hafen reviewed some important contributions of genetic approaches to the study of cell signaling. The format of the meeting provided a combined emphasis on the physiological roles of protein tyrosine kinases and phosphatases and how their actions are integrated to modulate signaling events in vivo. The sessions were based largely around physiological processes and cellular functions to try to provide the biological context for the data. The program included scientists from the United States, Europe, the Far East, and the South Pacific. Fourty-three speakers were selected to present their data in sessions that dealt with protein kinases and adaptor molecules, hematopoietic cell signaling, adhesion-mediated signaling, kinases and phosphatases, signaling in the immune system, signaling in development, and signaling abnormalities in disease. A variety of systems were described, with great progress reported in genetic and biochemical approaches to the characterization of physiological functions for tyrosine phosphorylation. In particular, exciting insights were provided into how signaling pathways may be abrogated in a variety of human disease states. The meeting continues to be successful and alternates with a conference with the same format held every other year at The Salk Institute so there is an annual Tyrosine Phosphorylation meeting.

This meeting was funded in part by the National Cancer Institute, a branch of the National Institutes of Health; and the National Science Foundation.

P. Simoncic, A. Cheng, M. Ibarra-Sanchez, A. Davy

N. Tonks

PROGRAM

Keynote Lectures
Chairperson: B. Neel, *Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts*
Lew Cantley, *Beth Israel Deaconess Medical Center, Harvard Medical School*
Ernst Hafen, *University of Zurich*

Kinases and Adaptors
Chairperson: J. Cooper, *Fred Hutchinson Cancer Research Center, Seattle, Washington*

Hematopoietic Cell Signaling
Chairperson: M. Roussel, *St. Jude Children's Research Hospital, Memphis, Tennessee*

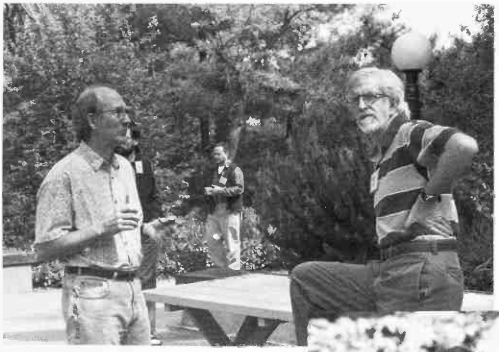
Adhesion-mediated Signaling
Chairperson: L. Van Aelst, *Cold Spring Harbor Laboratory*

Kinases and Phosphatases
Chairperson: J. Dixon, *University of Michigan, Ann Arbor*

Signaling in the Immune System
Chairperson: B. Sefton, *The Salk Institute, La Jolla, California*

Signaling in Development
Chairperson: D. Morrison, *NCI-Frederick Cancer Research and Development Center, Maryland*

Signaling Abnormalities in Disease
Chairperson: J. Brugge, *Harvard Medical School, Boston, Massachusetts*



L. Rohrschneider, B. Sefton

J. Cooper, R. Geahlen

Genome Sequencing and Biology

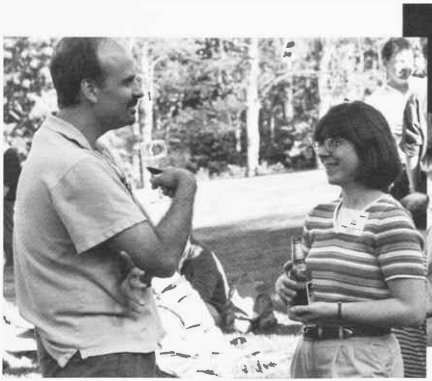
May 19–23 475 participants

ARRANGED BY **Mark Boguski,** National Center for Biotechnology Information
Richard Gibbs, Baylor College of Medicine
Stephen Brown, MRC Mouse Genome Centre, United Kingdom

This meeting marked the 12th 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 "postgenome 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." Just over 450 people from around the world attended the meeting, and 242 abstracts were presented describing a broad array of topics relating to the analysis of genomes from a number of different organisms.

The session topics included areas such as biological insights from phylogenomics, mapping methods and technologies, functional genomics, computational genomics, and 21st century genetics. This year's poster symposium featured 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



M. Boguski, K. Pruitt

R. Gibbs, D. Smith

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.

PROGRAM

Polymorphisms I: Discovery and Mapping
Chairpersons: D. Nickerson, *University of Washington, Seattle*; L. Kruglyak, *Fred Hutchinson Cancer Research Center, Seattle*

Polymorphisms II: Detection and Scoring
Chairpersons: P.-Y. Kwok, *Washington University, St. Louis, Missouri;* M. Dean, *National Cancer Institute-Frederick Cancer Research and Development Center, Maryland*

Bioinformatics I: Database Resources
Chairperson: B.F. Francis Ouellette, *University of British Columbia, Vancouver*

Comparative Genomics I
Chairpersons: M. Ashburner, *European Bioinformatics Institute, Hinxton, United Kingdom*; J. Nadeau, *Case Western Reserve University, Cleveland, Ohio*

Human Sequencing Poster Symposium
Chairpersons: E. Green, *NHGRI, National Institutes of Health, Bethesda, Maryland*; G. Schuler, *NCBI, National Institutes of Health, Bethesda, Maryland*

Bioinformatics II: Software and Algorithms
Chairperson: T. Matise, *Rockefeller University, New York, New York*

ELSI Panel Discussion
Moderator: F. Collins, *NHGRI, National Institutes of Health, Bethesda, Maryland*

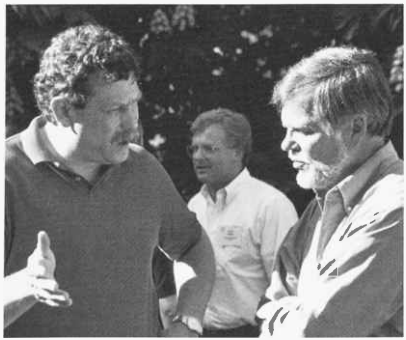
Functional Genomics I: Technologies and Infrastructure
Chairpersons: M. Mann, *University of Southern Denmark, Odense*; M. Lovett, *Washington University School of Medicine, St. Louis, Missouri*

Comparative Genomics II
Chairpersons: R. Martienssen, *Cold Spring Harbor Laboratory*; S. Rounsley, *Cereon Genomics, Cambridge, Massachusetts*

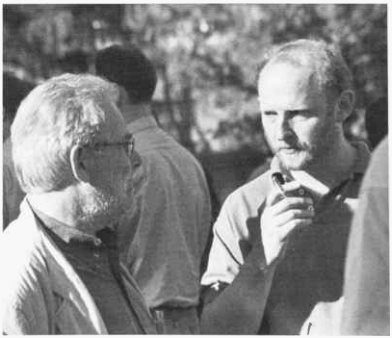
Bioinformatics III: Expression Technologies
Chairperson: A. Baxevanis, *NHGRI, National Institutes of Health, Bethesda, Maryland*

Keynote Speakers
Chairpersons: Richard Wilson, *Washington University Genome Sequencing Center;* Ira Herskowitz, *University of California, San Francisco*

Functional Genomics II: Applications
Chairpersons: S. Gullans, *Harvard Institutes of Medicine, Boston, Massachusetts*; S. Nelson, *University of California, Los Angeles*



E. Lander, J. Hudson



M. Ashburner, R. Martienssen

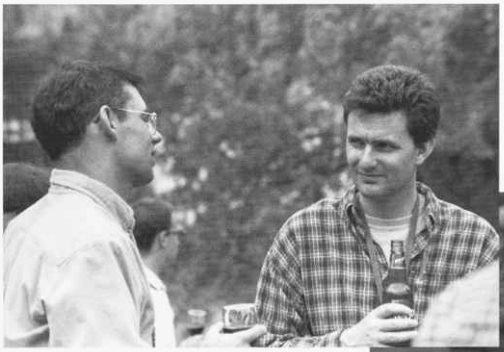
Retroviruses

May 25–30 453 participants

ARRANGED BY **Paul Bates,** University of Pennsylvania
Michael H. Malim, University of Pennsylvania

As always, many exciting new findings in diverse areas of retrovirology were presented. The traditionally strong sessions on viral entry, reverse transcription, integration, and accessory proteins were typically impressive. Increased interest in two general areas was also evident: host factors and their possible roles in intracellular aspects of replication, and structural biology. For instance, several groups working on virus assembly and early postentry events presented results indicative of crucial roles played by cellular proteins. We can expect these areas to provide future insight into virus replication and host cell biology. Several new structures were presented in a session devoted specifically to this topic. Particularly impressive were the cryo–electron microscopy analyses of RSV and HIV virion cores. In summary, retroviruses continue to be excellent systems for drawing together groups working in diverse areas using a variety of approaches.

Contributions from the Corporate Sponsors, Plant Corporate Associates, Corporate Affiliates, Corporate Contributors, and Foundations provided core support for this meeting.



P. Bieniasz, M. Malim

S. Hughes

PROGRAM

Entry
Chairpersons: M. Roth, *UMDNJ-Robert Wood Johnson Medical School, Piscataway;* E. Hunter, *University of Alabama, Birmingham*

Reverse Transcription
Chairpersons: B. Berkhout, *Academic Medical Center, Amsterdam, The Netherlands;* A.M. Skalka, *Fox Chase Cancer Center, Philadelphia, Pennsylvania*

Host Factors and Early Events
Chairpersons: D. Trono, *University of Geneva, Switzerland;* J. Luban, *Columbia University, New York*

Integration
Chairpersons: R. Katz, *Fox Chase Cancer Center, Philadelphia, Pennsylvania;* A. Engelman, *Dana-Farber Cancer Institute, Boston, Massachusetts*

Transcription and RNA Processing
Chairpersons: B. Cullen, *Howard Hughes Medical Institute, Duke University, Durham, North Carolina;* T. Hope, *Salk Institute for Biological Studies, La Jolla, California*

Assembly
Chairpersons: E. Freed, *NIAID, National Institutes of Health, Bethesda, Maryland;* M. Resh, *Memorial Sloan-Kettering Cancer Center, New York*

Accessory Factors
Chairpersons: O. Schwartz, *Institut Pasteur, Paris, France;* D. Rekosh, *University of Virginia, Charlottesville*

Pathogenesis
Chairpersons: H. Fan, *University of California, Irvine;* J. Dudley, *University of Texas, Austin*

Antivirals/Packaging/Vectors/Evolution
Chairpersons: A. Lever, *University of Cambridge, United Kingdom;* P. Cannon, *University of Southern California, Los Angeles*

Structures
Chairperson: W. Sundquist, *University of Utah, Salt Lake City*

Emerging Topics
Chairperson: J. Stoye, *National Institute for Medical Research, London, United Kingdom*

R. Tun, C. Ngian

A. Engelman, L. Mansky, E. Freed

SPECIAL CONFERENCE

New York Structural Biology Group

July 14 220 participants

ARRANGED BY **Barry Honig,** Columbia University, New York
Leemor Joshua-Tor, Cold Spring Harbor Laboratory
John Kuriyan, Rockefeller University

The summer meeting of the New York Structural Biology Discussion Group was the second in day-long meetings allowing structural biologists from all over the region to meet and discuss their latest results. The meeting was open to structural biologists from different disciplines, including crystallographers, spectroscopists, computational biologists, and biochemists, with 220 participants from academia and industry from the tristate area. The program featured 10 talks, a poster session, and a beach barbecue, which allowed a wonderful opportunity for informal interactions. This meeting complements the bimonthly evening meetings of the group held at Rockefeller University. No registration was required, and participants were encouraged to set up posters.

Financial support was provided by Hampton Research Inc., Molecular Structure Corporation, Bruker AXS Inc., Nonius, Inc., Protein Solutions Inc., Pfizer Inc., Hoffmann-La Roche Inc., and Cold Spring Harbor Laboratory.

PROGRAM

SESSION I

Chairperson: Ann McDermott, *Columbia University*

Wayne Hendrickson, *Columbia University*
Jannette Carey, *Princeton University*
Benoit Roux, *University of Montreal*

SESSION II

Chairperson: Andy Neuwald, *Cold Spring Harbor Laboratory*

Dan Raleigh, *State University of New York, Stony Brook*
Yuh-Min Chook, *Rockefeller University*
Jay Pandit, *Pfizer Inc.*

POSTER SESSION

SESSION III

Chairperson: Steve Roderick, *Albert Einstein College of Medicine*

Steve Burley, *Rockefeller University*
David Cowburn, *Rockefeller University*
Paula Fitzgerald, *Merck*
Barry Honig, *Columbia University*

FALL MEETINGS

Yeast Cell Biology

August 17–22 424 participants

ARRANGED BY **Brenda Andrews,** University of Toronto, Canada
Chris Kaiser, Massachusetts Institute of Technology
Mark Winey, University of Colorado, Boulder

The Yeast Cell Biology conference was the seventh biannual international meeting devoted to major aspects of cell biology in yeast. This conference is unique in having all major areas of cell biology represented at a single meeting organized around a simple eukaryotic organism, the budding yeast *Saccharomyces cerevisiae*.

A common interest in one organism, instead of one topic in cell biology, encourages extensive cross-fertilization of ideas, insights, and methodologies. Important insights were further gained by studies in the fission yeast *Schizosaccharomyces pombe,* as well as other yeasts. The meeting included a session on genome-wide expression analysis in yeast during critical cellular processes and at different parts of the life cycle. Major areas of interest included the functions of the actin and microtubule cytoskeleton with collaborating proteins, and the targeting and sorting of proteins in the secretory, endocytotic, and nuclear localization pathways. The coordination of several cellular processes was discussed in the context of the response to mating pheromone, meiosis, and the cell cycle, with the exit from mitosis being particularly important. All told, it was a very rich and exciting meeting, with 424 scientists in attendance presenting some 317 abstracts in the form of 111 talks and 206 posters.

Contributions from the Corporate Sponsors, Plant Corporate Associates, Corporate Affiliates, Corporate Contributors, and Foundations provided core support for this meeting.

C. Kaiser, D. Pellman

O. Cohen-Fix, Y. Guacci, M. Winey

PROGRAM

Chromosome Structure and Mating Pathway
Chairperson: J. Thorner, *University of California, Berkeley*

Actin and Polarized Growth
Chairperson: D. Botstein, *Stanford University School of Medicine, California*

Signaling
Chairperson: E. Elion, *Harvard Medical School, Boston, Massachusetts*

Early Secretion and Organelles
Chairperson: T. Fox, *Cornell University, Ithaca, New York*

Mitotic Exit/Cytokinesis and Meiosis
Chairperson: J. Pringle, *University of North Carolina, Chapel Hill*

Microtubule Cytoskeleton
Chairperson: M. Rose, *Princeton University, New Jersey*

Cell Cycle
Chairperson: G. Fink, *Whitehead Institute/MIT, Cambridge, Massachusetts*

Late Secretion
Chairperson: R. Fuller, *University of Michigan, Ann Arbor*

Functional Genomics
Chairperson: J. Berman, *University of Minnesota, St. Paul*

Nuclear Transport
Chairperson: M. Cyert, *Stanford University, California*

G. Fink

Resting up during poster session.

M. Solomon, C. Slayman

Eukaryotic mRNA Processing

August 25–29 273 participants

ARRANGED BY **Adrian Krainer,** Cold Spring Harbor Laboratory
James Manley, Columbia University
Timothy Nilsen, Case Western Reserve University

The second Eukaryotic mRNA Processing meeting was held this year and focused on the mechanisms and regulation of pre-mRNA splicing, 3′-end formation, editing, and turnover in yeast and multicellular eukaryotes.

The structural characterization of spliceosomal snRNP particles has progressed significantly from the identification and characterization of constituent polypeptides to the visualization of the three-dimensional structure of the U1 snRNP at 25-Å resolution by electron cryomicroscopy. Continued interest in understanding the integration of mRNA processing with transcription revealed new aspects of this link, such as the dependence of alternative splicing patterns on the particular promoter from which a pre-mRNA is transcribed in vivo. Links between RNA splicing, RNA export to the cytoplasm, and turnover were further studied by analyzing specific splicing-dependent complexes assembled on the mature mRNA, as well as proteins present at the exon-exon junctions.

Components responsible for recognition of the 3′ splice site AG dinucleotide in both mammalian *cis*-splicing and nematode *trans*-splicing were identified, one of which was shown to be required for discrimination between correct and incorrect 3′ splice sites and is conserved from yeast to humans. *cis*-spliced introns were reported for the first time in trypanosomes. Exonic splicing enhancers and silencers and the proteins that recognize them have been extensively characterized in several systems. The functions of several nematode SR proteins and of some of the kinases that phosphorylate them were probed in vivo using gene inactivation by RNAi. Mechanistic studies of the phenomenon of RNAi itself in *Drosophila* and trypanosomes were reported, including the development of the first in vitro system, which should allow biochemical dissection of this remarkable phenomenon. Novel RNA-RNA interac-

M. O'Connell, W. Keller

B. Bass, M. Moore

tions between the spliceosomal snRNAs and between snRNAs and specific sites on the substrate continue to be found.

Other presentations at the meeting described genetic, biochemical, and cell biological approaches applied to the study of diverse aspects of mRNA metabolism, including 3′-end processing mechanisms and regulation, splicing, editing, turnover, and transport in yeast, metazoans, plants, and viruses.

This meeting was funded in part by the National Cancer Institute and the National Institute of Child Health and Human Development branches of the National Institutes of Health, and by the National Science Foundation. The Laboratory thanks the RNA Society for its support of this meeting.

PROGRAM

snRNPs and SR Protein Kinases
Chairperson: C. Guthrie, *University of California, San Francisco*

Integration of Processing Pathways
Chairperson: J. Dahlberg, *University of Wisconsin, Madison*

Splicing Factors and Mechanisms of Enhancement
Chairperson: R. Reed, *Harvard Medical School, Boston, Massachusetts*

3′ End Formation
Chairperson: W. Keller, *Biozentrum, University of Basel, Switzerland*

Splicing Mechanisms and Signals
Chairperson: M. Rosbash, *Brandeis University/HHMI, Waltham, Massachusetts*

Splicing Regulation
Chairperson: D. Rio, *University of California, Berkeley*

RNA Stability and Editing
Chairpersons: R. Parker, *University of Arizona/HHMI, Tucson*; B. Bass, *University of Utah School of Medicine/HHMI, Salt Lake City*

M. Rosbash, A. Krainer, J. Manley, T. Nilsen

R. Sperling, J. Sperling, J. Dahlberg

Mechanisms of Eukaryotic Transcription

September 1–5 439 participants

ARRANGED BY **Nouria Hernandez,** Cold Spring Harbor Laboratory
Robert Kingston, Massachusetts General Hospital
Richard Treisman, Imperial Cancer Research Fund, London

Mechanisms of Eukaryotic Transcription was the sixth biennial Cold Spring Harbor meeting devoted to mechanisms of transcriptional regulation in eukaryotes. The conference focused on the structure and function of RNA polymerase, mechanisms of epigenetic regulation and chromatin remodeling, structure and function of regulatory complexes, mechanisms of activation, regulatory pathways, and mechanisms of promoter clearance and elongation. The conference was attended by an international audience of scientists who study transcription in widely different organisms. Significant advances in our understanding of transcription mechanisms were presented, perhaps most notably the first high-resolution crystal structure of a multisubunit RNA polymerase. A number of presentations also addressed the role of various multisubunit complexes in chromatin remodeling, the role of TFIID, in particular the TBP-associated factors in TFIID, and the role of other regulatory complexes in regulation of gene expression, and the mechanisms that govern promoter opening and transcription elongation.

N. Hernandez, Q. Zhou

D. Allis, R. Tjian, S. Triezenberg

This meeting was funded in part by the National Cancer Institute and the National Institute of Child Health & Human Development, branches of the National Institutes of Health; and the National Science Foundation.

PROGRAM

RNA Polymerase
Chairperson: K. Yamamoto, *University of California, San Francisco*

Epigenetic Regulation
Chairperson: B. Emerson, *The Salk Institute, La Jolla, California*

Chromatin Remodeling
Chairperson: I. Grummt, *German Cancer Research Center, Heidelberg*

Regulatory Complexes I
Chairperson: P. Verrijzer, *Leiden University Medical Centre, The Netherlands*

Regulatory Complexes II
Chairperson: L. Freedman, *Memorial Sloan-Kettering Cancer Center, New York*

Activation Mechanisms
Chairperson: J. Lis, *Cornell University, Ithaca, New York*

Regulatory Pathways
Chairperson: B. Dynlacht, *Harvard University, Cambridge, Massachusetts*

Promoter Clearance and Elongation
Chairperson: R. Roeder, *Rockefeller University, New York*

R. Young, R. Kingston

A. Rao, S. Tilghman, A. Chepelinsky

Workshop on Computational Biology: Bridging the Gap between Sequence and Function

September 7–9 127 participants

ARRANGED BY **Michael Q. Zhang,** Cold Spring Harbor Laboratory
Eugene V. Koonin, National Center For Biotechnology Center
Edward Uberbacher, Oak Ridge National Laboratory

The first Cold Spring Harbor Workshop on Computational Biology was held over two days in September. The primary focus of the meeting was "to bridge the gap between sequence and function." As the human genome project picks up speed to deliver a "rough draft" of the human genetic code by the dawn of the new century, deciphering and making use of massive amounts of information coming from genome sequencing and functional genomics/proteomics are becoming ever more challenging to all biologists. In an effort to foster greater collaboration between computational and experimental biologists, this workshop asked people to address the following questions: (1) What have we already learned from computational analysis of "genomic" information? (2) What do we hope to learn in the future for which we do not yet have enough data? (3) What concepts, methods, and infrastructure are missing that keep us from exploiting the genome information to the fullest?

The workshop concentrated primarily on biological rather than algorithmic aspects of computational biology. The topics covered a wide range of structural and functional issues in genomics and proteomics. Despite its brevity, the workshop attracted substantial international participation from laboratories in Russia, Japan, China, and South Africa, as well as from Europe and Canada. Among many distinguished session chairs (of whom half were female) and speakers, two special guest lectures were given by Lee Hood, University of Washington, and Terry Sejnowski, The Salk Institute. Many attendees praised this workshop for its quality and unique setting and expressed strong interest in this format being continued in the future.

This meeting was funded in part by the Alfred P. Sloan Foundation.

PROGRAM

Gene Finding/Polymorphisms and Evolution
Chairpersons: E. Uberbacher, *Oak Ridge National Laboratory, Tennessee*; J. Eppig, *Jackson Laboratory, Bar Harbor, Maine*

Promoters and Gene Regulation/Databases
Chairpersons: M. Kanehisa, *Kyoto University, Japan*; L. Landweber, *Princeton University, New Jersey*

General Interest
Chairpersons: M. Zhang, *Cold Spring Harbor Laboratory*; R. Durbin, *Sanger Centre, Cambridge, United Kingdom*

Protein Families/Structure Genomics
Chairpersons: E. Koonin, *NCBI, National Institutes of Health, Bethesda, Maryland*; L. Holm,*EMBL-EBI, Cambridge, United Kingdom*

Closing Remarks
Chairperson: E. Uberbacher, *Oak Ridge National Laboratory, Tennessee*

M. Zhang, B. Stillman

Eukaryotic DNA Replication

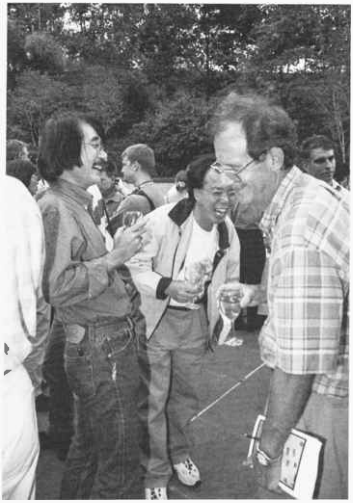
September 15–19 375 participants

ARRANGED BY **Thomas Kelly,** Johns Hopkins University School of Medicine
Bruce Stillman, Cold Spring Harbor Laboratory

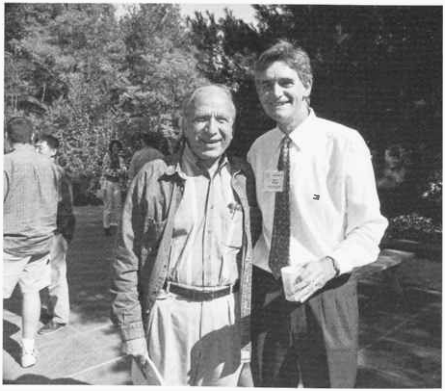
This was the seventh biannual meeting on eukaryotic DNA replication held at Cold Spring Harbor. Studies of eukaryotic DNA replication are advancing rapidly on many fronts, and this meeting is now established as the most important in the field. A total of 375 investigators participated in the 10 scientific sessions, and there were nearly 275 platform and poster presentations. Thus, interest in the mechanisms and regulation of DNA replication in eukaryotic cells remains extremely high, and the meeting is playing a key role in fostering the exchange of new ideas and experimental approaches.

A major goal of work in the field is to define the mechanisms of initiation of eukaryotic DNA replication at replication origins. The kinases that signal the triggering of initiation were further characterized. In addition, many labs have begun to consider the role of chromatin structure in defining origins of replication. Detailed biochemical characterization of a number of previously identified replication proteins was reported, and this work will undoubtedly have a bearing on the ultimate elucidation of the initiation process. Results from a number of novel model systems were described and added further clarity to the eukaryotic paradigm.

Essential funding for the meeting was provided by the National Cancer Institute and the National Institute of Environmental Health Sciences, branches of National Institutes of Health, and the National Science Foundation.



A. Matsukage, T. Kelly



J. Hurwitz, B. Stillman

PROGRAM

Early Events in Initiation and Unwinding
Chairperson: M. Wold, *University of Iowa College of Medicine, Iowa City*

Origin Recognition
Chairperson: J. Campbell, *California Institute of Technology, Pasadena*

Triggering Initiation
Chairperson: H. Araki, *National Institute of Genetics, Shizuoka, Japan*

Defining Origins
Chairperson: S. Bell, *Massachusetts Institute of Technology, Cambridge*

Polymerases and Accessory Proteins
Chairperson: A. Stenlund, *Cold Spring Harbor Laboratory*

RPA, Helicases, and Telomeres
Chairperson: C. Newlon, *University of Medicine and Dentistry of New Jersey, Newark*

Repair and Checkpoint Control
Chairperson: U. Hübscher, *University of Zurich, Switzerland*

Virus Replication and Polymerase
Chairperson: S. Forsburg, *The Salk Institute, La Jolla, California*

C. Newlon, C. Price, J. Hamlin

J. Diffley, C. Hardy

Microbial Pathogenesis and Host Response

September 22–26 268 participants

ARRANGED BY **P.T. Magee,** University of Minnesota
Stanley Maloy, University of Illinois, Urbana
Ronald Taylor, Dartmouth Medical School

Understanding microbial pathogenesis demands a detailed knowledge of the host response as well as of the pathogen itself and requires an interdisciplinary approach, integrating the fields of microbiology, eukaryotic cell biology, and immunology. The second Cold Spring Harbor Microbial Pathogenesis and Host Response meeting was planned to facilitate such integrations. The meeting attracted 268 international scientists who approach the study of bacterial and fungal pathogens from a broad range of perspectives and included 57 talks and 140 posters.

The first session dealt with adhesion of microbial pathogens to the eukaryotic host. Several sessions focused on how microbial pathogens sense different microenvironments in the host and regulate gene expression in response to these environments. Additional sessions focused on host defenses to microbial pathogens. One session focused on what we have learned about microbial virulence and the evolution of virulence from genome analysis. This session conveyed a variety of exciting new insights into the understanding of how microbial-virulence mechanisms evolve and suggested that with appropriate perspicacity, genomic analysis can provide clues to virulence determinants and their acquisition. The final oral session focused on emerging pathogens and novel environmental niches.

In addition to the oral sessions, the poster sessions presented a variety of new insights into bacterial and fungal pathogens and host defenses to microbial pathogens. Finally, Stanley Falkow (Stanford University) gave an inspiring, introspective keynote address on future challenges facing the field of microbial pathogenesis. He discussed the importance of the numerous pathogens that have not yet been adequately studied due to the lack of suitable molecular tools and argued that the application of new tools may make these organisms more pliable.

This meeting was funded in part by the National Institute of Allergy and Infectious Diseases, the U.S. Army, AstraZeneca R&D Boston, Infection; Burroughs Wellcome; Intrabiotics Pharmaceuticals, Inc.; and Pharmacia & Upjohn Inc. Contributions from the Corporate Sponsors, Plant Corporate Associates, Corporate Affiliates, Corporate Contributors, and Foundations also provided core support for this meeting.

J. Inglis, J. Witkowski, S. Maloy

J. Felton, R. Taylor

PROGRAM

Introduction
Chairperson: S. Maloy, *University of Illinois, Urbana*

Adhesion to the Host
Chairperson: P. Orlean, *University of Illinois, Urbana-Champaign*

Regulatory Responses to Environmental Cues
Chairperson: C. Lee, *Harvard Medical School, Boston, Massachusetts*

Virulence Determinants That Manipulate the Host Reponse
Chairperson: R. Taylor, *Dartmouth Medical School, Hanover, New Hampshire*

Survival in the Host
Chairperson: S. Libby, *North Carolina State University, Raleigh*

Host Defenses
Chairperson: B. Cookson, *University of Washington, Seattle*

Evolution of Pathogens
Chairperson: S. Maloy, *University of Illinois, Urbana*

Toxins and Superantigens
Chairperson: J. Scott, *Emory University, Atlanta, Georgia*

Future Perspectives
Chairperson: S. Falkow, *Stanford University, California*

Emerging Pathogens, Environmental Niches, and Model Systems
Chairperson: P. Fields, *Centers for Disease Control and Prevention, Atlanta, Georgia*

P.T. Magee, B. Magee, A. Casadevall

A.-M. Verheyden, T. Haq

Coffee break.

Programmed Cell Death

September 29–October 3 430 participants

ARRANGED BY **Hermann Steller,** HHMI/Massachusetts Institute of Technology
Craig Thompson, HHMI/University of Chicago
Eileen White, HHMI/Rutgers University

The third Cold Spring Harbor meeting on Programmed Cell Death again attracted a standing-room only crowd to hear the latest news in a fast-paced field. There were numerous seminal discoveries presented. Data from *C. elegans* demonstrated that Egl-1 functions by binding to Ced-9 and Ced-4 which causes Ced-4 to be released, whereupon it can activate Ced-3 and induce cell death. Genetic studies of *Drosophila* revealed that the IAP homologs function in the reaper pathway to regulate cell death. Furthermore, Bcl-2 and Apaf-1 homologs were also identified, and their role in fly development and cell death regulation was demonstrated. Regulation of mitochondrial function by mammalian Bcl-2 family members also resulted in heated discussions related to the different theories and data generated by competing groups. The precise role of mammalian ICAD/DFF45 and a new nuclease in apoptosis were reported. Data from numerous mice with mutations in apoptotic regulators were also presented, which enhance our understanding of apoptotic-signaling pathways. These and numerous other discoveries led to heated discussions at poster sessions and during question periods.

This meeting was funded in part by the National Cancer Institute, the National Institute on Aging, and the National Institute of Child Health and Human Development, branches of the National Institutes of Health; and the National Science Foundation.

K. Fladmark, M. Zigmond

D. Hockenbery, D. Faustman

PROGRAM

Invertebrate Development
Chairpersons: J. Abrams, *University of Texas Southwestern Medical Center, Dallas*; K. White, *Massachusetts General Hospital, Harvard Medical School, Charlestown*

Special Lecture
Speaker: H. Robert Horvitz, *Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge*

BCL-2 Family
Chairpersons: S. Cory, *Walter and Eliza Hall Institute of Medical Research, Parkville, Australia*; D. Green, *La Jolla Institute for Allergy and Immunology, California*

Special Lecture
Speaker: Stanley Korsmeyer, *Dana-Farber Cancer Institute, Harvard University Medical School, Boston, Massachusetts*

Caspase Regulation
Chairpersons: X. Wang, *University of Texas Southwestern Medical Center, Dallas*; J. Tschopp, *University of Lausanne, Switzerland*

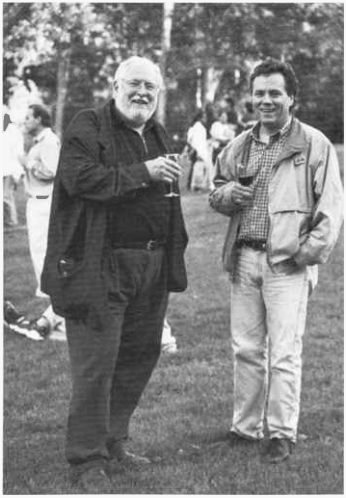
Receptor Signaling
Chairpersons: D. Wallach, *Weizmann Institute, Rehovot, Israel*; S. Nagata, *Osaka Bioscience Institute, Japan*

Transcription
Chairpersons: A. Wyllie, *University of Cambridge, United Kingdom*; B. Osborne, *University of Massachusetts, Amherst*

Signal Transduction
Chairpersons: G. Nuñez, *University of Michigan, Ann Arbor*; G. Evan, *Imperial Cancer Research Fund, London, United Kingdom*

Viral Regulators
Chairpersons: W. Wold, *St. Louis University School of Medicine, Missouri*; P. Friesen, *University of Wisconsin, Madison*

Caspase Action
Chairpersons: J. Yuan, *Harvard Medical School, Boston, Massachusetts; E. Alnemri*, Thomas Jefferson University, Philadelphia, Pennsylvania



P. Branton, S. Lowe

Neurobiology of *Drosophila*

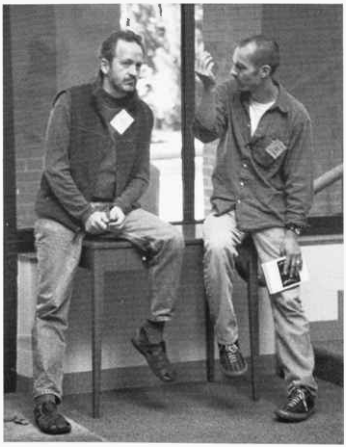
October 6–10 314 Participants

ARRANGED BY **Ulrike Heberlein,** University of California, San Francisco
Haig Keshishian, Yale University

As it is now traditional, the primary goal of this year's meeting on Neurobiology of *Drosophila* was to provide a forum for exchange of ideas, techniques, and new discoveries within the field of *Drosophila* neurobiology. The meeting consisted of platform and poster presentations by a mixture of junior and established investigators. The platform sessions were arranged to reflect the exciting advances that have been made in understanding the molecular mechanisms underlying neurophysiology, sensory systems, adult nervous system development and structure, behavior, cellular determination and differentiation, axon guidance, and synaptic plasticity and target recognition. The work presented included results of genetic, cell biological, molecular, neurophysiological and behavioral investigations of topics from nervous system development to nervous system function. The highlights of the meeting included presentations of such exciting new developments as the identification of a mechanosensory transduction channel, a gene encoding a taste receptor, new genes controlling circadian rhythms, and genes that control dendritic development and synaptic homeostasis. The Elkins plenary lecture, given by a recent Ph.D. graduate, focused on mechanisms of olfaction. The setting of the meeting provided ample opportunities for informal discussions. The high quality of presentations and the novel findings in many areas made it amply clear that this format, which is inclusive of all aspects of neurobiology, is extremely useful to both young and seasoned scientists because it provides an ideal opportunity to cover all the different aspects of *Drosophila* neuroscience.

This meeting was funded in part by the National Institute of Neurological Disorders and Stroke, and the National Institute of Child Health and Human Development, branches of the National Institutes of Health; and the National Science Foundation.

U. Heberlein, J. Hirsh, M. Rosbash



P. Taghert, M. Landgraf

PROGRAM

Neurophysiology
Chairperson: C.-F. Wu, *University of Iowa, Iowa City*

Sensory Systems
Chairperson: M. Mlodzik, *European Molecular Biology Laboratory, Heidelberg, Germany*

Adult NS Development and Structure
Chairperson: L. Restifo, *University of Arizona, Tucson*

Behavior
Chairperson: M. Rosbash, *Howard Hughes Medical Institute, Brandeis University, Waltham, Massachusetts*

Cellular Determination and Differentiation
Chairperson: A. Brand, *University of Cambridge, United Kingdom*

Axon Guidance and Target Recognition
Chairperson: R. Murphey, *University of Massachusetts, Amherst*

Elkins Memorial Lecture
Speaker: Peter J. Clyne, *Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, Connecticut*

Synaptic Plasticity and Target Recognition
Chairperson: G. Davis, *University of California, San Francisco*

J. Witkowski, H. Keshishian

P. Harin, J. Yin

J. Ewer, A. Chiba

WINTER BIOTECHNOLOGY CONFERENCES

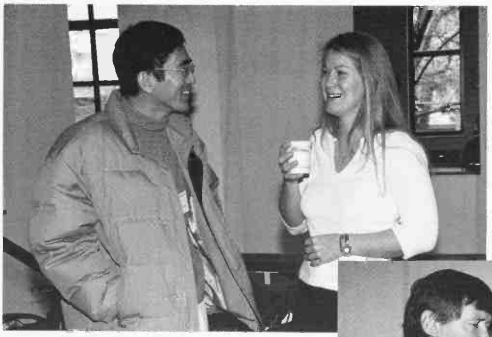
Molecular Approaches to Vaccine Design

December 2–5 196 Participants

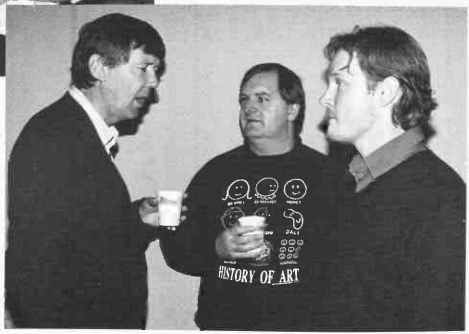
ARRANGED BY **Rahi Ahmed,** Emory University School of Medicine
Dennis Burton, Scripps Research Institute
Margaret Liu, Chiron Corporation

Molecular Approaches to Vaccine Design was a new meeting organized by Rafi Ahmed, Dennis Burton, and Margaret Liu. This meeting grew out of the previous Cold Spring Harbor Laboratory vaccine meetings initiated in 1983 by Bob Chanock and Richard Lerner. The emphasis of the meeting was on understanding the basic concepts underlying immune responses to infectious agents and how this knowledge could be applied to design new and more effective vaccines.

The meeting began with a session on HIV and an overview of progress to date and future prospects for an AIDS vaccine from Neal Nathanson, Director of the Office of AIDS Research at the National Institutes of Health. This was followed by a talk by Joe Sodroski from Harvard on strategies for producing stable HIV envelope trimers that might serve to elicit better antibody responses than the envelope molecule used to date. In the next session on mechanisms of protective immunity, Frank Chisari



J. Jin, K. Schuman



D. Burton, J. Monroe, J. Binley

from the Scripps Research Institute described how inflammatory cytokines can cure hepatitis B infected cells and Kim Hasenkrug from the Rocky Mountain Laboratories showed how all three immune cell types (CD4, CD8, and B cells) were required to fully protect against a retroviral challenge. After a lively and well attended poster session, the meeting then moved on to the controversial area of mucosal immunity. John Robbins from the National Institutes of Health argued the case for the importance of serum IgG in mucosal protection, and Jerry McGhee from the University of Birmingham argued for the significance of mucosally stimulated immune responses.

The mechanisms and requirements for T-cell memory were described by Rafi Ahmed from Emory University, and Dennis Burton from the Scripps Research Institute discussed the limitations of the molecules of natural infection in eliciting useful antibody responses and how vaccine design should perhaps look beyond these molecules. In a session on costimulation and dendritic cells, Antonio Lanzavecchia from the Basel Institute for Immunology described mechanisms of costimulation and T-cell activation and Jim Allison from the University of California at Berkeley discussed the role of CTLA-4 in T-cell responses. The meeting concluded with a session on vaccine delivery systems in which Margaret Liu from Chiron discussed DNA and alphavirus vectors, Bernie Moss from the National Institutes of Health described poxvirus vectors particularly in relation to SIV and James Galen from the University of Maryland presented a series of studies on molecules expressed in attenuated *Salmonella typhi*.

The meeting proved to be a unique forum in which to discuss how modern molecular and structural biology and immunology are beginning to contribute to an understanding of how vaccines work and how new vaccines can be designed.

This meeting was funded in part by Chiron Corporation.

PROGRAM

HIV
Chairperson: J. Moore, *Aaron Diamond AIDS Research Center, New York*

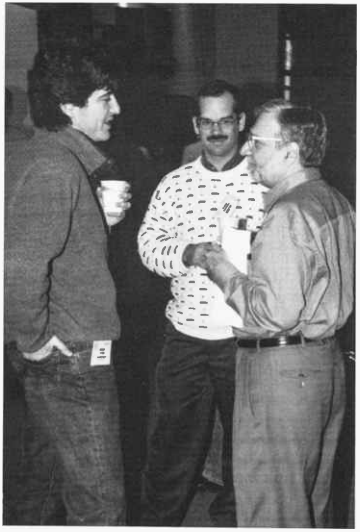
Mechanisms of Protective Immunity
Chairperson: D. Burton, *Scripps Research Institute, La Jolla, California*

Mucosal vs. Systemic Immunity
Chairperson: F. Brown, *USDA, Plum Island Animal Disease Center, New York*

Immune Memory
Chairperson: R. Ahmed, *Emory University School of Medicine, Atlanta, Georgia*

Costimulation and Dendritic Cells
Chairperson: J. Allison, *University of California, Berkeley*

Delivery Systems
Chairperson: M. Liu, *Chiron Corporation, Emeryville, California*



R. Ahmed, A. Catazaro, J. Luhan

Physiological Genomics and Rat Models

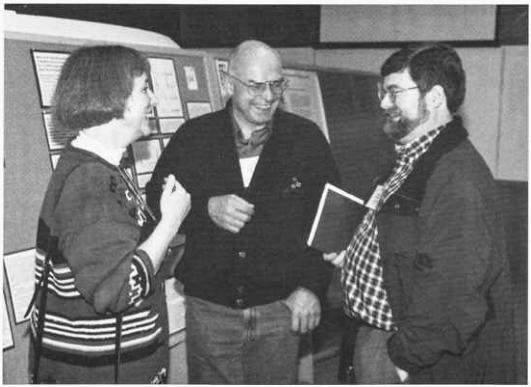
December 9–12 151 participants

ARRANGED BY **Douglas Vollrath,** Stanford University School of Medicine
Howard Jacob, Medical College of Wisconsin

This first meeting on Physiological Genomics and Rat Models was a landmark for two reasons. First, there had not been a rat-specific meeting at the Laboratory in many decades (earlier in the 20th century, the Laboratory had a rich history of rat research). Second, it brought together and cemented a growing field. Nearly 150 scientists from around the world met and discussed research in the areas related to cloning in the rat and the use of genetics, genomics, and physiology to unravel the genetic basis of autoimmunity, cancer, cardiovascular diseases, diabetes, neuroscience, and technology platforms (from databases to microarrays), to name but a few topics. The significance of this meeting was that it was the first time a "rat" meeting had been held that brought together researchers from such a broad range of fields. Traditionally, investigators using rats have clustered by disease, not by organism of interest.

The laboratory rat, *Rattus norvegicus*, was the first mammalian species domesticated for scientific research, with work dating back to before 1850. From this auspicious beginning, the rat has become the most widely studied experimental animal model for biomedical research. Since 1966, nearly 900,000 research articles about rats have been published, most focused on evaluating the biology and/or the pathobiology of the rat. In contrast to its central role in the study of behavior, biochemistry, neurobiology, physiology, and pharmacology, the rat has lagged far behind the mouse as a genetic "model" organism, until recently. A major goal of this meeting was to bring together the disparate groups and link them via genetics and genomics. The meeting appears to have had a catalytic effect on the field. Not only were the meeting attendees extremely pleased with the meeting, but they voted to hold it annually, with the Laboratory hosting every other year. We expect this field of research to play a major role in the era of functional genomics.

Contributions from the Corporate Sponsors, Plant Corporate Associates, Corporate Affiliates, Corporate Contributors, and Foundations provided core support for this meeting.



L. Blankenhorn, H. Hedrich, A. MacMurray

PROGRAM

Genomics
Chairpersons: D. Vollrath, *Stanford University School of Medicine, California;* J. Nadeau, *Case Western Reserve University, Cleveland, Ohio*

Model I
Chairpersons: J. Rapp, *Medical College of Ohio, Toledo;* M. Printz, *University of California, San Diego*

Transgenic
Chairpersons: J. Mullins, *University of Edinburgh, United Kingdom;* M. Paul, *Institute of Clinical Pharmacology and Toxicology, Berlin, Germany*

Expression
Chairpersons: T. Aitman, *MRC Clinical Sciences Centre, London, United Kingdom;* G. Cicila, *Medical College of Ohio, Toledo*

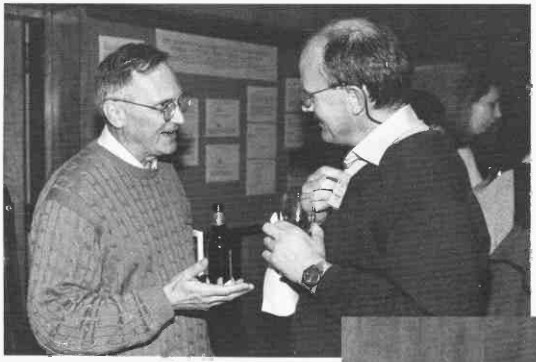
Bioinformatics
Chairpersons: J. Eppig, *Jackson Laboratory, Bar Harbor, Maine;* G. Levan, *Goteborg University, Sweden*

Forum on the Future of Rat Research

Transgenic Mini-course
Chairperson: J. Mullins, *University of Edinburgh, United Kingdom*

Model II
Chairpersons: M. LaVail, *UCSF School of Medicine, California;* C. Walker, *University of Texas M.D. Anderson Cancer Center, Smithville*

Model III
Chairpersons: H. Jacob, *Medical College of Wisconsin, Milwaukee;* A. Lernmark, *University of Washington, Seattle*



A. Cowley, Jr., M. Michalkiewicz

J. Eppig, A. Reiner

Cycling through the Cancer Cell, Fishing for Oncogenes: A Symposium in Celebration of the 60th Birthday of Harold E. Varmus

December 17–19 75 participants

ARRANGED BY **Titia de Lange** **Steve Hughes** **Roel Nusse** **Suzanne Ortiz**

The Cycling through the Cancer Cell, Fishing for Oncogenes symposium was a private celebration of Harold Varmus' 60th birthday. A significant portion of the symposium was dedicated to scientific presentations, as science has been the major focus of Harold's career, reflecting his wide range of interests. Apart from the science, Harold Varmus' career has had many other aspects: scholar of medieval literature, physician, researcher, teacher, and, most recently, NIH director, where he managed the largest and most complex scientific enterprise in the world.

Harold's training as a physician directed him toward basic science, but basic science with the underlying idea that there are important diseases that cannot yet be effectively treated. This led to a long and fruitful collaboration with Mike Bishop, resulting in the discovery of cellular oncogenes and the Nobel prize. This was a period of great intellectual ferment, with dozens of exciting discoveries, some of which involved the discovery of new oncogenes, others elucidating the rules that govern the replication of retroviruses.

Winning the Nobel prize could have been the capstone on what was already a remarkable career. However, a few years after receiving the prize, Harold took on the directorship of the NIH, during a difficult time for American science. Funding, in particular funding for extramural research, was exceptionally tight. The relationship between Congress and the NIH leadership was at a low ebb, and the future looked anything but bright. Harold has effected a remarkable transformation in the institution and in its relationship to Congress and the public. The best leadership creates respect for the institution from the outside and respect for the leader within the organization. Few expected that someone who had never managed an organization larger than a research laboratory could revitalize NIH. Harold's leadership has set a new standard and, we hope, points the way for other eminent scientists to follow in his footsteps as NIH director. It is, furthermore, a measure of Harold's love of basic science that, as director of NIH, he maintained an active laboratory whose research program involves both oncogenes and retroviruses.

Harold Varmus has now left NIH to become the head of Memorial Sloan-Kettering Cancer Center in New York. This will allow him to continue to work to bring the discoveries of basic research into the clinic.

PROGRAM

Development
Chairperson: M. Bishop

Retroviruses
Chairperson: D. Baltimore

Cell Biology
Chairperson: A. Huang

Cancer Biology I
Chairperson: P. Vogt

Cancer Biology II
Chairperson: B. Alberts

R. Block, C. Varmus, J. Varmus, E. Bloch, H. Varmus

POSTGRADUATE COURSES

The 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 course faculty from many laboratories around the world and supplement this staff with a series of seminar speakers.

Advanced Molecular Cytogenetics

MARCH 9–15

INSTRUCTORS **Ried, T.,** Ph.D., National Cancer Institute, NIH, Bethesda, Maryland
Schrock, E., Ph.D., National Cancer Institute, NIH, Bethesda, Maryland

ASSISTANTS **Janish, C.,** Applied Spectral Imaging, Carlsbad, California
Veldman, T., Georgetown University, Washington, D.C.

This course focused on advanced molecular cytogenetic methods for the visualization of chromosome aberrations. Emphasis was placed on applications of spectral karyotyping (SKY) for the refinement of clinical and tumor cytogenetic diagnostics. In addition, the course covered comparative genomic

hybridization (CGH) and novel, sensitive methods for visualization of FISH-experiments (tyramides). 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 molecular biologists who intend to use advanced molecular cytogenetic methods in mammalian chromosome analysis. Among the methods presented were the preparation of tagged DNA probes, fluorescence in situ hybridization 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 for hands-on experience. The laboratory portion of the course was supplemented by invited lectures by Drs. Ollie-P. Kallioniemi, Cynthia Morton, Janet D. Rowley, Robert L. Strausberg, and David C. Ward, who presented updates in the field of molecular cytogenetic research and diagnostics and genomics. The course was supported by the National Cancer Institute, Applied Spectral Imaging, Chroma Technology, Leica Imaging Systems, and Vysis.

PARTICIPANTS

Andersen, C., M.S., Danish Cancer Society, Aarhus, Denmark
Cabal-Manzano, R., M.D., Georgetown University Medical Center, Washington, D.C.
Cote, G.B., Ph.D., Sudbury General Hospital, Ontario, Canada
Crolla, J.A., Ph.D., Salisbury District Hospital, United Kingdom
Hsu, B., M.S., Tsan Yuk Hospital, Hong Kong, China
Kerr, J., Ph.D., The DuPont Merck Pharmaceutical Company, Wilmington, Delaware
Lindemann, G.W., Ph.D., Roche Diagnostics, Berkeley, California
Myska, A.E., Ph.D., Armed Forces Radiobiology Research Institute, Rockville, Maryland
Neal, D.R., B.S., Children's Hospital Medical Center of Akron, Ohio
O'Connor, S.J., M.Sc., The General Infirmary at Leeds, United Kingdom
Ohmido, N., Ph.D., Hokuriku National Agricultural Experimental Station, Joetsu, Japan
Parada, L.A., M.Ph., Lund University Hospital, Sweden
Reddy, V., Ph.D., Osmania University, Hyderabad, India
Schumacher, V., B.S., University of Duesseldorf, Germany
Wan, T.S.K., Ph.D., University of Hong Kong, China
Watson, M.L., Ph.D., University of Texas Southwestern Medical Center, Dallas

SEMINARS

Garini, Y., Applied Spectral Imaging, Carlsbad, California. Spectral imaging.
Kallioniemi, O.-P., National Institutes of Health, Bethesda, Maryland. Genome screening.
Morton, C., Brighams & Women's Hospital, Boston, Massachusetts. FISH tales from the clinical cytogenetics laboratory.
Rowley, J., University of Chicago Medical Center, Illinois. Chromosome translocations: Dangerous liasions.
Schrock, E., National Institutes of Health, Bethesda, Maryland. Cancer cytogenetics revisited: Comparative genomic hybridization and spectral karyotyping.
Strausberg, R.L., National Institutes of Health, Bethesda, Maryland. Genes, genomes, and the molecular profiling of cancer.
Ward, D., Yale University School of Medicine, New Haven, Connecticut: Mutation analysis using rolling circle amplification.

Advanced Genome Sequence Analysis

MARCH 17–30

INSTRUCTORS
Chen, E., Ph.D., PE-Applied Biosystems, Foster City, California
Gibbs, R., Ph.D., Baylor College of Medicine. Houston, Texas
Mardis, E., Ph.D., Washington University School of Medicine, St. Louis, Missouri
McCombie, W.R., Ph.D., Cold Spring Harbor Laboratory
McPherson, J., Ph.D., Washington University School of Medicine, St. Louis, Missouri
Muzny, D., Ph.D., Baylor College of Medicine, Houston, Texas
Wilson, R., Ph.D., Washington University School of Medicine, St. Louis, Missouri
Zuo, L., Ph.D., AxyS Pharmaceuticals, Inc., La Jolla, California

ASSISTANTS
Dedhia, N., Cold Spring Harbor Laboratory
De la Bastide, M., Cold Spring Harbor Laboratory
Fitzgerald, M., Genome Therapeutics Corporation, Waltham, Massachusetts
Habermann, K., Cold Spring Harbor Laboratory
Huang, E., Cold Spring Harbor Laboratory
Hutchison, D., AxyS Pharmaceuticals, Inc., La Jolla, California
Johnson, D., Washington University School of Medicine, St. Louis, Missouri
Dugan, S., Baylor College of Medicine, Houston, Texas
Ma, P., PE-Applied Biosystems, Foster City, California
Miner, T., Washington University School of Medicine, St. Louis, Missouri
Nhan, M., Washington University School of Medicine, St. Louis, Missouri
Preston, R., Cold Spring Harbor Laboratory
Schutz, K., Cold Spring Harbor Laboratory
See, L.H., Cold Spring Harbor Laboratory
Shah, R., Cold Spring Harbor Laboratory
Spiegel, L., Cold Spring Harbor Laboratory
Vil, D., Cold Spring Harbor Laboratory

This course focused on obtaining and analyzing genomic DNA sequence data and on large-scale sequencing, presenting students with important information on the technical and project management aspects of genomic DNA sequencing projects as well as the computational analysis of the sequence. This was achieved by carrying out a large-scale sequencing project during the class, with a special emphasis on the technical nuances of large-scale sequencing. Increases in sequencing efficiency now permit us to carry out the production phase of the student's sequencing project in less time. As a result, the course increased its emphasis on two areas: the finishing process and the analysis of DNA sequence (both computational and experimental). Last year's course included a module on sequence-based polymorphism analysis. This year, the course expanded the polymorphism analysis to include sequencing of regions from multiple individuals and additional SNP analysis. This curriculum allowed students to learn advanced techniques and principles of genomic DNA sequence analysis, from data generation to analysis of sequence variations in populations.

PARTICIPANTS

Blomstergren, A., M.S., Royal Institute of Technology, Stockholm, Sweden
Bonds, W.D., Ph.D., Yale University, New Haven, Connecticut
Chen, Z., M.D., Baylor College of Medicine, Houston, Texas
Dharakul, T., M.D., Mahidol University, Bangkok, Thailand
Gan, W., Ph.D., National Human Genome Research Institute, Gaithersburg, Maryland
Hadd, A.G., Ph.D., Molecular Dynamics Research and Development, Sunnyvale, California
Lin, L., B.S., Stanford University, California
Liu, K., Ph.D., Huazhong Agricultural University, Wuhan, China
Mullen, L.M., B.S., SmithKline Beecham Clinical Laboratories, Van Nuys, Califorinia
Peters, S.O., Ph.D., United Kingdom Human Genome

Mapping Resource Center, Cambridge
Rajagopal, R., B.S., Biomaterials Technology Center, St. Paul, Minnesota
Scarcez, T., Eng., M.S., Eurogentec, Herstal, Belgium
Skopicki, H., Ph.D., Columbia University College of Physicians & Surgeons, New York
Snoeyenbos-West, O.L., M.S., Ph.D., University of Massachusetts, Amherst
Stinnett, S.W., M.S., Glaxo Wellcome, Research Triangle Park, North Carolina
Vanavichit, A., Ph.D., Kasetsart University, Nakorn Pathom, Thailand

SEMINARS

Bashkin, J., Molecular Dynamics, Sunnyvale, California. DNA sequencing by capillary array electrophoresis.
Eisen, J., Stanford University, Stanford, California. How evolutionary methods can benefit genome analysis and vice versa: The uses of a composite phylogenomic approach.
Eisen, M., Stanford University School of Medicine, California. Making biological sense of genome-wide expression data.
Galperin, M., National Institutes of Health, Bethesda, Maryland. Functional annotation of microbial genomes: Evolutionary approaches (PSI-BLAST, COGs, and metabolic reconstructions).
Gordon, D., University of Washington, Seattle. Semi-automated finishing with the phred/phrap/consed system.
Green, E., National Institutes of Health, Bethesda, Maryland. Mapping and sequencing a human chromosome: How and why.
Halushka, M., Case Western Reserve University, Cleveland, Ohio. SNPs and genetic analysis.
Kwok, P., Washington University School of Medicine, St. Louis, Missouri. SNP discovery and scoring.
Marra, M., Washington University Genome Sequencing Center, St. Louis, Missouri. High throughput fingerprinting of large insert bacterial clones.
Ross-MacDonald, P., Bristol-Myers Squibb, Princeton, New Jersey. Beyond the yeast genome sequence.

Protein Purification and Characterization

April 7–20

INSTRUCTORS
Burgess, R., Ph.D., University of Wisconsin, Madison
Courey, A., Ph.D., University of California, Los Angeles
Lin, S.-H., Ph.D., University of Texas/M.D. Anderson Cancer Center, Houston
Mische, S., Ph.D, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, Connecticut

ASSISTANTS
Bhaskar, V., University of California, Los Angeles
Chen, G., University of California, Los Angeles
Gharahdaghi, F., Rockefeller University, New York
Phan, D., University of Texas/M.D. Anderson Cancer Center, Houston
Pietz, B., University of Wisconsin, Madison
Thompson, N., University of Wisconsin, Madison
Weinberg, C., Rockefeller University, New York

This course was intended for scientists who are not familiar with techniques of protein isolation and characterization. It was a rigorous program that included laboratory work all day and a lecture with a discussion session every evening. Each student became familiar with each of the major techniques in protein purification by performing four separate isolations of (1) a regulatory protein from muscle tissue, (2) a sequence-specific DNA-binding protein, (3) recombinant protein overexpressed in *Escherichia coli*, and (4) a membrane-bound receptor. Students used a variety of bulk fractionation, electrophoretic, and chromatographic techniques, 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 pre-

sented for solubilizing proteins from inclusion bodies and refolding them into active monomeric forms. The methods of protein characterization used included immunological and biochemical assays, protein-protein interaction studies by far-Western analysis, peptide mapping, amino acid analysis, protein sequencing, and mass spectrometry. Emphasis was placed on strategies of protein purification and characterization rather than on automated instrumental analysis. Guest lecturers discussed protein structure, modification of proteins, methodologies for protein purification and characterization, and applications of protein biochemistry to cell and molecular biology.

PARTICIPANTS

Brix, J., B.S., Ph.D., University of Freiburg, Germany
Brock, H., B.S., Ph.D., University of British Columbia, Canada
Canhoto, A., B.S., Ph.D., Dana-Farber Cancer Institute, Boston, Massachusetts
Chadwick, B., B.S., Ph.D., Harvard Medical School, Boston, Massachusetts
Cimprich, K., B.S., Ph.D., Stanford Univeristy Medical School, California
De Wulf, P., B.S., M.S., Ph.D., Harvard University, Boston, Massachusetts
Gordon, C., B.S., Ph.D., Medical Research Council, Edinburgh, United Kingdom
Haggarty, S., B.S., Harvard University, Cambridge, Massachusetts
Iftner, T., B.S., Ph.D., University of Tübingen, Germany
Karpova, A., B.S., M.S., Harvard University, Boston, Massachusetts
Lydall, D., B.S., Ph.D., University of Manchester, United Kingdom
Schild, D., B.A., Ph.D., Lawrence Berkeley National Laboratory, Berkeley, California
Sharon, M., B.S., Ph.D., Weizmann Institute of Science, Rehovot, Israel
Sepehri, S., B.S., Cold Spring Harbor Laboratory
Skene, K., B.S., Ph.D., University of Dundee, United Kingdom
Voytas, D., A.B., Ph.D., Iowa State University, Ames

SEMINARS

Alber, T., University of California, Berkeley. Origins of specificity in protein structure/structural polymorphisms in regulatory proteins.
Baker, T., Massachusetts Institute of Technology, Cambridge. Protein machines in DNA transposition.
Burgess, R., University of Wisconsin, Madison. Overview of protein purification, immunoaffinity purification. Biochemical studies of RNA polymerase–sigma factor interactions.
Courey, A., University of California, Los Angeles. Protein-protein interactions in dorsal-ventral pattern formation in *Drosophila*.
Guidotti, G., Harvard University, Cambridge, Massachusetts. Membrane proteins and extracellular ATP.
Joshua-Tor, L., Cold Spring Harbor Laboratory. Protein structure using a three-dimensional visualization system.
Lin, S.-H., University of Texas/MD Anderson Cancer Center, Houston. C-CAM: A novel tumor suppressor with anti-angiogenesis activity.
Mische, S., Boehringer Ingelheim Pharmaceuticals, Inc. Ridgefield, Connecticut. Microanalytical protein preparation and characterization.
Neuwald, A., Cold Spring Harbor Laboratory. Protein sequence alignment to find protein superfamilies.
Stillman, B., Cold Spring Harbor Laboratory. Biochemical approaches to understanding replication of the eukaryotic cell.

Cell and Developmental Biology of *Xenopus*

APRIL 10–20

INSTRUCTORS **Krieg, P.,** Ph.D., University of Texas, Austin
Moody, S., Ph.D, George Washington University Medical Center, Washington, D.C.

ASSISTANTS **Cleaver, O.,** University of Texas, Austin
Kenyon, K., George Washington University Medical Center, Washington, D.C.
Kroll, K., Harvard University, Boston, Massachusetts
Zorn, A., Welcome/CRC Institute, Cambridge, United Kingdom

The frog *Xenopus* is an important vertebrate model for studies of maternal factors, molecular mechanisms of tissue inductions, and regulation of cell fate decisions. In addition, *Xenopus* oocytes and embryos provide a powerful system in which to conduct a number of cell biological and gene regulation assays. This course provided extensive laboratory exposure to the biology, manipulation, and use of oocytes and embryos of *Xenopus*. Intensive laboratory sessions were supplemented by daily lectures and demonstrations from experts in cellular, experimental, and molecular development. Areas

covered included (1) care of adults; (2) oocyte isolation and embryo production; (3) stages of embryonic development and anatomy; (4) whole-mount in situ hybridization and immunocytochemistry; (5) microinjection of eggs and oocytes with lineage tracers, DNA constructs, mRNA, and antisense oligonucleotides; (6) micromanipulation of embryos, including explant and transplantation assays; (7) preparation of transgenic embryos; and (8) use of *Xenopus tropicalis* for genetic analyses. This course was suited both for investigators who had no previous experience with *Xenopus* and for those who had worked with *Xenopus* and wished to learn new and cutting-edge techniques. All participants had current training in molecular biology and some knowledge of developmental biology.

PARTICIPANTS

Cutler, R., B.S., Ph.D., Sugen, Inc., South San Francisco, California
Greenwood, J., B.S., Columbia University College of Physicians & Surgeons, New York
Krishnan, P., B.A., Ph.D., Eli Lilly & Co., Indianapolis, Indiana
Lansbery, K., BA., Washington University, St. Louis, Missouri
Mullegger, J., M.S., Austrian Academy of Sciences, Salzburg
Mueller, P., B.S., Ph.D., University of Chicago, Illinois
Nagai, T., B.S., Ph.D., Riken, Ibaraki, Japan
Olmedo, E., B.S., Ph.D., University of Barcelona, Spain
Ori, M., B.S., University of Pisa, Italy
Ossipova, O., B.S., M.S., University of Goettingen, Germany
Park, C.Y., B.S., Ph.D., State University of New York, Stony Brook
Pierreux, C., B.S., Ph.D., Imperial Cancer Research Fund, London, United Kingdom
Pignoni, F., B.S., Ph.D., Harvard Medical School, Boston, Massachusetts
Roesijadi, G., B.S., M.S., Ph.D., University of Maryland, Solomons
Tietge, J., B.S., M.S., U.S. Environmental Protection Agency, Duluth, Minnesota
Wayman, G., B.S., Ph.D., Oregon Health Sciences University, Portland

SEMINARS

Christian, J., Oregon Health Sciences University, Portland. The role of CaM kinases in cardiovascular development.
Grainger, R., University of Virginia, Charlottesville. *Xenopus tropicalis:* A new model for vertebrate developmental genetics.
Krieg, P., University of Texas, Austin. Development of the heart and blood vessels.
Keller, R., University of Virginia, Charlottesville. Early embryonic anatomy and morphogenesis of *Xenopus*.
King, M.L., University of Miami, Florida. Localized maternal RNAs: Understanding the how and why.
Kroll, K., Harvard Medical School, Boston, Massachusetts. Neural cell fate determination in the early embryo.
Moody, S.A., George Washington University, Washington, D.C. Maternal control of dorsal cell fates in the blastula.
Smith, J., National Institute for Medical Research, London, United Kingdom: T targets.
Thomsen, G., State University of New York, Stony Brook. Ubiquitination in TGFβ signaling and *Xenopus* pattern formation.
Zorn, A., Wellcome Trust, Cambridge, United Kingdom. Sox proteins are negative regulators of Wnt signaling.

Developmental Neurobiology

June 9–22

INSTRUCTORS **Bargmann, C.,** Ph.D., University of California, San Francisco
Burden, S., Ph.D., New York University Medical Center, New York
Cline, H., Ph.D., Cold Spring Harbor Laboratory

The aim of this lecture course was to discuss principles and recent advances in developmental neurobiology. Prospective students had a background in neurobiology or molecular biology. Major topics considered included determination, proliferation, and differentiation of neural cells; trophic interactions in neural development; gradients and compartments; guidance of axons to targets; and the formation of synapses. The topics were considered within the context of the development of both invertebrate and vertebrate neural systems.

PARTICIPANTS

Abrell, S., B.S., M.S., Max Planck Institute, Gottingen, Germany
Akerman, C., M.A., University of Oxford, United Kingdom
Austin, C., B.S., Ph.D., University of Newcastle, United Kingdom
Bingham, S., B.S., University of Missouri, Columbia
Fraidakis, M., M.D., Karolinska Institute, Stockholm, Sweden
Furutani-Seiki, M., M.D., Ph.D., University of Freiburg, Germany
Holmberg, J., B.S., M.S., Karolinska Institute, Stockholm, Sweden

Lu, Q., B.S., Ph.D., Dana Farber Cancer Institute, Boston, Massachusetts
Noveroske, J., B.S., M.S., Baylor College of Medicine, Houston, Texas
Nutt, C., B.S., Ph.D., Yale University, New Haven, Connecticut
Ong, M.T., B.S., M.S., University of Geneva, Switzerland
Peters, M., B.A., Harvard Medical School, Boston, Massachusetts
Rusten, T.E., M.S., European Molecular Biology Laboratory, Heidelberg, Germany
Saura, C., B.S., Ph.D., Johns Hopkins University, Baltimore, Maryland
Singer, J., B.S., Ph.D., National Institutes of Health, Bethesda, Maryland
Solecki, D., B.S., State University of New York, Stony Brook
Talamillo, A., B.S., Ph.D., Medical Research Council, Edinburgh, United Kingdom
Toresson, H., M.S., Lund University, Sweden
Wallen, A., M.S., Karolinska Institute, Stockholm, Sweden
Yeomans, H., B.S., University of Sheffield, United Kingdom

SEMINARS

Ben-Ari, Y., Maternité de Port-Royal, Paris, France. Sequential expression of GABA and glutamate synapses in the developing hippocampal network.
Barde, Y., Max Planck Institute, Martinsried, Germany. Neurotrophins.
Bargman, C., University of California, San Francisco. Pathfinding in *C. elegans*—Olfaction.
Barres, B., Stanford Medical School, California. Glial differentiation.
Burden, S., New York University Medical Center, New York. Neuromuscular synapse formation.
Cepko, C., Harvard Medical School, Boston, Massachusetts. Retinal differentiation.
Cline, H., Cold Spring Harbor Laboratory. Synaptic plasticity.
Davis, G., University of California, Berkeley. Modulation of synapses in *Drosophila*.
Fox, K., University of Wales, Cardiff, United Kingdom. Somatosensory system.
Jan, Y.-N., University of California, San Francisco. Neurogenesis and asymmetric cell divisions.
Jessell, T., Columbia University, New York. Neurogenesis in the spinal cord.
Lemke, G., Salk Institute, La Jolla, California. Regulation of Schwann cell differentiation.
Lichtman, J., Washington University, St. Louis, Missouri. Editing of synaptic connections.
Mombaerts, P., Rockefeller University, New York. Olfaction.
O'Leary, D., Salk Institute, La Jolla, California. Pathfinding in the visual system.
Poo, M.-M., University of California, San Diego. Growth cone guidance.
Raff, M., University College London, United Kingdom. Gliogenesis/apoptosis.
Serafini, T., University of California, Berkeley. Synapse formation in the CNS.
Shatz, C., University of California, Berkeley. Visual system development.
Stern, C., Columbia University, New York. Neural introduction.
Van Vactor, D., Harvard Medical School, Boston, Massachusetts. Pathfinding.
Walsh, C., Harvard Institute of Medicine, Boston, Massachusetts. Neuronal migration.

Advanced Bacterial Genetics

June 9–29

INSTRUCTORS **Bassler, B.,** Ph.D., Princeton University, New Jersey
Manoil, C., Ph.D., University of Washington, Seattle
Slauch, J., Ph.D., University of Illinois, Urbana

ASSISTANTS **Gallagher, L.,** University of Washington, Seattle
Jankiraman, A., University of Illinios, Urbana
Schauder, S., Princeton University, New Jersey

The laboratory course presented logic and methods used in the genetic dissection of complex biological processes in bacteria. The methods presented included mutagenesis using transposons, mutator strains, and chemical and physical mutagens; mapping mutations using genetic and physical techniques; generation and analysis of gene fusions; molecular cloning; polymerase chain reaction; Southern blot analysis; epitope insertion mutagenesis; and site-directed mutagenesis. A key component of the course was the use of sophisticated genetic methods in the analysis of pathogenic and "undomesticated" bacteria. Invited lecturers described the use of genetic approaches to study biological processes in a variety of bacteria.

PARTICIPANTS

Camacho, C., Margarita, M.S., Leiden University, The Netherlands
Englen, M., B.A. Ph.D., U.S. Department of Agriculture, Athens, Georgia
Judd, E., B.A., Stanford University, California
Kishony, R., B.S., Nuclear Research Center Negev, Beer-Sheva, Israel
Kostelidou, K., B.S., Ph.D., University of Birmingham, United Kingdom
Knivt, K., B.S., M.S., Gothenburg University, Goteborg, Sweden
Lenz, L., B.A., Ph.D., University of California, Berkeley
Morin, P., B.S., Ph.D., Albert Einstein College of Medicine, Bronx, New York
Navarro, F., B.S., Ph.D., Hospital de Sant Pau, Barcelona, Spain
Philp, J., B.S., Ph.D., Napier Polytechnic University, United Kingdom
Prince, H., B.A., Dartmouth College, Hanover, New Hampshire
Ramos, A., B.S., Ph.D., Instituto de Tecnologia Quimica e Biologica, Oeiras, Portugal
Rudnick, P., B.S., University of Arizona, Tucson
Songsivilai, S., B.S., M.D., Ph.D., Mahidol University, Bangkok, Thailand
Stroppolo, M.E., B.S., Ph.D., University of Roma "Tor Vergata," Italy
Xavier, K., B.S., Instituto de Tecnologia Quimica e Biologica, Oeiras, Portugal

SEMINARS

Craig, N., Johns Hopkins Medical School, Baltimore, Maryland. Tn7: A smarter transposon.
DeBoer, P., Case Western Reserve University, Cleveland, Ohio. Formation and positioning of the division apparatus in *E. coli*.
Ehrmann, M., University of Konstanz, Germany. Site-specific proteolysis in vivo: A genetic method to study protein structure and function.
Kolter, R., Harvard Medical School, Boston, Massachusetts. Genetic dissection of biofilm development.
Losick, R., Harvard University, Cambridge, Massachusetts. Asymmetric division and cell fate in a bacterium.
Parkinson, J., University of Utah, Salt Lake City. Dissecting the three-protein brain of *E. coli*.
Phillips, G., Iowa State University, Ames. Genetic analysis of the bacterial signal recognition particle.
Pogliano, K., University of California, San Diego. Moving and fusing membranes during *Bacillus subtilis* sporulation.
Reznikoff, W., University of Wisconsin, Madison. Molecular analysis of the Tn*5* transposition machine. Using Tn*5* in vitro transposition as a genome analysis tool.
Segall, A., San Diego State University, California. Using recombination to probe the folded structure of the bacterial chromosome.
Silhavy, T., Princeton University, New Jersey. Parallel pathways perceive periplasmic problems.

Molecular Embryology of the Mouse

June 9–29

INSTRUCTORS	**Nagy, A.,** Ph.D., Samuel Lunenfeld Research Institute/Mount Sinai Hospital, Ontario, Canada **Tam, P.,** Ph.D., Children's Medical Research Institute, Sydney, Australia
CO-INSTRUCTORS	**Gossler, A.,** Ph.D., The Jackson Laboratory, Bar Harbor, Maine **Ang, S.-L.,** Ph.D., Institute of Genetics, Molecular and Cellular Biology, Strasbourg, France
ASSISTANTS	**Gad, J.,** Children's Medical Research Institute, Sydney, Australia **Hadjantonakis, A.K.,** Samuel Lunenfeld Research Institute/Mount Sinai Hospital, Ontario, Canada

This intensive laboratory-lecture course was designed for biologists interested in applying their expertise to the study of mouse embryonic development. Laboratory components provided an introduction into the technical aspects of working with and analyzing mouse embryos, and lectures provided the conceptual basis for contemporary research in mouse development. Experimental techniques included in vitro culture and manipulation of pre- and postimplantation embryos; transgenesis by DNA microinjection; embryo transfer; the establishment, culture, and genetic manipulation of embryonic stem cells; production of chimeras by aggregation with and injection of embryonic stem cells; and the analysis of development by whole-mount in situ hybridization, skeletal preparation, and transgene expression.

PARTICIPANTS

Agate, R., B.S., University of California, Los Angeles
Chan, A., B.S., Ph.D., University of Texas/M.D. Anderson Cancer Center, Houston
Cunningham, D., B.A., Ph.D., Children's Hospital Research Foundation, Columbus, Ohio
Duate, A., D.V.M., Ph.D., University of Lisbon, Portugal
Feng, G.-S., B.S., Ph.D., Indiana University School of Medicine, Indianapolis
Gong, S.-G., M.S., M.A., Ph.D., University of Michigan School of Dentistry, Ann Arbor
Hyatt, B., B.S., B.A., Ph.D., Children's Hospital Medical Center, Cincinnati, Ohio
Lindemann, L., B.S., Max Planck Institute, Munich, Germany
Luetteke, N., B.A., Ph.D., University of North Carolina, Chapel Hill
Pabon-Pena, L., B.S., Vanderbilt University, Nashville, Tennessee
Robson, P., B.S., Ph.D., Children's Hospital of Philadelphia, Pennsylvania
Wilhelm, M., B.S., Karolinska Institute, Stockholm, Sweden
Wright, T., B.S., Ph.D., University of Utah, Salt Lake City
Xavier-Neto, J., M.D., Ph.D., Massachusetts General Hospital, Charlestown

SEMINARS

Ang, S.-L., Institute of Genetics, Molecular and Cellular Biology, Strasbourg, France. Establishment of anterior-posterior polarity in the mouse embryo.
Behringer, R., University of Texas/M.D. Anderson Cancer Center, Houston. Gene targeting 101 and conditional genetic manipulation. Genetic regulation of mouse axis formation and transgenic mouse methods in biomedical research.
De Crombrugghe, B., University of Texas/M.D. Anderson Cancer Center, Houston. Skeletal development.
Gossler, A., The Jackson Laboratory, Bar Harbor, Maine. Genetic analysis of somitogenesis in the mouse.
Harvey, R., Victor Chang Cardiac Research Institute, Darlinghurst, Australia. Heart development.
Hogan, B., Vanderbilt University Medical School, Nashville, Tennessee. BMPs: Germ cells, lungs, eyes, and kidneys. How to cope with a pleiotypic phenotype.
Jenkins, N., National Cancer Institute, NIH, Frederick, Maryland. Mouse genetics.
Joyner, A., New York University Medical Center, New York. Genetic approaches in mouse to study CNS patterning.
Koopman, P., University of Queensland, Brisbane, Australia. In situ hybridization. Eye of newt, hair of mouse, sox factors, and the control of organogenesis.
Lovell-Badge, R., National Institute for Medical Research, London, United Kingdom. Introduction to embryo manipulation and animal experiments. Mammalian sex determination.
Maeda, N., University of North Carolina, Chapel Hill. Modeling artherosclerosis in mice.
Magnuson, T., Case Western Reserve University, Cleveland, Ohio. Genetic approaches to development.
Martin G., University of California, San Francisco: Use of site-specific DNA recombinases to study FGF functions in development.
McLaren, A., Wellcome/CRC Institute, Cambridge, Massachusetts. Germ cells.
McMahon, A., Harvard University, Cambridge, Massachusetts. SHH signaling.
Nagy, A., Mt. Sinai Hospital/ Samuel Lunenfeld Research Institute, Ontario, Canada. ES cells and chimeras. Gene targeting 101 and conditional genetic manipulation.
Papaioannou, V., Columbia University, New York. The T-box gene family in vertebrate development.
Rossant, J., Samnuel Lunenfeld Research Institute, Ontario, Canada. Regulation of extraembryonic development.
Smithies, O., University of North Carolina, Chapel Hill. HOM recombination and disease models.
Solter, D., Max Planck Institute, Freiburg, Germany. From egg to blastocyst—A hard road to follow. Hows and whys of imprinting and cloning.
Tam, P., Children's Medical Research Institute, Wentworthville, Australia. Postimplantation development: Organogenesis to early fetus. Embryological tools. Gastrulation and morphogenesis: Building the body plan.

Physiological Approaches to Ion Channel Biology

June 9–29

INSTRUCTORS **Otis, T.,** Ph.D., University of California, Los Angeles
Ribera, A., Ph.D., University of Colorado Health Sciences Center, Denver
Trussell, L., Ph.D., Oregon Health Sciences University, Portland

ASSISTANTS **Brenowitz, S.,** Oregon Health Sciences University, Portland
Lim, I., University of Wisconsin, Madison

This intensive laboratory-lecture course introduced students to electrophysiological approaches tor the study of ion channels in their native environments. Hands-on exercises included patch-clamp recording of ion channel activity in acutely isolated or cultured cells or neurons in brain slice preparations. The advantages and disadvantages of different methods and preparations were considered in order to match experimental approaches to scientific questions. Similarly, various methods for ligand-drug application were demonstrated. A primary goal was to identify properties of ion channels that allow neurons and nonneuronal cells to carry out their unique physiological or systems-level roles. These properties were related to information previously gained from molecular cloning and heterologous

expression of ion channel genes. Areas of particular interest included channels that (1) are activated by neurotransmitter at central and peripheral synapses, (2) interact with other channels to shape the response properties of neurons, (3) respond to neuromodulators with changes in functional properties, or (4) are developmentally required and regulated. The research interests of guest lecturers complemented and expanded this list. This course was intended for students with specific plans to apply these techniques to a defined problem, and students were encouraged to bring their preparation to the course for preliminary studies. Guest lecturers included Meyer Jackson, Wade Regehr, Paul Fuchs, Vince Dionne, Gerard Borst, Irwin Levitan, Robert Manilow, Bruce Bean, and John Huguenard.

PARTICIPANTS

Johnson, J., B.S., Ph.D., University of California, Los Angles
Kielland, A., B.S. M.S., University of Oslo, Norway
Kuehl-Kovarik, C., B.S., Ph.D., Colorado State University, Ft. Collin
Kurschner, C., M.S., Ph.D., St. Jude's Children Research Hospital, Memphis, Tennessee
Parameshwaran, S., B.S., M.S., University of Maryland, College Park
Perruccio, L., B.A., New York Medical College, Valhalla
Soong, T.-W., B.S., Ph.D., National Neuroscience Institute, Singapore
Storjohann, L., B.S., M.S., University of Utah, Salt Lake City
Tombola, F., M.S., Ph.D., University of Padua, Italy
Wu, F.-F., B.S., University of Pittsburgh, Pennsylvania
Yamada, E., M.D., M.S., Ph.D., University of Texas, Houston
Yang, H., B.S., M.S., Johns Hopkins University, Baltimore, Maryland

SEMINARS

Bean, B., Harvard Medical School, Boston, Massachusetts. Ionic mechanisms of spontaneous activity of central neurons.
Borst, G., Max Planck Institute, Heidelberg, Germany. Physiology of a presynaptic terminal in the CNS, I. Physiology of a presynaptic terminal in the CNS, II.
Dionne, V., Boston University, Woods Hole, Massachusetts. Odor modulated conductances in olfactory receptor neurons.
Fuchs, P., Johns Hopkins University Medical School, Baltimore, Maryland. Hair cell physiology—A molecular mechanism for electrical tuning. Efferent control of the cochlea—Cholinergic inhibition of the cells.
Huguenard, J., Stanford University Medical Center, California. Differential ion channel expression in neuronal subclasses: Cellular and circuit consequences.
Jackson, M., University of Wisconsin, Madison. Roles for ion channels in regulating neurosecretion from peptidergic nerve terminals. Cable properties of neurons.
Levitan, I., Brandies University, Waltham, Massachusetts. Ion channel modulation—A role for regulatory protein complexes.
Malinow, R., Cold Spring Harbor Laboratory. Probing synaptic transmission and plasticity in hippocampal slices using acute recombinant expression techniques.
Otis, T., University of California, Los Angeles. Glutamate transporters: Ion channels that actually do some useful work.
Regehr, W., Harvard Medical School, Boston, Massachusetts. Calcium control of synaptic transmission I. Calcium control of synaptic transmission II.
Ribera, A., University of Colorado, Denver. Regulation of ion channels in embryonic neurons: Using forward and reverse genetics to identify physiological mechanisms.
Trussell, L., University of Wisconsin, Madison. Gating kinetics of glutamate receptor channels and synaptic function.

Molecular Mechanisms of Human Neurodegenerative Diseases

June 24–30

INSTRUCTORS: **Aguzzi, A.,** Ph.D., University Hospital of Zurich, Switzerland
Gandy, S., M.D., Ph.D., New York University, Orangeburg

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, and it focused principally on the specific hypotheses and approaches driving current research. 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, neurofibrillary tangle diseases (tauopathies), Lewy body diseases (cortical Lewy body disease, Parkinson disease synucleinopathies), prion diseases, and polyglutamine repeat disorders. Taking advantage of small class size and extensive discussion, invited faculty lecturers examined critical issues in their areas of expertise. Overviews were provided, and course participants did not need to be familiar with neurological diseases. 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

Amet, L., B.S., Ph.D., Edinburgh University, United Kingdom
Barnes, N., B.S., Wake Forest University School of Medicine, Winston-Salem, North Carolina
Cabelli, D., B.A., Ph.D., Brookhaven National Laboratory, Upton, New York
Carnicero, A., B.S., M.S., University of Murcia, Spain
Cotman, S., B.S., Ohio State University, Columbus
DeFeo, A., B.A., Ph.D., Mercy College, Dobbs Ferry, New York
Gearhart, D., B.S., Ph.D., Medical College of Georgia, Augusta
Glatzel, M., B.S., M.D., University Hospital Zurich, Switzerland
Grant, E., B.S., Ph.D., Organon Laboratories, Lanarkshire, Scotland, United Kingdom
Gupta, A., M.D., Harvard Medical School, Boston, Massachusetts
Heikkila, H., B.S., M.S., University of Helsinki, Finland
Hughes, R., B.S., Ph.D., University of Washington, Seattle
Indyk, J., B.S., State University of New York, Stony Brook
Jankowsky, J., B.A., California Institute of Technology, Pasadena
Kalender, A., B.S., M.S., SmithKline Beecham Pharmaceuticals, Essex, United Kingdom
Kornuc, M., B.A., M.S., AMGEN, Thousand Oaks, California
Kuan, C.-Y., M.D., Ph.D., Yale University, New Haven, Connecticut
Oluwatosin, Y., B.S., Ph.D., ASTRA Arcus USA, Inc., Worcester, Massachusetts
Sohur, U., B.S., Ph.D., Vanderbilt University School of Medicine, Nashville, Tennessee
Van Deerlin, V., B.S., M.S., M.D., Ph.D, University of Pennsylvania, Philadelphia
Van Sweiten, J., Ph.D., AZR Dijkzigt, Rotterdam, The Netherlands
White, J., B.S., Scripps Research Institute, La Jolla, California
Yvert, G., B.S., University Louis Pasteur, Illkirch Strasbourg, France

SEMINARS

Aguzzi, A., University of Zurich Hospital, Switzerland. Transgenic models of prion diseases.
Borchelt, D., Johns Hopkins University, Bethesda, Maryland. Transgenic models of Alzheimer's disease.
Cleveland, D., University of California, San Diego. Mechanisms of motor neuron growth and death: Neurofilaments, SOD1, and Lou Gehrig's disease.
Gandy, S., New York University, Orangeburg. Vesicle biology of Aβ generation.
Ghiso, J., New York University Medical Center, New York. Cerebrovascular amyloidosis.
Glockshuber, R., Institut für Molekularbiologie und Biophysik, Zurich, Switzerland. Prion disease.
Hardy, J., Mayo Clinic, Jacksonville, Florida. Genetic dissection of neurodegeneration.
Holtzman, D., Washington University School of Medicine, St. Louis, Missouri. Potential roles of apoE in the normal and diseased nervous system. Astrocyte-secreted apoE/lipoproteins: Characterization and role in amyloid β metabolism.
Koo, E., University of California, San Diego. Trafficking and endosomal processing of APP and Aβ. Modulation of β-catenin by presenilins.
Lee, V., University of Pennsylvania, Philadelphia. Tauopathies.
Macdonald, M., Massachusetts General Hospital, Charlestown. Genetics and models of Huntington's disease.
Morris, J., Jewish Hospital of St. Louis, Missouri. Are Alzheimer's disease and aging dichotomous or continuous? Age-associated memory impairment, mild cognitive impairment, and incipient Alzheimer's disease: Clinicopathologic studies.
Orr, H., University of Minnesota, Minneapolis. Pathogenesis of a polyglutamine neurodegenerative disease, SCA1.
Selkoe, D., Harvard Medical School, Boston, Massachusetts. Clinical, pathological, and biochemical underpinnings of Alzheimer's disease. Genotype-to-phenotype relationships in Alzheimer's disease.
Tagle, D., National Institutes of Health, Bethesda, Maryland. Approaches to understanding the pathogenesis of Huntington's disease.
Trojanowski, J., University of Pennsylvania, Philadelphia. Molecular and cellular mechanisms of brain degeneration in synucleinopathies.
Tanzi, R., Harvard Medical School, Charlestown, Masssachusetts. Using genetics to develop molecular models for Alzheimer's disease.

Arabidopsis Molecular Genetics

July 2–22

INSTRUCTORS **Barton, K.,** Ph.D., University of Wisconsin, Madison
Deng, X.W., Ph.D., Yale University, New Haven, Connecticut
Grossniklaus, U., Ph.D., Friedrich Miescher Institute, Basel, Switzerland

ASSISTANTS **Fernandez, A.,** University of Wisconsin, Madison
Huck, N., Friedrich Miescher Institute, Basel, Switzerland
Shiu, S., University of Wisconsin, Madison

This course provided an intensive overview of topics in plant growth, genetics, physiology, and development, focusing on molecular genetic approaches to understanding plant biology. It emphasized recent results from research using *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 wished 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. Seminar topics included plant morphology and anatomy, plant development, including development of flowers, roots, meristems, and leaves, male and female gametophytes, and embryos; perception of light and

photomorphogenesis; and synthesis, function, and perception of hormones. Lectures describing bioinformatics tools available to the *Arabidopsis* community and the 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 and included studies of *Arabidopsis* development, mutant analysis, in situ detection of RNA, histochemical staining, transient gene expression, applications of green fluorescent protein fusions, protein interaction and detection, techniques commonly used in genetic and physical mapping, map-based cloning, and mutant isolation by mouse genetics. Important topics in genetics were discussed in a series of four workshops.

PARTICIPANTS

Boudonck, K., M.S., Ph.D., John Innes Centre, Norwich, United Kingdom
Brembu, T., B.S., M.S., Norwegian University of Science and Technology, Trondheim, Norway
Costa Nunes, J., B.S., University of Oxford, United Kingdom
Date, S., B.S., M.S., University of Texas, Austin
Edwards-Stuart, A., B.A., University of Oxford, United Kingdom
Giuli, P., B.A., M.S., University "La Sapienza" of Rome, Italy
Huntley, R., B.S., Ph.D., University of Cambridge, United Kingdom
Kalbina, I., M.S., Goteborg University, Sweden
Kmieciak, M., B.S., M.S., Adam Mickiewicz University, Poland
Lorkovic, Z., B.S., Ph.D., Friedrich Miescher Institut, Basel, Switzerland
Matzke, A., B.S., M.S., Ph.D., Austrian Academy of Sciences, Salzburg
Naested, H., B.A., M.S., Copenhagen University, Denmark
Racicot, V., B.A., M.S., NEN Life Science Products, Boston, Massachusetts
Sohlenkamp, C., B.S., M.S., Max Planck Gesellschaft, Golm, Germany
Sponsel, V., B.S., Ph.D., University of Texas, San Antonio
Van Wijk, K., M.S., Ph.D., University of Stockholm, Sweden

SEMINARS

Barton, K., University of Wisconsin, Madison. Embryogenesis.
Benfey, P., New York University, New York. Root development.
Briggs, W., Carnegie Institute, Stanford, California. Blue light signal transduction.
Cande, Z., University of California, Berkeley. Meiosis.
Chory, J., Salk Institute, La Jolla, California. Brassinosteroids.
Cohen, J., U.S. Department of Agriculture, Beltsville, Maryland. Biochemistry of *Arabidopsis*.
Deng, X.-W., Yale University, New Haven, Connecticut. Light regulation and transcriptional control.
Dengler, N., University of Toronto, Ontario, Canada. Anatomy of *Arabidopsis*. Vascular anatomy and its development.
Grossniklaus, U., Friedrich Miescher Institute, Basel, Switzerland. The female gametophyte. Pollen development and fertilization.
Jack, T., Dartmouth College, Hanover, New Hampshire. Flower development.
Jackson, D., Cold Spring Harbor Laboratory. Leaf and meristem development.
Kerk, N., Yale University, New Haven, Connecticut. Auxin and the quiescent center.
Martienssen, R., Cold Spring Harbor Laboratory. Use of enhancer traps.
McCourt, P., University of Toronto, Ontario, Canada. Abscisic acid and giberellins.
Palmenberg, A., University of Wisconsin, Madison. Bioinformatics.
Poething, S., University of Pennsylvania, Philadelphia. Time and plant development.
Richards, E., Washington University, St. Louis, Missouri. Epigenetics.
Spector, D., Cold Spring Harbor Laboratory. Imaging theory and practice.
Sussex, I., Yale University, New Haven, Connecticut. Introduction to plant development.
Sussman, M., University of Wisconsin, Madison. Reverse genetics.
Theologis, A., University of California, Berkeley. The *Arabidopsis* genome. Molecular biology of auxin.
Von Arnim, A., University of Tennessee, Knoxville. Subcellular targeting.
Wessler, S., University of Georgia, Athens. Transposable elements.
Weigel, D., Salk Institute, La Jolla, California. Flowering.

Molecular Cloning of Neural Genes

July 2–22

INSTRUCTORS
Boulter, J., Ph.D., University of California, Los Angeles
Darnell, R., M.D., Ph.D., Rockefeller University, New York
Dulac, C., Ph.D., Harvard University, Cambridge, Massachusetts
Lai, C., Ph.D., The Scripps Research Institute, La Jolla, California
Lavery, D., Ph.D., Glaxo Wellcome Research Development, Lausanne, Switzerland
Rupp, F., Ph.D., Johns Hopkins University, Baltimore, Maryland

CO-INSTRUCTORS
Amrein, H., Duke University, Durham, North Caroline
Schwab, M., The Scripps Research Institute, La Jolla, California

ASSISTANTS
Chang, E., Harvard University, Boston, Massachusetts
Fung, E., The Scripps Research Institute, La Jolla, California
Gu, C., Johns Hopkins University, Bethesda, Maryland
Kong, H., Skirball Institute/New York University, New York
Samson, M., Harvard University, Boston, Massachusetts

This intensive laboratory-lecture course was intended to teach neuroscientists current approaches to molecular neurobiology. The course consisted of daily lectures and laboratory exercises on the practice of molecular neurobiology, with an emphasis on modern approaches to cloning and analyzing the expression of neural genes. A series of evening research seminars by invited speakers focused on the ways these molecular techniques have been successfully applied. In the past, evening seminar topics have included expression cloning; single cell cloning; subtractive cDNA cloning strategies; and genetic and mechanistic studies of neurologic disease, aquisition of cell identity, and axon guidance in the developing nervous system. The laboratory portion of the course began with instruction in a series of basic molecular biological techniques and rapidly advanced to more sophisticated methodologies. Students learned to prepare genomic, phage, and plasmid DNAs and total and poly(A)$^+$ RNAs, and to generate and screen cDNA libraries. Additional topics and methods covered included restriction mapping, agarose and polyacrylamide gel electrophoresis, Northern and Southern blotting, subcloning, oligonucleotide primer design, a selection of PCR-based techniques, and the use of nucleotide and protein sequence databases. Gene expression studies included the production of fusion proteins in bacteria and mammalian cell transfection. Advanced techniques included the construction of cDNA libraries from single cells and the use of subtractive cDNA methods to clone genes expressed in limited populations of cells.

PARTICIPANTS

Bhattacharyya, S., B.S., California Institute of Technology, Pasadena
Butler, A., B.A., Ph.D., The Salk Institute, La Jolla, California
Chow, R., B.A. M.D., Ph.D., University of Edinburgh, United Kingdom
Corwin, J., B.S., Ph.D., University of Virginia, Charlottesville
Dutt, A., M.S., Columbia University, New York
Gray, P., B.S., University of California, Los Angeles
Irving, C., B.S., Ph.D., King's College London, United Kingdom
Jakeman, L., B.A., Ph.D., Ohio State University, Columbus
Johansson, C., B.A. D.D.S., Karolinska Institute, Stockholm, Sweden
McGinty, J., B.A., Ph.D., East Carolina University School of Medicine, Greenville, North Carolina
Molnar, Z., M.D., Ph.D., University of Lausanne, Switzerland
Momma, S., M.S., Karolinska Institute, Stockholm, Sweden
Owens, D., B.A., Columbia University, New York
Tuttle, R., B.S., Ph.D., The Salk Institute, La Jolla, California
Udolph, G., B.S., Ph.D., National University of Singapore
Xiong, Z., M.D., Ph.D., University of Toronto, Canada

Barres, B., Stanford University, California. Neuron-glial interactions in the developing rat optic nerve.
Bettler, B., Novartis, AG, Basel, Switzerland. Molecular insights into GABA-B receptor physiology.
Chao, M., Skirball Institute/New York University, New York. Cell death and survival receptors in the nervous system.
Darnell, R., Rockefeller University, New York. Functional studies of neuron-specific proteins cloned as paraneoplastic neurologic disease antigens.
Dulac, C., Harvard University, Cambridge, Massachusetts. Molecular analysis of pheromone sensory coding in the mammalian brain.
Lemke, G., The Salk Institute, La Jolla, California. Mutation of the Tyro 3 family of receptors.
Stein, L., Cold Spring Harbor Laboratory. Sequence analysis resources on the World Wide Web.
Weinmaster, G., University of California, Los Angeles. Notch signal transduction in mammalian cells.

Neurobiology of *Drosophila*

July 2–22

INSTRUCTORS **Broadie, K.,** Ph.D., University of Utah, Salt Lake City
De Belle, S., Ph.D., University of Nevada, Las Vegas
Taylor, B., Ph.D., Oregon State University, Corvallis
Tear, G., Ph.D., King's College, London, United Kingdom

ASSISTANTS **Alldredge, A.,** University of Nevada, Las Vegas
Feathersone, D., University of Utah, Salt Lake City
Myat, A., King's College, London, United Kingdom

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 addressing 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. Students had hands-on experience with a variety of experimental preparations that are used in the investigation of current neurobiological questions. The lectures and laboratories focused on both the development of the nervous system and its role in controlling larval and adult behaviors. In addition

to an exposure to the molecular genetic approaches available in *Drosophila*, students learned a variety of techniques, including embryo in situ hybridization, labeling of identified neurons, electrophysiological recording from nerves and muscles, and the analysis of larval and adult behavior. Collectively, the course provided a comprehensive and practical introduction to modern experimental methods for studying the *Drosophila* nervous system.

PARTICIPANTS

Babu, K., B.S., National University of Singapore
Lee, R., B.S., University of California, Los Angeles
Lloyd, T., B.S., Baylor College of Medicine, Houston, Texas
Lohr, R., M.S., Johannes Gutenberg-University Mainz, Germany
McGuire, S., B.A., Baylor College of Medicine, Houston, Texas
Oh, P., B.A., Stanford University, California
Ren, X., B.S., University of California, Berkeley
Speese, S., B.S., University of Utah, Salt Lake City
Tavosanis, G., M.D., European Molecular Biology Laboratory, Heidelberg, Germany
Wildonger, J., B.A., University of California, San Francisco
Wolf, F., B.S., Ph.D., University of California, San Francisco
Wucherpfennig, T., B.S., M.S., MPI for Biophysical Chemistry, Goettingen, Germany

SEMINARS

Blau, J., Rockefeller University, New York. Neurobiology of rhythmicity in *Drosophila*.
Brand, A., The Wellcome/CRC Institute, Cambridge, United Kingdom. The role of asymmetric determinants in the specification of neural cell fate.
Broadie, K., University of Utah, Salt Lake City. Overview of *Drosophila* physiology. The neuromuscular junction: Synaptogenesis, neurotransmission, and synaptic plasticity.
De Belle, S., University of Nevada, Las Vegas. Behavior-genetic analysis in *Drosophila*. Neuroanatomy of odor learning and memory in *Drosophila*.
Hartenstein, V., University of California, Los Angeles. An introduction to *Drosophila* development.
Ito, K., National Institute for Basic Biology, Aichi, Japan. Identification and lineage tracing of postembryonic neuroblasts.
Kernan, M., State University of New York, Stony Brook. Hearing and touch reception in *Drosophila*.
Murphey, R., University of Massachusetts, Amherst. *Drosophila* neural circuits.
O'Dell, K., University of Glasgow, United Kingdom. Courtship and its neurobiological basis in *Drosophila*.
Prokop, A., Johannes Gutenberg-University Mainz, Germany. Determination and analysis of embryonic neuroblast lineage. An ultrastructural investigation of synaptogenesis.
Restifo, L., University of Arizona, Tucson. Development of the pupal central nervous system in *Drosophila*.
Sokolowski, M., University of Toronto, Canada. Neurobiology of foraging behavior in *Drosophila*.
Stark, W., St. Louis University, Missouri. Phototransduction: Light reception and processing in *Drosophila*.
Strauss, R., Max Planck Institute, Tübingen, Germany. Central processing of motor behavior in *Drosophila*.
Tear, G., King's College London School of Medicine, London, United Kingdom. Axon pathfinding in the embryo.
Treisman, J., New York Medical Center/Skirball Institute, New York. Development of the adult visual system.
Tully, T., Cold Spring Harbor Laboratory. Learning and memory in *Drosophila:* Psychology meets biology.
Zhong, Y., Cold Spring Harbor Laboratory. Plasticity in central and peripheral synapses.
Zinsmaier, K., University of Pennsylvania, Philadelphia. Genetic control of the synaptic vesicle cycle.

The Biology of Memory: From Molecules to Behavior

July 7–20

INSTRUCTORS **Byrne, J.,** Ph.D., University of Texas, Houston
Eichenbaum, H., Ph.D., Boston University, Massachusetts
Pearson, K., Ph.D., University of Alberta, Edmonton, Canada
Squire, L., Ph.D., University of California, San Diego

This lecture course provided an introduction to cell, molecular, and systems approaches to learning and memory. It was suited for graduate students in molecular biology, neurobiology, and psychology as well as research workers interested in an introduction to this field. Topics ranged from behavioral considerations of learning and memory to gene regulation in the nervous system. The lectures provided an intensive coverage of six selected areas: (1) an introduction to modern behavioral studies of learning and memory, (2) an overview of the cell biology of neuronal plasticity and second messenger systems, (3) the regulation of gene expression, (4) cellular and molecular mechanisms of simple forms of learning and memory in invertebrates and vertebrates, (5) cellular and molecular mechanisms of long-term potentiation and depression in various regions of the mammalian brain, and (6) systems approaches to learning in vertebrates and humans.

PARTICIPANTS

Chin, J., B.A., B.S., University of Texas, Houston
Dragoi, G., M.S., M.D., Rutgers University, Newark, New Jersey
Fortin, N., B.S., Ph.D., Boston University, Massachusetts
Fullgrabe, C., B.A., M.A., Fondation des Etats-Unis, Paris, France
Gray, N., B.S., Mayo Graduate School, Rochester, Minnesota
Hristov, K., M.S., University of Oxford, United Kingdom
Kim, D.G., M.D., Ph.D., Yonsei University, Seoul, South Korea
Libbey, M., B.A., Boston University, Massachusetts
Lucic, V., B.S., Ph.D., California Institute of Technology, Pasadena
Mehren, J., B.S., Brandeis University, Waltham, Massachusetts
Petersson, P., M.S., Lund University, Sweden
Petkov, C., B.S., University of California, Davis
Petrulis, A., B.S., Ph.D., Boston University, Massachusetts
Stark, C., B.A., Ph.D., University of California, San Diego
Stewart, D., B.S., Imperial College, London, United Kingdom
Tapechum, S., M.D., University of Edinburgh, United Kingdom
Taubenfeld, S., B.A., Brown University, Providence, Rhode Island
Volkar, V., M.D., University of Helsinki, Finland

SEMINARS

Ball, G., Johns Hopkins University, Baltimore, Maryland. Ethological approaches to learning.
Byrne, J., University of Texas, Houston. Introduction to the cellular study of learning. Overview of membranes and synaptic transmission. Nonassociative learning in *Aplysia* I. Nonassociative learning in *Aplysia* II.
Davis, M., Emory University, Atlanta, Georgia. Fear conditioning.
Eichenbaum, H., Boston University, Massachusetts. Role of LTP and LTD in learning. Memory systems in rodents.
Gilbert, C., Rockefeller University, New York. Dynamics of adult visual cortex.
Greenough, W., University of Illinois, Urbana. Morphological correlates of learning and experience.
Griffith, L., Brandies University, Waltham, Massachusetts. Genetic approaches to study learning in *Drosophila*.
Holland, P., Duke University, Durham, North Carolina. Introduction to learning—Theory I. Introduction to learning—Theory II.
Kaczmarek, L., Yale University, New Haven, Connecticut. Structure, function, and modulation of ion channels.
Kennedy, M., California Institute of Technology, Pasadena. Overview of second-messenger systems and their role in learning and memory I. Overview of second-messenger systems and their role in learning and memory II.
Linden, D., Johns Hopkins University, Baltimore, Maryland. Long-term depression.
Lisberger, S., University of California, San Francisco. Adaptive modifications of the VOR.
Lu, B., National Institutes of Health, Betheda, Maryland. Long-term potentiation II.
Malinow, R., Cold Spring Harbor Laboratory. Long-term potentiation I.
Mayford, M., University of California, San Diego. Gene-knocking/transgenic approaches to study learning.
McGaugh, J., University of California, Irvine. Modulation of memory.
Menzel, R., Freie University, Berlin, Germany. Memory phases in honeybees.
Posner, M., Cornell University, New York. Neuropsychology of cognition.
Sahley, C., Purdue University, West Lafayette, Indiana. Nonassociative and associative learning in the leach.
Sweatt, D., Baylor College of Medicine, Houston, Texas. Long-term potentiation III.
Thompson, R., University of Southern California, Los Angeles. Classical conditioning of the nictitating membrane response.
Squire, L., University of California, San Diego. Memory systems in nonhuman primates. Human memory and disorders of memory.

Structure, Function, and Development of the Visual System

July 22–August 4

INSTRUCTORS **Bonhoeffer, T.,** Ph.D., Max Planck Institute, Munchen, Germany
Fitzpatrick, D., Ph.D., Duke University Medical Center, Durham, North Carolina
McAllister, K., Ph.D., The Salk Institute, La Joila, California
Usrey, M., Ph.D., Harvard Medical School, Boston, Massachusetts

This lecture-discussion course explored the functional organization and development of the visual system as revealed by the use of a variety of anatomical, physiological, and behavioral methods. It was designed for graduate students and more advanced researchers who wished to gain a basic understanding of the biological basis of vision and to share in the excitement of the latest developments in this field. Topics included phototransduction and neural processing in the retina; functional architecture of striate cortex; cellular basis of cortical receptive field properties; the anatomy, physiology, and perceptual significance of parallel pathways; functional parcellation of extrastriate cortex; the role of patterned neuronal activity in the development of central visual pathways; and molecular mechanisms of development and plasticity in the visual system.

PARTICIPANTS

Alliston, E., B.S., New York University, New York
Barbarosie, M., B.S., Ph.D., Brown University, Providence, Rhode Island
Boudreau, E., B.A., Baylor College of Medicine, Houston, Texas
Briggs, F., B.A., The Salk Institute, La Jolla, California
Burkitt, G., B.S., Ph.D., Rockefeller University, New York
Chisum, H., B.S., Duke University, Durham, North Carolina
Cueva, J., B.S., University of California, Los Angeles
Duffy, K., B.A., McMaster University, Ontario, Canada
Fiser, J., M.S., Ph.D., University of Rochester, New York
Florer, F., M.S., Ph.D., Marymount College and New York University, Tarrytown
Furtado, D., B.S., M.S., University of Rio de Janeiro, Brazil
Godinho, L., B.S., Howard Florey Institute, Victoria, Australia
Kara, P., B.S., M.S., Ph.D., Harvard Medical School, Boston, Massachusetts
Kohly, R., B.S., M.A., York University, Toronto, Canada
Macknik, S., B.A., Ph.D., Harvard Medical School, Boston, Massachusetts
Martinez, L., B.A., Ph.D., Rockefeller University, New York
Martinez-Conde, S., B.A., Ph.D., Harvard Medical School, Boston, Massachusetts
Oberdorfer, M., B.A., Ph.D., National Institutes of Health, Bethesda, Maryland
Sermasi, E., M.A., Ph.D., International School for Advanced Studies, Trieste, Italy
Tropea, D., B.S., International School for Advanced Studies, Trieste, Italy
Vaz, A.M., M.D., Max Planck Institute, Munich, Germany

SEMINARS

Adelson, T., Massachusetts Institute of Technology, Cambridge. Psychophysics and human imaging.
Assad, J., Harvard Medical School, Boston, Massachsuetts. Extrastriate cortex.
Bear, M., Brown University, Providence, Rhode Island. Development.
Bonhoeffer, T., Max Planck Institute, Munich, Germany. Striate cortex.
Chapman, B., University of California, Davis. Development.
Dacey, D., University of Washington, Seattle. Retina.
DeYoe, T., Medical College of Wisconsin, Milwaukee. Psychophysics and human imaging.
Feller, M., National Institutes of Health, Bethesda, Maryland. Development.
Ferster, D., Northwestern University, Evanston, Illinois. Striate cortex.
Fitzpatrick, D., Duke University, Durham, North Carolina. Striate cortex.
Gross, C., Princeton University, New Jersey. The history of the visual system.
Hubel, D., Harvard Medical School, Boston, Massachusetts. Striate cortex.
Maunsell, J., Baylor College of Medicine, Houston, Texas. Extrastriate cortex.
McAllister, K., Salk Institute, La Jolla, California. Development.
Miller, K., University of California, San Francisco. Development.
Movshon, T., New York University, New York. Extrastriate cortex.
Reid, C., Harvard Medical School, Boston, Massachusetts. Striate cortex.
Rieke, F., University of Washington, Seattle. Retina.
Shadlen, M., University of Washington, Seattle. Extrastriate cortex.
Singer, W., Max Planck Institute, Frankfurt, Germany. Striate cortex.
Usrey, M., Harvard Medical School, Boston, Massachusetts. Striate cortex.

Eukaryotic Gene Expression

July 27–August 16

INSTRUCTORS **Gill, G.,** Ph.D., Harvard Medical School, Boston, Massachusetts
Gilmour, D., Ph.D., Pennsylvania State University, University Park
Goodrich, J., Ph.D., University of Colorado, Boulder
Lees, J., Ph.D., Massachusetts Institute of Technology, Cambridge

ASSISTANTS **Benjamin, L.,** Pennsylvania State University, University Park
Kotovsky, I., Harvard Medical School, Boston, Massachusetts
Kugel, J., University of Colorado, Boulder
Verona, R., Massachusetts Institute of Technology, Cambridge

This course was designed for students, postdocs, and principal investigators 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. Cell-free extracts for in vitro transcription were prepared and RNA levels were measured by primer extension analysis. An emphasis was placed on biochemical studies of protein-DNA and protein-protein interactions. A detailed characterization of the DNA-binding properties of a site-specific transcription factor was carried out using electrophoretic mobility shift and DNase I footprinting assays. These assays were used to study protein-DNA interactions in crude extracts and using recombinant proteins purified in class. Chromatography and coimmunoprecipitation methods

were used to investigate protein-protein interactions. Over the past few years, the gene regulation field has begun to emphasize the importance of in vivo approaches to studying protein-DNA and protein-protein interactions. Students were therefore exposed to in vivo footprinting, mapping of DNase I hypersensitive sites, and 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 gene regulation and technical approaches to their solution.

PARTICIPANTS

Beere, H., B.S., Ph.D., La Jolla Institute for Allergy and Immunology, San Diego, California
Cuff, M., B.S., Ph.D., University of Liverpool, United Kingdom
Damberg, M., M.S., Ph.D., Uppsala University, Sweden
Esumi, N., M.D., Ph.D., Johns Hopkins University, Baltimore, Maryland
Ford, J., B.A., M.D., Stanford University, California
Hamilton, N., B.S., Meharry Medical College, Nashville, Tennessee
Hollowell, G., B.S., Ph.D., National Institutes of Health, Bethesda, Maryland
Huot, T., B.S., M.S., Imperial Cancer Research Fund, London, United Kingdom.
Jonsson, C., B.S., Karolinska Institute, Stockholm, Sweden
Kimura, A., B.S., M.S., University of Tokyo, Japan
Koentges, G., B.A., Ph.D., Harvard University, Cambridge, Massachusetts
Lepique, A., B.S., M.S., University of San Paolo, Brazil
Luce, M., B.S., Ph.D., Yale University, New Haven, Connecticut
McTiernan, C., B.A., Ph.D., University of Pittsburgh, Pennsylvania
Reddi, P., B.S., Ph.D., University of Virginia, Charlottesville
Zhang, J., B.M., M.D., Beth Israel Deaconess Medical Center, Boston, Massachusetts

SEMINARS

Archer, T., National Institutes of Health, Bethesda, Maryland. Posttranslational modification of histones, chromatin remodeling, and activated transcription.
Bentley, D., University of Colorado, Denver. The role of the pol II CTD in transcription and RNA processing.
Blackwell, K., Harvard Medical School, Boston, Massachusetts. Activation and repression of zygotic transcription in the early C. *elegans* embryo.
Burley, S., Rockefeller University, New York. X-ray crystallographic studies of eukaryotic transcription factors.
Carey, M., University of California, Los Angeles. Enhanceosome-mediated transcription complex assembly.
Gill, G., Harvard Medical School, Boston, Massachusetts. Transcription factors that function in the *Drosophila* nervous system.
Gilmour, D., Pennsylvania State University, University Park. Transcription regulation of the hsp70 heat shock gene of *Drosophila*.
Goodrich, J., University of Colorado, Bolder. Mechanisms of human RNA polymerase II transcription.
Freedman, L., Memorial Sloan-Kettering Cancer Center, New York. DRIPS: A multiprotein coactivator complex for nuclear receptors and beyond.
Hernandez, N., Cold Spring Harbor Laboratory. Structure and function of $SNAP_c$, a core promoter binding factor involved in RNA polymerase II and III snRNA gene transcription.
Lees, J., Massachusetts Institute of Technology, Cambridge. E2F in proliferation and development.
Price, D., University of Iowa, Iowa City. Factors controlling the elongation phase of transcription by RNA polymerase II.
Reese, J., Pennsylvania State University, University Park. Molecular and genetic analysis of yeast TATA-binding protein associated factors (TAFs).
Smale, S., University of California/Howard Hughes Medical Institute, Los Angeles. Nuclear architecture and nucleosome remodeling in the immune system.
Workman, J., Pennsylvania State University, University Park. Transcription regulation by SWI/SNF and HAT complexes.
Young, R., Massachusetts Institute of Technology, Cambridge. The new genetics of gene expression.
Zaret, K., Brown University, Providence, Rhode Island. Opening up specific chromatin domains in development.

Imaging Structure and Function in the Nervous System

July 27–August 16

INSTRUCTORS **Denk, W.,** Ph.D., Lucent Technologies Bell Laboratory, Murray Hill, New Jersey
Halpain, S., Ph.D., Scripps Research Institute, La Jolla, California
Kay, S., Ph.D., Scripps Research Institute, La Jolla, California

ASSISTANTS **Arnth-Jensen, N.,** University of Zurich, Switzerland
Littlefield, R., Scripps Research Institute, La Jolla, California
Oheim, M., Ecole Supérieure de Physique et Chimie Industrielles, Paris, France
Ozer, R., Scripps Research Institute, La Jolla, California

Advances in light microscopy and digital image processing and the development of a variety of powerful fluorescent probes present expanding opportunities for investigating the nervous system, from synaptic spines to networks in the brain. This intensive laboratory and lecture course provided participants with the theoretical and practical knowledge they need to use emerging imaging technologies. The primary emphasis of the course was on vital light microscopy. Students learned the principles of light microscopy, as well as the use of different types of electronic cameras, laser scanning systems, functional fluorophores, delivery techniques, and digital image-processing software. In addition to transmitted light microscopy for viewing cellular motility, the course examined a variety of molecular probes of cell function, including calcium-sensitive dyes, voltage-sensitive dyes, photoactivated ("caged") compounds, and exocytosis tracers. Issues arising in the combination of imaging with electrophysiological

methods were covered. Particular weight was given to multiphoton laser scanning microscopy and to newly available biological fluorophores, especially green fluorescent protein (GFP) and its variants. A spectrum of neural and cell biological systems, including living animals, brain slices, and cultured cells, was used. Participants had a strong background in the neurosciences or in cell biology.

PARTICIPANTS

Bringuier, V., M.S., Ph.D., Medical Research Council Laboratory of Molecular Biology, Cambridge, United Kingdom
Diamond, J., B.S., Ph.D., Oregon Health Sciences University, Portland
Harris, R., B.S., Ph.D., National Institutes of Health, Bethesdsa, Maryland
Kuperman, R., B.S., University of California, San Francisco
Luscher, C., M.D., University of California, San Francisco
Mikko, I., B.A., New York University, New York
Orger, M., B.A., University of California, San Francisco
Roesch, H., B.S., Max Planck Institute, Munich, Germany
Scolnick, E., M.D., Merck Research Laboratories, West Point, Pennsylvania
Sharma, G., M.S., Ph.D., University of Colorado Health Sciences Center, Denver
Uchida, N., B.S., Ph.D., RIKEN Brain Science Institute, Saitama, Japan
Usdin, M., B.S., Stanford University, California
Van Roessel, P., B.S., M.S., University of Cambridge, United Kingdom
Zeng, H., B.S., Ph.D., Massachusetts Institute of Technology, Cambridge

SEMINARS

Aoki, C., New York University, New York. EM: Imaging macromolecular organization.
Betz, W., University of Colorado, Denver. Imaging neurotransmitter release.
Bonhoeffer, T., Max Planck Institute, Munich, Germany. Intrinsic imaging.
Brand, A., Wellcome/CRC Institute, Cambridge, United Kingdom. Imaging neuronal development in flies.
Connor, J., University of New Mexico, Albuquerque. Calcium imaging in brain slices.
Day, R., University of Virginia, Charlottesville. Studies of protein-protein interaction using FRET in vivo.
Denk, W., Lucent Technologies Bell Labs, Murray Hill, New Jersey. Optical physics. Lasers. Reviews of optical principles. Introduction to multiphoton imaging.
Fetcho, J., State University of New York, Stony Brook. Calcium imaging in zebrafish.
Forscher, P., Yale University, New Haven, Connecticut. Principles of DIC—DIC imaging of growth cones.
Halpain, S., Scripps Research Institute, La Jolla, California. Fixation and staining: Immunocytochemistry. Gene, protein, and probe delivery into neurons.
Helmchen, F., Lucent Technologies Bell Labs, Murray Hill, New Jersey. Introduction to calcium imaging.
Jay, D., Tufts University, Boston, Massachusetts. Chromophore-assisted laser inactivation.
Keller, E., Zeiss Microscopes, Thornwood, New York. Introduction to the light microscope.
Kleinfeld, D., University of California, San Diego. Imaging blood flow in the living brain.
Lichtman, J., Washington University, St. Louis, Missouri. Introduction to confocal microscopy. Imaging long-term changes in synapses using confocal microscopy.
Loew, L., University of Connecticut Health Sciences Center, Farmington. Voltage-sensitive dyes.
Matus, A., Friedrich Miescher Institute, Basel, Switzerland. Imaging dendritic spine motility in living neurons.
Moomaw, L., Hamamatsu Photonics, Bridgewater, New Jersey. Cameras and detectors.
Schafer, W., University of California, San Diego. Imaging neuronal development in frog.
Svoboda, K., Cold Spring Harbor Laboratory. Application of multiphoton imaging.
Taylor, L., Cellomics, Inc., Pittsburgh, Pennsylvania. Fluorescence and the genomic revolution.
Tsien, R., University of California, San Diego. Imaging cameleons in *C. elegans* and other new applications of fluorescent probes.
Waterman-Storer, C., Scripps Research Institute, La Jolla, California. Combining DIC and fluorescence microscopy for live cell imaging. Fluorescent speckle microscopy.
Weiss, S., Lawrence Berkeley National Lab, Berkeley, California. Q-Dots.

Yeast Genetics

July 27–August 16

INSTRUCTORS **Burke, D.,** Ph.D., University of Virginia, Charlottesville
Dawson, D., Ph.D., Tufts University, Boston, Massachusetts
Stearns, T., Ph.D., Stanford University, California

ASSISTANTS **Frank, J.,** University of Virginia, Charlottesville
Preble, A., University of Colorado, Boulder
Shanks, R., Tufts University, Boston, Massachusetts

The major laboratory techniques used in the genetic analysis of yeast were studied in this course, including the isolation and characterization of mutants, tetrad analysis, complementation, and mitotic recombination. All students carried out the micromanipulation used in tetrad analysis. They studied molecular genetic techniques, including yeast transformation, gene replacement, analysis of gene fusions, and generation of mutations in cloned genes. 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

Boddy, C., B.S., Ph.D., Scripps Research Institute, La Jolla, California
Chan, D., M.A., M.D., Ph.D., Massachusetts Institute of Technology/Whitehead Institute, Cambridge
Fabrizio, P., B.S., Ph.D., University of Southern California, Los Angeles
Galindo-Castro, I., B.S., Ph.D., Polar Technology Center, Venezuela
Gendron, C., B.S., Ph.D., Max Planck Institute, Rostock, Germany
Ideker, T., B.S., M.S., University of Washington, Seattle
Jonson, L., M.S., Copenhagen University Hospital, Denmark
March, T., B.S., University of Idaho, Moscow
Monje Casas, F., B.S., University of Cordoba, Spain
Newman, J., B.S., Massachusetts Institute of Technology/Whitehead Institute, Cambridge
Otterstedt, K., M.S., Goteborg University, Sweden
Pannunzio, A., B.S., McGill University, Montreal, Canada
Perlstein, D., B.S., Massachusetts Institute of Technology, Cambridge
Schuller, D., M.S., University of Minho, Braga, Portugal
Yao, G., B.S., University of Wisconsin, Madison

SEMINARS

Amon, A., Massachusetts Institute of Technology, Cambridge. Regulation of mitotic exit in yeast.
Boeke, J., Johns Hopkins University, Baltimore, Maryland. The Ty1 elements of yeast.
Fink, G., Whitehead Institute, Cambridge, Massachusetts. Invasive growth.
Hopper, A., Pennsylvania State University, Hershey. Evidence for nuclear tRNA charging and its role in nuclear export of endogenous tRNAs in budding yeast.
Konopka, J., State University of New York, Stony Brook. Activation and regulation of the α-factor mating pheromone receptor.
Lichten, M., National Cancer Institute, NIH, Bethesda, Maryland. Meiotic recombination.
Lundblad, V., Baylor College of Medicine, Houston, Texas. Tethering telomerase to the telomere: Analysis of in vivo regulators.
Meluh, P., Memorial Sloan-Kettering Cancer Institute, New York. Centromere assembly and function.
Pellman, D., Dana Farber Cancer Institute, Boston, Massachusetts. Neoclassicism in yeast cell biology.
Roof, D., University of Pennsylvania, Philadelphia. Molecular motors.
Smith, M., University of Virginia, Charlottesville. Histone structure and function.
Snyder, M., Yale University, New Haven, Connecticut. Yeast genomics.
Weinert, T., University of Arizona, Tucson. DNA damage checkpoint.
Winey, M., University of Colorado, Boulder. Budding yeast spindle pole body duplication.

Brain Mapping

August 6–12

INSTRUCTORS **Mazziotta, J.,** M.D., Ph.D., University of California, Los Angeles
Toga, A., Ph.D., University of California, Los Angeles

The aim of this lecture course was to describe the rapidly evolving developments in methods used for mapping the structure and function of the brain, both to understand its normal function and to evaluate neurological, neurosurgical, and psychiatric disease states. This course described new and traditional methods, including magnetic resonance imaging (including functional, spectroscopic, and angiographic approaches), positron emission tomography, electrophysiological techniques, optical intrinsic signal imaging, digital approaches to conventional postmortem neuroanatomical investigations, and experimental design, data analysis, statistical approaches, visualization, and stereotaxy. The course was not designed to simply describe methods, but rather to discuss how brain-mapping strategies can be used in combination with biological models for understanding the structure and function of the brain. Findings relevant to the function of the visual, motor, language, memory, and cognitive brain systems, as well as diseases that adversely affect them, were discussed. Students developed specific hypotheses and experimental designs for mock execution, and they went on a field trip to an imaging

laboratory. They toured an active PET laboratory and a 4-tesla MRI center, both at Brookhaven National Laboratory. Invited speakers included world leaders in each of the respective brain mapping subspecialties.

PARTICIPANTS

Bussey, T., Ph.D., National Institutes of Health, Bethesda, Maryland
Capaday, C., Ph.D., Université Laval Robert Giffard, Quebec, Canada
Cash, D., B.S., University of London, Kings College, United Kingdom
Giedd, J., M.D., National Institutes of Health, Bethesda, Maryland
Holm, L., Ph.D., European Molecular Biology Laboratory, Cambridge, United Kingdom
Jacobs, M., B.A., National Institutes of Health, Bethesda, Maryland
Margolis, D., B.S., Brown University, Providence, Rhode Island
Pinsk, M., B.S., National Institutes of Health, Bethesda, Maryland
Speer, A., M.D., National Institutes of Health, Bethesda, Maryland

SEMINARS

Bandettini, P., National Institutes of Health, Bethesda, Maryland. MRI.
Belliveau, J., Massachusetts General Hospital, Charleston. Vision.
Grafton, S., Emory University, Atlanta, Georgia. Motor systems.
Fletcher, P., Heinrich Hein Universitat, Dussseldorf, Germany. Language and memory.
Mayberg, H., University of Toronto, Ontario, Canada. Emotions and mood disorders.
Mazziotta, J., University of California, Los Angeles. PET. ATLASES.
Petersen, S., Washington University, St. Louis, Missouri. Experimental design.
Petrides, M., McGill University, Quebec, Canada. Cortex: Structure and function.
Pons, T., Wake Forest University, Winston-Salem, North Carolina. Plasticity.
Simpson, G., Albert Einstein College of Medicine, Bronx, New York. EEG—MEG—ERP.
Toga, A., University of California, Los Angeles. Microtechniques: OIS, histology, cryomacrotome.
Woods, R., University of California, Los Angeles. Spatial issues, statistical issues.
Woolsey, T., Washington University, St. Louis, Missouri. Hemodynamics.
Zeffiro, T., Sensor Systems, Inc., Sterling, Virginia. Data analysis.

Advanced In Situ Hybridization and Immunocytochemistry

October 13–26

INSTRUCTORS **Ochs, R.,** Ph.D., Precision Therapeutics, Pittsburgh, Pennsylvania
Murray, J., M.D., Ph.D., University of Pennsylvania, Philadelphia
Spector, D., Ph.D., Cold Spring Harbor Laboratory
Ried, T., Ph.D., National Institutes of Health, Bethesda, Maryland
Schrock, E., Ph.D., Institut für Molekulare Biotechnologie, Jena, Germany

ASSISTANTS **Binnie, A.,** Sir William Dunn School of Pathology, Oxford, United Kingdom
Howard, T., Cold Spring Harbor Laboratory

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 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 was in need of microscopic approaches and for the cell biologist who was not familiar with the practical application of the advanced techniques presented. Among the methods present-

ed 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. Several experimental protocols were presented in each method, allowing the students to assess the relative merits of each and to relate them to their own research. Students were encouraged to bring nucleic acid or antibody probes to the course, which were used in addition to those provided by the instructors. The laboratory portion of the course was supplemented by invited lecturers, who presented up-to-the-minute reports on current methods and research using the techniques being presented in the course.

PARTICIPANTS

Abramsson, A., M.S, Ph.D., Goteborg University, Sweden
Craig, E., B.S., Albert Einstein College of Medicine, Bronx, New York
Di Daniel, E., B.A., International School for Advanced Studies, Trieste, Italy
Hemken, P., B.S., M.A., Ph.D., Abbott Laboratories, Abbott Park, Illinois
Holmberg, S., M.S., Uppsala University, Sweden
Jones, G., B.S, Ph.D., University of Kentucky, Lexington
Karpova, A., B.S., M.S., Harvard Medical School, Boston, Massachusetts
Kisielow, J., B.S., M.S., Basel Institute for Immunology, Switzerland
Margotti, E., B.S., Ph.D., International School for Advanced Studies, Trieste, Italy
O'Doherty, U., B.A., M.D., Ph.D., University of Pennsylvania, Philadelphia
Ozcan, T., B.S., M.D., Yale University, New Haven, Connecticut
Raghib, A., B.S., University College London, United Kingdom
Roethy, W., M.S., Columbia University, New York
Soultanakis, R., B.A., University of Vermont, Burlington
Tuttle, J., B.S., Ph.D., Indiana University, Indianapolis
Wegel, E., B.S., M.S., John Innes Centre, Norwich, United Kingdom

SEMINARS

Day, R.N., University of Virginia Health Sciences Center, Charlottesville. Visualizing protein interactions in living cells using digitized GFP imaging and FRET microscopy.
Dernburg, A., Stanford University School of Medicine, California. In situ hybridization in embryos.
Ernst, L., Carnegie Mellon University, Pittsburgh, Pennsylvania. Filters and fluorochromes for fluorescence microscopy.
Martone, M., University of California at San Diego, La Jolla. Electron microscopy as a tool in cell and molecular biology.
Matera, G., Case Western Reserve University, Cleveland, Ohio. Fluorescence in situ hybridization.
Murray, J., University of Pennsylvania, Philadelphia. Basic introduction to light and fluorescence microscopy. Principles of confocal microscopy and deconvolution.
Pederson, T., University of Massachusetts Medical School, Worcester. RNA dynamics in living cells.
Ried, T., National Institutes of Health/NCI, Bethesda, Maryland. CGH and SKY.
Singer, R.H., Albert Einstein College of Medicine, Bronx, New York. Cytoplasmic organization of mRNA.
Spector, D.L., Cold Spring Harbor Laboratory. Immunocytochemistry.
White, J., University of Wisconsin, Madison. Multi-photon microscopy.

Genome Informatics

October 13–26

INSTRUCTORS **Rozen, S.,** Ph.D., Massachusetts Institute of Technology/Whitehead Institute, Cambridge
Stein, L., Ph.D., Cold Spring Harbor Laboratory

ASSISTANT **Sachidanandam, R.,** Cold Spring Harbor Laboratory

The desktop computer is rapidly becoming an indispensable tool in the biologists' toolchest. The success of the human genome project has created an explosion of information: billions of bits of biological information stashed electronically in databases around the globe just waiting for the right key to unlock them. New technologies such as DNA microarrays and high-throughput genotyping are creating an information overload that the traditional laboratory notebook cannot handle. To exploit the information revolution in biology, biologists must move beyond canned Web interfaces and Excel spreadsheets. They must take charge of the data by creating their own software to fetch, manage, and integrate them. The goal of this course was to provide biologists with the tools needed to deal with this changing landscape. Designed for students and researchers with little prior knowledge of programming, this two-week course taught the fundamentals of the UNIX operating system, Perl scripting, dynamic Web page development with the CGI protocol, and database design. The course combined formal lectures with hands-on experience in which students worked to solve a series of problem sets

drawn from common scenarios in biological data acquisition, integration, and laboratory workflow management. Students were also encouraged to pose problems using their own data and to work together with faculty to solve them.

PARTICIPANTS

Brown, J., B.S., Ph.D., SmithKline Beecham Pharmaceuticals, Collegeville, Pennsylvania
Caffrey, J., B.A., Ph.D., National Institutes of Health, Research Triangle Park, North Carolina
Caporale, L., B.S., Ph.D., Independent Research, New York
Chen, T., B.S., B.A., Ph.D., Forsyth Institute, Boston, Massachusetts
Davis, B., B.A., Ph.D., Proteome, Inc., Beverly, Massachusetts
Glass, J., B.S., M.A., Eli Lilly & Company, Indianapolis, Indiana
Gomez, M., B.S., Ph.D., Public Health Research Institute, New York
Halgren, R., B.S., Ph.D., Michigan State University, East Lansing
Harris, T., B.A., Ph.D., University of Utah, Salt Lake City
Huang, L., B.S., Ph.D., University of California, San Francisco
Kissinger, J., A.B., Ph.D., University of Pennsylvania, Philadelphia
McCombie, W.R., B.A., Ph.D., Cold Spring Harbor Laboratory
Rabinowicz, P., B.S., M.S., Ph.D., Cold Spring Harbor Laboratory
Sana, T., B.S., Ph.D., DNAX Research Institute, Palo Alto, California
Stein, J., B.S., Ph.D., Cereon Genomics, Cambridge, Massachusetts
Stoddard, S., B.S., Ph.D., Archer Daniels Midland Company, Decatur, Illinois
Udar, N., B.S., Ph.D., Jules Stein Eye Institute, Los Angeles, California
Viriyakosol, S., B.S., Ph.D., University of California, San Diego
Walhout, A., M.D., Ph.D., Massachusetts General Hospital, Charlestown

SEMINARS

Bader, J., CuraGen Corporation, New Haven, Connecticut.
Access to gene expression data.
Birney, E., EMBL-European Bioinformatics Institute, Cambridge, United Kingdom. Perl Scripting 5: Installing and creating Perl modules. Perl Scripting 6: Objects. Sequence alignment. Web-based protein analysis resources.
Chervitz, S., Neomorphics, Inc., Berkeley, California.
Introduction to BioPerl. Similarity searching with BioPerl.
Introduction to neomorphics tools.
Eddy, S., Washington University School of Medicine, St. Louis, Missouri. RNA sequence/structure analysis.
Gish, W., Washington University School of Medicine, St. Louis, Missouri. The BLAST/FAST family of sequence-similarity tools I. The BLAST/FAST family of sequence-similarity tools II.
Jamison, C., National Institutes of Health, Bethesda, Maryland. Web-based nucleotide analysis resources.
Leach, M., CuraGen Corporation, New Haven, Connecticut.
CGI Scripting 1: Basic. CGI Scripting 2: Advanced.
Marth, G., Washington University School of Medicine, St. Louis, Missouri. Basic sequence manipulation: Primer picking, repeat masking, vector finding.
Peitzsch, R., Pfizer, Inc., Groton, Connecticut. SQL databases.
Rehban, M., AstraZeneca, Inc., Cambridge, Massachusetts.
Web design and usability.
Rozen, S., Whitehead Institute for Biomedical Research, Cambridge, Massachusetts. Using the UNIX operating system 2: Command pipes, redirection, periodic tasks.
Review of Perl essentials: Arrays, hashes, etc.
Review of database essentials.
Review of Perl essentials: Control constructs.
Perl scripting 4: Perl references.
Providing Web information services 1.
Introduction to databases. The UNIX DBM family.
Sachidanandam, R., Cold Spring Harbor Laboratory. Perl scripting 3: Perl basics: Arrays and hashes.
Snoody, J., Oak Ridge National Laboratory, Oak Ridge, Tennessee. Gene prediction software.
Stein, Lincoln, Cold Spring Harbor Laboratory.
Using the UNIX Operating System 1: Files, directories, commands, text editor.
Perl Scripting 1: Perl basics: Operators, functions.
Perl Scripting 2: Perl basics: Loops, input/output, regular expressions.
Introduction to the Web: HTML, HTTP, FTP.
Extracting information from Web pages: Client-side FTP and HTTP techniques.

Macromolecular Crystallography

October 13–26

INSTRUCTORS **Furey, W.,** Ph.D., Veteran's Administration Medical Center, Pittsburgh, Pennsylvania
Gilliland, G., Ph.D., National Institute of Standards and Technologies, Rockville, Maryland
McPherson, A., Ph.D., University of California, Irvine
Pflugrath, J., Ph.D., Molecular Structure Corporation, The Woodlands, Texas

ASSISTANT **Chu, S.,** National Institute of Standards and Technology, 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, crystal freezing, synchrotron data collection, data reduction, multiple isomorphous replacement, multiwavelength anomalous diffraction phase determination, solvent flattening, molecular replacement, electron density interpretation, structure refinement, molecular graphics, noncrystallographic symmetry averaging, simulated annealing, and coordinate desposition. Participants learned through extensive hands-on experiments. They crystallized and determined a protein structure, heard lectures on the theory, and had informal discussions about what is behind the techniques.

PARTICIPANTS

Caffrey, M., B.S., Ph.D., Ohio State University, Columbus
Domaille, P., B.S., Ph.D., DuPont Pharmaceuticals Company, Wilmington, Delaware
Dottorini, T., B.S., Institute of Research and Molecular Biology, Pomezia, Italy
Harvey, S., B.S., Ph.D., University of Alabama, Birmingham
Manning, N., B.S., M.S., Brookhaven National Laboratory, Upton, New York
Mapelli, M., B.S., Ph.D., European Molecular Biology Laboratory, Heidelberg, Germany
Mark, B., B.S., University of Alberta, Edmonton, Canada
Mesecar, A., B.S., Ph.D., University of Illinois, Chicago
Rife, C., B.A., Vanderbilt University, Nashville, Tennessee
Rosen, M., B.S., Ph.D., Cornell Medical College, New York
Sorensen, T., M.S., University of Aarhus, Denmark
Spicer, E., B.S., Ph.D., Medical University of South Carolina, Charleston
Stevens, A., B.S., M.S., Monsanto Company, St. Louis, Missouri
Tang, W.-J., B.S., Ph.D., University of Chicago, Illinois
Tsai, S.-C., B.S., Ph.D., University of California, San Francisco
Zarembinski, T., B.A., Ph.D., Argonne National Laboratory, Illinois

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Adam, P., Yale University/Howard Hughes Medical Institute, New Haven, Connecticut. Simulated annealing refinement.
Gilliland, G., National Institute of Standards and Technology, Rockville, Maryland. Deposition and querying the protein data bank.
Hung, L.-W., Lawrence Berkeley National Laboratory, Berkeley, California. Practical aspects of MAD methods.
Joshua-Tor, L., Cold Spring Harbor Laboratory. Bleomycin hydrolase: Intriguing structure, enigmatic activities, and links to diseases.
Richardson, David and Jane, Duke University, Durham, North Carolina. The importance of packing hydrogens when making a rotamer library.
Vaughn, D., Cold Spring Harbor Laboratory. In the Apaf-1 CARD game, two pair is more than good enough.
Weeks, C., Hauptman-Woodward Medical Research Institute, Inc. Buffalo, New York. Finding seleniums with SnB.
Xu, R., Cold Spring Harbor Laboratory. A novel structure of human mitochondrial protein p32.

Positional Cloning: Contig to Candidate Gene

October 13–26

INSTRUCTORS	**Jacob, H.,** Ph.D., Medical College of Wisconsin, Milwaukee **Silverman, G.,** M.D., Ph.D., Harvard Medical School, Boston, Massachusetts **Spencer, F.,** Ph.D., Johns Hopkins University, Baltimore, Maryland
CO-INSTRUCTORS	**Baxevanis, A.,** National Human Genome Research Institute, Bethesda, Maryland **Parimoo, S.,** Johnson & Johnson, Skillman, New Jersey
ASSISTANTS	**Kwitek-Black, A.,** Medical College of Wisconsin, Milwaukee **Mills, D.,** Brown University, Providence, Rhode Island

This laboratory-based course was designed for investigators to use genetic and physical mapping tools or functional assays to isolate genes of interest, with emphasis on procedures for isolating and evaluating 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 embryonic stem cells 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 was 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 prominent investigators who use many of the procedures taught in the course. All participants gave presentations about their own research topics, which were used as the basis for structured discussions of how to apply current technologies to specific research projects.

PARTICIPANTS

Amaral, E., B.S., Ph.D., Universdade Estadual Paulista, Sao Jose Rio Preto, Brazil
Arieff, Z., B.S., M.S., University of Western Cape, Bellville, South Africa
Beebe, A., B.A., Ph.D., DNAX Research Institute, Palo Alto, California
Gallego Sanchez, G., B.S., International Center for Tropical Agriculture, Palmira, Colombia
Ikea, J., B.S., M.S., Ph.D., International Institute for Tropical Agriculture, Ibadan, Nigeria
Krusell, L., MS., University of Aarhus, Denmark
Nunes, I., B.S. O.D., Ph.D., Columbia University, New York
Phaneuf, D., B.S., Ph.D., Laval University, Ste-Foy, Quebec, Canada
Pogue-Geille, B.A., Ph.D., University of Pittsburgh, Pennsylvania
Raman, C.S., B.S., Ph.D., University of California, Irvine
Rattink, A., M.S., Wageningen Agricultural University, The Netherlands
Rithidech, K., B.S., Ph.D., State University of New York, Stony Brook
Roden, L., B.S., Ph.D., University of Warwick, Coventry, United Kingdom
Ron, M., B.S., Ph.D., Volcani Research Center, Bet-Dagan, Israel
Sobrido, M., B.S., M.D., Ph.D., University of California, Los Angeles
Velickovic, M., B.S., Wellington School of Medicine, Wellington, New Zealand

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Birren, B., Whitehead Institute/Massachusetts Institute of Technology, Cambridge, Massachusetts. From genome to clones and BAC again.
Eddy, R., Lawrence Berkeley and Women's Hospital, Boston, Massachusetts. Sifting sequence for function: Exploiting the mouse.
deJong, P., Children's Hospital of Oakland Research Institute, Oakland, California. Construction of YAC/BAC shuttle libraries by recombination.
Green, E., National Institutes of Health, Bethesda, Maryland. Mapping and sequencing mammalian chromosomes: How and why.
Hengartner, M., Cold Spring Harbor Laboratory. Positional cloning in *C. elegans*.
Kwiatkowski, D., Brigham and Women's Hospital, Boston, Massachusetts. Methods for mutation/SNP detection: HD gels, SSCP, DGGE, DHPLC.
Meltzer, P., National Institutes of Health, Bethesda, Maryland. Gene expression profiling with cDNA microarrays.
Parimoo, S., Johnson & Johnson CPWW, Skillman, New Jersey. Identification of gene defect in asebia mouse.
Reeves, R., Johns Hopkins University School of Medicine, Baltimore, Maryland. The face of Down syndrome: Meeting complex genetic traits head-on.
Roe, B., University of Oklahoma, Norman. The Human Genome Project: So many bases, so little time—A view from the trenches.
Schuler, G., National Institutes of Health, Bethesda, Maryland. EST mapping.
Trask, B., University of Washington School of Medicine, Seattle. Fluorescence in situ hybridization (FISH) and genome analysis.

Making and Using DNA Microarrays

October 20–November 2

INSTRUCTORS **Brown, P.,** Ph.D., Stanford University, California
Eisen, M., Ph.D., Stanford University, California
DeRisi, J., Ph.D., University of California, San Francisco

ASSISTANTS **Alizadeh, A.,** Stanford University, California
Diehn, M., Stanford University, California
Spellman, P., Stanford University, California

A DNA microarray is a simple, inexpensive, and versatile tool for experimental explorations of genome structure, gene expression programs, gene function, and cell and organismal biology. In this hands-on course, students were guided through the processes of building a robot for printing DNA microarrays, preparing DNA samples and slides to be used for printing microarrays, printing DNA microarrays, designing and conducting experiments for analysis by DNA microarray hybridization, and analyzing, displaying, and interpreting data. Experimental applications covered in the course included systematic studies of global gene expression programs, inferring gene function by using microarrays, genotyping, and measuring changes in gene copy number. Students who completed this course were able to set up their own independent facility for printing and experimental use of DNA microarrays. Guest instructors presented state-of-the-art technology, experimental applications, and interpretation of large genomic data sets.

PARTICIPANTS

Bahler, J., B.S., Ph.D., Imperial Cancer Research Fund, London, United Kingdom
Chinnaiyan, A., B.S., M.D., Ph.D., University of Michigan, Ann Arbor
Collingwood, D., B.S., Ph.D., University of Washington, Seattle
Futcher, B., B.S., Ph.D., Cold Spring Harbor Laboratory
Hager, J., B.S., Ph.D., Yale University, New Haven, Connecticut
Hinton, J., B.S., Ph.D., University of Oxford, United Kingdom
Kaltschmidt, C., B.S., Ph.D., University of Frieburg, Germany
Kocarek, T., B.S., Ph.D., Wayne State University, Detroit, Michigan
Lagerstrom-Fermer, M., B.S., Ph.D., University of Uppsala, Sweden
Lorenz, M., B.A., B.S., M.S., Ph.D., National Cancer Institute, NIH, Gaithersburg, Maryland
Love, D., B.S., Ph.D., University of Auckland, New Zealand
Marron, M., B.S., B.A., Ph.D., University of Florida, Gainsville
Mittal, V., B.S., Ph.D., Cold Spring Harbor Laboratory
Notterman, D., A.B., M.D., Princeton University, Princeton, New Jersey
Ryan, M., B.S., Ph.D., New York State Department of Health, Albany
Veeraraghavan, S., B.S., Ph.D., Scripps Research Institute, La Jolla, California

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Pollack, J., Stanford Medical Center, Stanford, California. CGH using cDNA microarrays.
Botstein, D., Stanford University School of Medicine, California. Designing experiments using micro-arrays.
Blalock, B., Affymetrix Technology, Santa Clara, California. Gene expression and SNP analysis utilizing Affymetrix GeneChip technology.
Miller, L., National Cancer Institute, NIH, Gaithersburg, Maryland. Experiences with using microarrays and micro-arrayers.
Kallioniemi, O.-P., National Human Genome Research Institute/NIH, Bethesda, Maryland. cDNA and tissue microarrays.
Young, R., Massachusetts Institute of Technology, Cambridge. The new genetics of gene expression.

Caenorhabditis elegans

November 2–15

INSTRUCTORS **Hengartner, M.,** Ph.D., Cold Spring Harbor Laboratory
Jorgensen, E., Ph.D., University of Utah, Salt Lake City
Plasterk, R., Ph.D., Netherlands Cancer Institute, Amsterdam

ASSISTANTS **Harris, T.,** University of Utah, Salt Lake City
Korswagen, H.C., University Hospital Utrecht, The Netherlands
Tharin, S., B.S., M.S., Cold Spring Harbor Laboratory

This course was designed to familiarize investigators with *C. elegans* as an experimental system, with an emphasis on both classical genetic analysis and reverse genetic approaches. A major goal was to teach students how to successfully exploit the information generated by the *C. elegans* genome project. The course was suited for those who had current training in molecular biology and some knowledge of genetics but had no experience with *C. elegans*, as well as students with some prior worm experience who wished to expand their repertoire of expertise. The following topics were covered both in the laboratory and by lectures from experts in the field: worm pushing, *C. elegans* databases and worm bioinformatics, anatomy and development, forward genetics, chemical and transposon mutagenesis, generation of transgenic animals, expression pattern analysis, reverse genetics, construction

and screening of deletion libraries, and RNA inactivation. The course was designed so that students would know enough about the most important attributes of the *C. elegans* system to embark on their own research projects after returning to their home institutions.

PARTICIPANTS

Bobinnec, Y., B.S., Ph.D., Kyoto University, Japan
Dreier, L., B.S., Ph.D., Harvard University, Boston, Massachusetts
Fuellekrug, J., B.S., Ph.D., European Molecular Biology Laboratory, Heidelberg, Germany
Gomez, M., B.S., Ph.D., Hoffman-LaRoche, Basel, Switzerland
Grill, S., B.S., European Molecular Biology Laboratory, Heidelberg, Germany
Haycraft, C., B.S., M.S., University of Alabama, Birmingham
Manoil, C., A.B., Ph.D., University of Washington, Seattle
Morely, J., B.S., Northwestern University, Evanston, Illinois
Payvar, F., M.S., Vanderbilt University, Nashville, Tennessee
Petersen, C., B.S., Ph.D., Vanderbilt University, Nashville, Tennessee
Rocha, J., B.S., Babraham Institute, Cambridge, United Kingdom
Samuel, A., B.A., Ph.D., Harvard University, Cambridge, Massachusetts
Sonnichsen, B., B.S., Ph.D., European Molecular Biology Laboratory, Heidelberg, Germany
Steele, R., B.S., Ph.D., University of California, Irvine
Sun, Y., M.D., Ph.D., Parke-Davis Pharmaceutical Research, Ann Arbor, Michigan
Wong, G., B.S., Ph.D., University of Kuopio, Finland

SEMINARS

Anderson, P., University of Wisconsin, Madison. mRNA surveillance worm biochemistry.
Bargmann, C., University of California, San Francisco. Nervous system function—Use of GFP for reporters.
Hengartner, M., Cold Spring Harbor Laboratory. Programmed cell death suppression genetics.
Johnson, C., Axys Pharmaceuticals, South San Francisco, California. *C. elegans* as a model system for the human.
Jorgensen, E., University of Utah, Salt Lake City. Synaptic transmission—Nomenclature, forward genetics.
Kimble, J., University of Wisconsin, Madison. Germ line development.
Mello, C., University of Massachusetts Medical Center, Worcester. Early embryonic development RNA inhibition.
Montgomery, M., Macalester College, St. Paul, Minnesota. Gene expression—Reporter gene constructs, ectopic expression.
Plasterk, R., Netherlands Cancer Center, Amsterdam. Reverse genetics, genome analysis.
Stein, L., Cold Spring Harbor Laboratory. The *C. elegans* genome.
White, J., University of Wisconsin, Madison. Worm cell biology microscopy.

Phage Display of Combinatorial Antibody Libraries

November 2–15

INSTRUCTORS **Barbas, C.,** Ph.D., Scripps Research Institute, La Jolla, California
Siegel, D., M.D., Ph.D., University of Pennsylvania, Philadelphia
Silverman, G., M.D., University of California, La Jolla

ASSISTANTS **Fuller, R.,** Scripps Research Institute, La Jolla, California
Goodyear, C., Glasgow Caledonian University, United Kingdom

Recent advances in the generation and selection of antibodies from combinatorial libraries allow for the rapid production of antibodies from immune and nonimmune sources. This intensive laboratory-lecture course focused on the construction of combinatorial antibody libraries expressed on the surface of phage and on the selection of desired antibodies from the library. Students learned theoretical and practical aspects of constructing combinatorial libraries from immune and nonimmune sources as well as the construction of synthetic antibody libraries. Antibodies were selected from the library by panning. Production, purification, and characterization of Fab fragments expressed in *E. coli* were also covered. The lecture series was presented by a number of invited speakers and emphasized PCR of immunoglobulin genes, the biology of filamentous phage and the utility of surface expression libraries, expression of antibodies in *E. coli* and mammalian cells, antibody structure and function, catalytic anti-

bodies, directed protein evolution, the immunobiology of antibody activity, and recent results on the use of antibodies in therapy. The theory behind and practical implications of selecting from phage displayed libraries of random peptides, cDNA products, and semisynthetic proteins were also explored.

PARTICIPANTS

Anderson, M., M.A., Ph.D., University of Alabama, Birmingham
Bull, P., B.S., Ph.D., University of Oxford, United Kingdom
Denolf, P., B.S., Ph.D., Plant Genetic Systems (AgrEvo), Gent, Belgium
Fernandez, P.-A., B.S., M.D., Ph.D., University College London, United Kingdom
Concalves, J., B.S., Ph.D., University of Lisbon, Portugal
Gould, M., B.A., Ph.D., Universidad Autonoma de Baja California, Ensenada, Mexico
Graslund, S., M.S., Royal Institute of Technology, Stockholm, Sweden
Gross, G., B.A., M.S., Ph.D., MIGAL-Galilee Technology Center, Rosh-Pina, Israel
Olee, T., B.S., Ph.D., Scripps Research Institute, La Jolla, California
Schober, M., B.S., Wayne State University, Detroit, Michigan
Stephano, J., B.S., M.S., Ph.D., Universidad Autonoma de Baja California, Ensenada, Mexico
Thisted, T., B.S., Ph.D., University of Pennsylvania, Philadelphia
Von Buedingen, H.C., M.D., University of California, San Francisco
Weiss, K., B.A., Elan Pharmaceuticals, South San Francisco, California
Werner, D., M.S., University of Potsdam, Luckenwalde, Germany
Yu, J., B.S., Ph.D., Korean Institute of Science and Technology, Seoul

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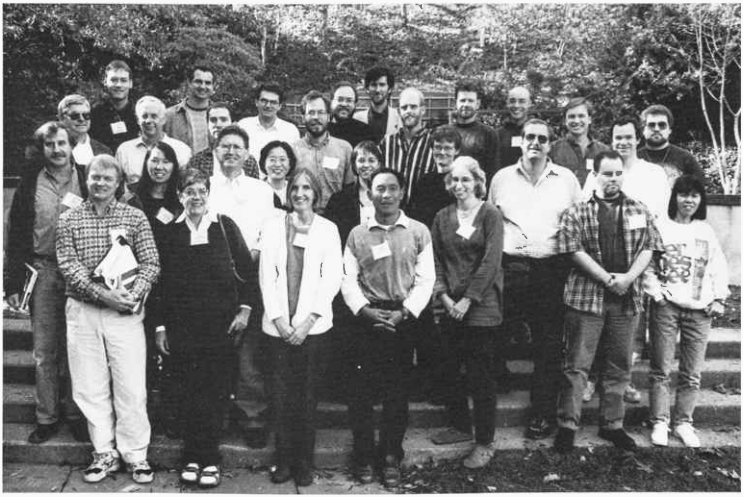
Barbas, C., The Scripps Research Institute, La Jolla, California. Phage display of antibodies and zinc finger proteins.
Lowman, H., Genentech, Inc., South San Francisco, California. SAR of peptides using phage.
Pasqualini, R., La Jolla Cancer Research Institute, California. In vivo panning.
Sanz, I., University of Rochester Medical Center, New York. Generation and features of antibody diversity.
Scott, J., Simon Fraser University, Burnaby, British Columbia, Canada. Phage display of peptides.
Siegel, D.L., University of Pennsylvania Medical Center, Philadelphia. Cell surface selection of combinatorial Fab libraries.
Silverman, G.J., University of California, San Diego, La Jolla. Repertoire cloning of SLE autoantibodies.
Webster, R., Duke University Durham, North Carolina. The biology of filamentous phage.
Wilson, I., The Scripps Research Institute, La Jolla, California. Structural biology of the immune system.

Computational Genomics

November 4–9

INSTRUCTORS **Pearson, W.,** Ph.D., University of Virginia, Charlottesville
Smith, R., Ph.D., SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania

Beyond BLAST and FASTA—This course presented a comprehensive overview of the theory and practice of computational methods for gene identification and characterization from DNA sequence data. The course focused on approaches to 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. This year, the course made extensive use of local World Wide Web pages to present problem sets and computing tools to solve those sets. Students used Windows and Mac workstations attached to a UNIX server; participants were comfortable using the UNIX operating system, the GCG sequence-analysis package, and a UNIX text editor. The course was designed for biologists seeking advanced training in biological sequence analysis, computer core directors and staff for molecular biology or genetics resources, and scientists in other disciplines, such as computer science, who wished to survey current research problems in biological sequence analysis.



PARTICIPANTS

Andrews, B., B.S., Astra Research Center Boston, Cambridge, Massachusetts
Crawford, M., B.A., Ph.D., Proteome, Inc., Beverly, Massachusetts
Cutler, G., B.A., Ph.D., Tularik, Inc., South San Francisco, California
Dauner, M., B.S., Swiss Federal Institute of Technology, Zurich, Switzerland
Edwards, S., B.S., Ph.D., National Library of Medicine, NIH, Bethesda, Maryland
Elsik, C., B.S., M.S., Texas A&M University, College Station
Fassler, J., B.S., Ph.D., University of Iowa, Iowa City
Fischer Lindahl, K., M.S., Ph.D., University of Texas Southwestern Medical Center, Dallas
Gosink, M., Parke Davis Pharmaceuticals, Ann Arbor, Michigan
Grigor, M., B.S., Ph.D., AgResearch, Hamilton, New Zealand
Hong-geller, E., B.A., Ph.D., Cornell University, Ithaca, New York
Howard, K., B.A., Ph.D., MRC Laboratory, London, United Kingdom
Jang, W., B.S., Ph.D., National Institutes of Health, Rockville, Maryland
Kelly, M., B.S., M.S., SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania
Lyster, P., B.S, Ph.D., University of Maryland, College Park
McCutcheon, J., B.S., University of Utah, Salt Lake City
Mount, S., B.A., Ph.D., University of Maryland, College Park
Peng, H.-M., B.S., Ph.D., Texas A&M University, College Station
Prades, C., DEA, Ph.D., Rhone Poulenc Rorer, Evry, France
Roof, D., B.A., Ph.D., University of Pennsylvania, Philadelphia
Rubin, G., B.S., Ph.D, University of California, Berkeley
Selinger, D., B.S., Ph.D., University of Arizona, Tucson
Semple, C., B.S., Ph.D., MRC Laboratory, London, United Kingdom
Vanavichit, A., B.S, Ph.D., Kasetsart University, Nakom Pathom, Thailand

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Atschul, S., National Library of Medicine, Bethesda, Maryland. Statistics of patterns and profiles.
Eddy, S., Washington University School of Medicine, St. Louis, Missouri. Blocks, motifs, domains, and other protein databases. Multiple sequence comparison with hidden Markov models.
Lawrence, C., Wadsworth Center, Albany, New York. Bayesian approaches to sequence alignment/motif identification.
Overton, C.G., University of Pennsylvania, Philadelphia. Genome databases.
Pearson, W., University of Virginia, Charlotteville. Protein evolution—Biology. Algorithms for pairwise sequence comparison.
Smith, R., SmithKline Beecham, King of Prussia, Pennsylvania. Introduction to multiple sequence comparison.
Stormo, G., Washington University Medical School, St. Louis, Missouri. Identifying sites in unaligned sequences. Eukaryotic gene finding.
Zhang, M., Cold Spring Harbor Laboratory. Competing on yeast promoters.

The Laboratory would like to acknowledge the generosity of the following companies who loaned equipment and reagents to the various courses:

5 Prime-3 Prime
ALA Scientific Instruments, Inc.
Ambion
Amersham Life Science Incorporated
Amersham Pharmacia Biotech, Inc.
A.M.P.I.
Applied Precision
Applied Scientific Instrumentation, Inc.
Applied Spectral Imaging
Axon Instruments
Becton Dickinson Labware
Bio-Rad Laboratories
Boehringer Mannheim Corp.
Brinkmann Instruments
Burleigh Instruments, Inc.
Calbiochem-Novabiochem
Carl Zeiss
CBS Scientific
Chroma Technology Corporation
Clontech Laboratories, Inc.
Codonics Inc.
Coherent Laser
Corning Costar Corporation
Corning Inc.
Cygnus Technology, Inc.
Dagan Corporation
Delaware Diamond Knoves
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Havard Apparatus Inc.
Hewlett Packard
Hitatchi Denski America LTD.
ICN Biomedicals
Improvision-Image Processing and Vision Co. LTD.
Instrutech
Invitrogen Corporation
Jackson Immunoresearch Labs
Jouan, Inc.
Kopf Instruments
Leica, Inc.
McMaster Carr
Micro Video Instruments
Millipore
MJ Research, Inc.
Modulation Optics, Inc.
Molecular Devices Corporation
Molecular Dynamics
Molecular Probes
Nalge Nunc International
Narishige International USA, Inc.
NEN Life Science Products
Newark Electronics
New England Biolabs
Newport Corporation
Nikon
Novagen
Novex
Olympus America, Inc.
Operon Technologies Inc.
Optronics Engineering
Pel-Freez Biologicals
Perkin-Elmer Applied Biosystems
PerSeptive Biosystems
Pfanstiehl Laboratories, Inc.
Pharmacia Biotech
Polysciences
Promega Corporation
Qiagen Inc.
Robbins Scientific Corporation
SD Instruments
Savant Instruments
Scanalytics Inc.
Schleicher & Schuell
Sigma-Aldrich
Sony Medical Systems
Specialty Media
Spectra-Physics Lasers, Inc.
Stratagene
Sutter Instruments
Tamargo Associates
Techne, Inc.
Technical Manufacturing Corp.
Technical Products International
USB Corporation
Universal Imaging Corporation
Upstate Biotechnology Inc.
Vector Laboratories
VWR Scientific Products
Vysis Inc.
Wallac, Inc.
Warner Instrument, Corp.
Western Technology Marketing, Inc.

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. Joseph Fetcho, Department of Neurobiology and Behavior, SUNY, Stony Brook. Imaging, ablations, and behavior: Optical studies of neuronal circuits in zebrafish. (Host: Hollis Cline)

Dr. Charles Sherr, HHMNI/St. Jude Children's Research Hospital. The ARF-p53 pathway. (Host: Scott Lowe)

Dr. Dafna Bar-Sagi, Department of Molecular Genetics and Microbiology, SUNY, Stony Brook. Ras signaling and growth control. (Host: Winship Herr)

Dr. Stuart Kim, Stanford University. MAP kinase signaling in *C. elegans*. (Host: Terri Grodzicker)

February

Dr. Pat Brown, HHMI/Stanford University, Department of Biochemistry. Watching a genome in action. (Host: Michael Zhang)

Dr. James Manley, Columbia University. A genetic approach to gene expression in vertebrate cells. (Host: Terri Grodzicker)

Dr. Eric Davison, California Institute of Technology. Gene regulatory circuitry in the embryogenesis of sea urchin. (Host: Andy Neuwald)

Dr. Charles Craik, University of California, San Francisco. Reverse biochemistry: Using macromolecular protease inhibitors to dissect complex biological processes. (Host: Leemor Joshua-Tor)

March

Dr. Julian Downward, Imperial Cancer Research Fund. Mechanisms by which Ras oncogenes control cell proliferation and survival. (Host: Linda Van Aelst)

Dr. Sue McConnell, Stanford University. The determination of axonal connectivity in the developing brain. (Host: Hollis Cline)

Dr. Ron Desrosiers, Harvard Medical School. Mutations that allow immunologic control of simian immunodeficiency virus. (Host: Jacek Skowronski)

Dr. Pamela Bjorkman, HHMI/California Institute of Technology. MHC homologs in immune recognition. (Host: Leemor Joshua-Tor)

Dr. George Tokiwa, Orchid Biocomputer, Inc. From CSHL to life in a start-up biotech company ... A personal experience. Career pathways for the biology Ph.D. seminar.

April

Dr. Thomas Stossel, American Cancer Society Professor of Medicine. Machinery of cell crawling. (Host: Linda Van Aelst)

Dr. David Tank, Bell Laboratories, Lucent Technologies. Cellular and circuit mechanisms of persistent neural activity. (Host: Karel Svoboda)

October

Dr. Jeffrey Rothstein, Johns Hopkins University. Glutamate transporters: Regulation and dysregulation-associated neurodegeneration. (Host: Adrian Krainer)

Dr. Susan McConnell, Stanford University, Department of Biological Science. The Determination of neuronal connectivity in the developing brain. (Host: Holly Cline)

Dr. Rolf Sternglanz, SUNY, Stony Brook. Studies on transcriptional silencing in yeast. (Host: Adrian Krainer)

November

Dr. Richard Flavell, Yale University School of Medicine. The role of caspases in cell death in vivo. (Host: Yuri Lazebnik)

Dr. Luc Montagnier, Queens College. Apoptosis and AIDS. (Host: Michael Hengartner)

Dr. Richard A. Young, Whitehead Institute for Biomedical Research. Genome-wide expression analysis: Mindless data collection? (Host: Michael Zhang)

December

Dr. Todd Sacktor, Associate Professor of Physiology, Pharmacology, and Neurology, SUNY Downstate. Protein kinase Mζ and the maintenance of late-LTP. (Host: Jerry Yin)

Dr. Mark Ginsberg, The Scripps Research Institute. Integrins: The inside story. (Host: Linda Van Aelst)

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

Bruce Futcher: The cell-cycle-regulated genes of yeast, in shades of green and red.
Ilya Ioshikhes (Zhang Lab); Periodical distribution of transcription factor sites in promoter regions and connection with chromatin structure.
Keiichi Shibahara (Stillman Lab): Mechanisms of replication-coupled chromatin assembly by CAF-1.
Hong-Xiang Liu (Krainer Lab): Finding exons in a sea of introns: The nature and function of exonic splicing enhancers.

February

Ueli Grossniklaus: Sex, parental conflicts, and infanticide (developmental genetics of plant reproduction).
Athena Lin (Lowe Lab): Premature senescence induced by oncogenic *ras*.
Lincoln Stein: Browsing the *C. elegans* genome.
Robert Lucito (Wigler Lab): Genetic analysis using genomic representations.

March

Rob Babb (Herr Lab): Mechanisms of combinatorial transcriptional regulation: It takes two (or three) to tango.
Grace Chen (Stenlund Lab): Unraveling the mechanics of origin recognition by the papillomavirus E1 DNA helicase.
Daan Van Aalten (Joshua-Tor Lab): Bent, flipped, and straight: A dancing dynamic DNA duplex duo.

April

Paul Mintz (Spector Lab): Everything you ever wanted to know about nuclear speckles.
Christine Berthier (Helfman Lab). Choreographing actin cytoskeleton dynamics in nonmuscle cells.
David Jackson: Two ways of talking: Channels and receptors for cell-to-cell communication in plants.

October

Josh Dubnau (Tully Lab): Functional genomics of memory formation.
Shiv Grewal: Genetics of epigenetics: Where Mendel's gene is more than a DNA moeity.
Herman Wijnen (Futcher Lab): Transcriptional control at the start of the cell cycle.

November

Catherine Kidner (Martienssen Lab): The role of ARGONAUTE in leaf development"

December

Joe Rodriguez (Lazebnik Lab): APAF1 and caspase-9 form an active holoenzyme.
Suzanne Tharin (Hengartner Lab): HUNCS, DrUNCS, and axon guidance in *C. elegans*.
Benjamin Boettner (Van Aelst Lab): AF-6, A component of cell-cell adhesion complexes: An investigation in man and flies.

UNDERGRADUATE RESEARCH

Co-Program Directors: Michael Hengartner, Ph.D./Leemor Joshua-Tor, Ph.D.

Program Administrator: Jane Reader

An important aspect of the summer program at the Laboratory is the participation of college undergraduate students in active research projects under the supervision of full-time laboratory staff members. The program was initiated in 1959. Since that year, 534 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 520 applicants, took part in the program:

Kelly Brown, Harding University
Sponsor: National Science Foundation
Advisor: **Dr. Bruce Stillman**
Human CDC6: An essential gene for DNA replication.

Kevin Christie, College of William and Mary
Sponsor: National Science Foundation
Advisor: **Dr. Andrew Neuwald**
A computational system for comprehensive sequence analysis for protein domains.

Heather Cosel-Pieper, New York University
Sponsor: Jephson Educational Trust
Advisor: **Dr. Michael Hengartner**
Toward an understanding of apoptosis in *C. elegans*.

Adriano Costa de Alcantara, Universidad Federal da Bahia
Sponsor: Frederica Von Stade
Advisor: **Dr. Michael Zhang**
First steps in building up a *C. elegans* promoter database.

Top Row: Fazila Pinar Erciyas; Keith Wu; Catherine Merrick; Marco Mangone; François St-Pierre; Heather Cosel-Pieper; Daniellla Dumitriu; Rebecca Ewald; Satoshi Kawashima; Andrew Cotton; Kevin Christie; Trevor Yeung; Ben Lehner; Maithreyi Krishnaswami; Michael Verzi; Kelly Brown; Benjamin de Bivort
Bottom Row: Adriano Costa de Alcantara; Fernando Ontiveros-Llamas; Justin Cross; Jamil Scott; Silja Kuusk; Megan Sullivan; Natashe Thorne; Sashay Franklyn; Bryce Portier

Andrew Cotton, Harvard University
Sponsor: James D. Watson Fund
Advisor: **Dr. Richard McCombie**
An ASN.1 to XML converter.

Justin Cross, University of Cambridge
Sponsor: The Olney Fund
Advisor: **Dr. Linda Van Aelst**
The role of Rap and AF-6/canoe in the control of cell morphology and adhesion.

Benjamin de Bivort, Duke University
Sponsor: Jephson Educational Trust
Advisor: **Dr. Yi Zhong**
Proteins in learning and memory: Morphology of the *Drosophila* neuromuscular junction.

Daniella Dumitriu, University of California, Santa Barbara
Sponsor: Burroughs Wellcome
Advisor: **Dr. Hollis Cline**
Behavioral assessment of visual acuity development in *Xenopus* tadpoles.

Fazila Pinar Erciyas, Bogazici University
Sponsor: Emanuel Ax Fund
Advisor: **Dr. Rui-Ming Xu**
Purification and crystallization of *S. cerevisiae* ORC1-BAH domain.

Rebecca Ewald, King's College London
Sponsor: Burroughs Wellcome
Advisor: **Dr. Scott Lowe**
Comparison of gene expression profiles of p53-mediated growth arrest and p53-mediated senescence.

Sashay Franklyn, Harvard University
Sponsor: National Science Foundation
Advisor: **Dr. Shiv Grewal**
Characterization of Clr6 histone deacetylase.

Satoshi Kawashima, University of Kobe School of Medicine
Sponsor: Shakespeare Fund
Advisor: **Dr. Yuri Lazebnik**
Epitope mapping by protein fragmentation.

Maithreyi Krishnaswami, Hobart and William Smith Colleges
Sponsor: The Bliss Memorial Fund
Advisor: **Dr. David Jackson**
Regulation of shoot morphogenesis in plants: Studying an altered phyllotaxy in maize.

Silja Kuusk, Tartu University
Sponsor: Burroughs Wellcome
Advisor: **Dr. Adrian R. Krainer**
In vitro selection for exonic splicing silencers.

Ben Lehner, University of Cambridge
Sponsor: Burroughs Wellcome
Advisor: **Dr. Rob Martienssen**
Molecular characterization of the gene *Argonaute* in *A. thaliana* and *S. pombe*.

Marco Mangone, University of Rome
Sponsor: The Read Fund
Advisor: **Dr. Lincoln Stein**
In silico mapping of human single nucleotide polymorphisms.

Catherine Merrick, Cambridge University
Sponsor: Burroughs Wellcome
Advisor: **Dr. Marja Timmermans**
Analysis of the *leafbladeless1* mutant of maize.

Fernando Ontiveros-Llamas, National Autonomous University of Mexico.
Sponsors: Glass Fund/Libby Fund
Advisor: **Dr. David L. Spector**
Ultrastructural visualization of a genetic locus and the pathway followed by its RNA.

Bryce P. Portier, Texas A&M University
Sponsor: National Science Foundation
Advisor: **Dr. Bruce Futcher**
Exploring the active site of a cyclin-dependent kinase.

Jamil B. Scott, Tennessee State University
Sponsor: National Science Foundation
Advisor: **Dr. William Tansey**
Characterization of the transcriptional repressor region in Myc.

François St-Pierre, University of Cambridge (Trinity College)
Sponsor: Burroughs Wellcome
Advisor: **Dr. Leemor Joshua-Tor**
Investigating the active site of human bleomycin hydrolase.

Megan R. Sullivan, Indiana University
Sponsor: National Science Foundation
Advisor: **Dr. Tim Tully**
Testing two approaches of concurrent spatial and temporal control of gene expression in *Drosophila*.

Natasha Thorne, University of Massachusetts
Sponsor: National Science Foundation
Advisor: **Dr. Gregory Hannon**
Construction of a cDNA library of secreted and cell surface proteins: A strategy to identify diagnostic markers for breast cancer.

Michael Verzi, The College of New Jersey
Sponsor: National Science Foundation
Advisor: **Dr. Grigori Enikolopov**
Alternative splicing of the *Drosophila* nitric oxide synthase gene.

Keith Wu, Cambridge University
Sponsor: Garfield Fund
Advisor: **Dr. Winship Herr**
Role of the VP16 core and transcriptional activating regions in HSV virion formation.

Trevor Ming-Yee Yeung, Clare College, University of Cambridge
Sponsor: James D. Watson Fund
Advisor: **Dr. David Helfman**
An investigation into one postulated mechanism regulating the distribution of tropomyosin in human SV80 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. Older students can enroll in more advanced programs, such as Marine Biology and Nature Photography.

During the summer of 1999, a total of 350 students participated in 26 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

The Banbury Center program continues to be as eclectic and exciting as ever. The year was filled with more meetings than ever before—a record 23 of them! Laboratory scientists used the Center for seven in-house meetings, and local community groups came here on eight occasions. Together with the five neurobiology courses, there was hardly a week when the Center was not in use.

Not surprisingly, 1999 was also a record year for the number of visitors to Banbury Center: 667 participants attended the 23 meetings. The demographics of our participants remain much the same: 25% of visitors to Banbury Center came from abroad, with the United Kingdom, Germany, and Canada leading the way. Of the American scientists, those from New York, Massachusetts, and California together accounted for more than 32% of the total. However, participants were drawn from no fewer than 42 states.

This is the first year that we have been able to use the Meier House to accommodate participants, which proved to be wonderful. Now the number of participants that we can house on the Banbury estate matches the number we can have in the Conference Room—we do not have to transport people between the Center and the main campus.

Biological and biomedical research is becoming ever more interdisciplinary, and as it does so, it also becomes ever more difficult to categorize the topics of Banbury Center meetings. A meeting may deal with the same phenomenon in a range of organisms, or many different strategies may be used to study one phenomenon in a single species. Nevertheless, the meetings followed the general themes for Banbury Center meetings—molecular biology, genetics, neurobiology, science policy, and education—and, as always, the topics were important and, on occasion, controversial.

Meier House provides accommodations for meeting participants at Banbury Center.

Human Genetic Disorders

Banbury Center has a long-standing interest in human genetics, and our meetings have helped promote research on many disorders. We have developed close relationships with a number of the foundations supporting such research, and scientists from three of these came to the Center in 1999.

The first meeting on neurofibromatosis was held at Banbury in 1990, and we have followed the significant advances in the field as researchers supported by the National Neurofibromatosis Foundation have returned for other meetings. This year, the Foundation turned to the painful and disfiguring nerve sheath tumors—plexiform neurofibromas—that grow along the length of nerves. They are an important target for trials of potential therapeutic agents, but first, a reliable scheme must be developed for assessing the size and growth rate of these tumors. A project is just beginning to do this, and the meeting *Natural History of Plexiform Neurofibromas in NF1*, organized by Bruce R. Korf (Children's Hospital, Boston) and funded by the U.S. Army Medical Research, was held to advance planning. It brought together all the principal investigators for each section of the project as well as representatives from each center that is recruiting patients for the study. The main goal of the meeting was to work out the final arrangements for recruitment of study subjects, to make any necessary modifications of the protocol, and to present some of the pilot data already being collected.

The Ehlers-Danlos syndrome (EDS) is a genetically heterogeneous group of disorders affecting connective tissue. Although the molecular basis of some forms of EDS is known, the pathogenesis of the disorders is largely unknown. This makes diagnosis and differentiating among the forms of EDS difficult, and it delays the search for treatments. Our meeting, *The Clinical and Biological Basis of the Ehlers-Danlos Syndrome*, organized by Peter Byers (University of Washington) and Petros Tsipouras (University of Connecticut) was funded by the Ehlers-Danlos Foundation. It addressed the diagnostic difficulties by reviewing what is known of the natural history of the various forms of EDS and discussing molecular mechanisms. In addition, participants presented data on the molecules that make up the extracellular matrix and that might be candidate molecules for the differing types of EDS.

The DNA-repair disorder ataxia telangectasia has reached a stage that is both a great advance and a great challenge. It is known now that the protein involved is a protein kinase called ATM, and the challenge is to discover precisely what this protein does and capitalize on this to develop treatments. *The Molecular Neurobiology of ATM* meeting, organized by Stephen Elledge (Baylor College of Medicine), Nathaniel Heintz (Rockefeller University), and Eugene Johnson (Washington University Medical School) and funded by the A-T Children's Project, was designed to tackle this challenge. Participants reviewed data on the structure of the ATM protein and the proteins with which it interacts, as well as its localization and possible function in neurons. One session was devoted to discussing gene therapy and stem cell therapy in ATM.

Strategies for Inactivation of Mutant Genes was also devoted to moving from knowledge of the fundamental molecular pathogenesis of a disorder to therapies. The meeting resulted from a collaboration between the two foundations that funded it: the Amyotrophic Lateral Sclerosis Association and the Hereditary Diseases Foundation. Organized by Robert Brown (Massachusetts General Hospital), Robert Nussbaum (National Human Genome Research Institute), Ethan Signer (Massachusetts Institute of Technology), and Nancy Wexler (Columbia University), the meeting was devoted to examining whether interventions might be possible at the nucleic acid level rather than at the protein level. This far-sighted approach involved participants using a variety of tools that modify gene expression, including antisense, ribozymes, RNA interference, DNA excision, and peptide nucleic acids.

It has been extraordinarily difficult to determine the factors, genetic and otherwise, that contribute to the pathogenesis of schizophrenia. The first Banbury Center meeting on the genetics of schizophrenia was held in 1988, and 11 years later, Arthur Pardee (Dana-Farber Cancer Institute) and Ann Goodman (The Nathan S. Kline Institute for Psychiatric Research) organized the meeting *Molecular Neurobiological Mechanisms in Schizophrenia—Seeking a Synthesis*. It was an ambitious program seeking to bring together studies ranging from diagnosis, through genetics and cell biology, to potential leads for therapies.

Genomics

In 1999, there were only two meetings concerned with the technical aspects of genomics, which is unusual for Banbury Center. Sequence data are being generated at an extraordinary rate and sequencing itself is no longer the bottleneck in genomics. Rather, making sense of all the sequences—finding genes and understanding what they do—has become the limiting step in using the data. Finley Austin (Merck Genome Research Institute) and Robert Strausberg (National Cancer Institute) organized the meeting *Functional Genomics: Technology Development and Research Applications* to examine this problem. Funded by the Merck Genome Research Institute and the National Cancer Institute, participants, drawn from technological as well as biological fields, assessed the current state-of-the-art strategies in functional genomics both in their own and other areas. We hoped that the meeting would promote interactions among investigators who use different approaches in different research fields.

The second genomics meeting, *Comparative Plant Genomics*, was organized by Ben Burr (Brookhaven National Laboratory), John Doebley (University of Minnesota), and Rob Martienssen (Cold Spring Harbor Laboratory). "Model" plant species are being sequenced in the hope and expectation that gene discovery and functional analysis in these species will speed the application of genomics to economically important plants. The first complete sequence of a plant—*Arabidopsis thaliana*—will be finished in late 2000, and it seemed the right time to think more carefully about the assumptions implicit in the "model" genome approach. This meeting reviewed critically the question of the usefulness of plant model genomes; e.g., the extent to which syntenic relationships will be useful in identifying genes and quantitative trait loci, and whether comparative analysis of the genes involved in common pathways will reveal functional relationships.

Using Biological Knowledge

The common theme to this set of otherwise disparate topics was the use of biological knowledge to help solve biomedical problems.

The conference *What Are Stem Cells? From Embryo to Adult Tissues* brought together scientists studying multipotent cells of the embryo and adult tissues, including hematopoietic, mesenchymal, neural, pancreatic, endothelial, hepatic, and germline systems. The organizers were Dan Marshak (Osiris Pharmaceuticals Inc.), Roland Scollay (Systemix Inc.), and Irv Weissman (Stanford University). The meeting was funded by Osiris Pharmaceuticals and Systemix. Participants were chosen so that a whole range of interesting questions relating to stem cell biology could be explored, including the biological definition of a stem cell, what constitutes clonality, how stem cells retain lineage commitment and proliferative capacity, and how differentiation is initiated.

Xenotransplantation: A Scientific Basis for Risk Assessment dealt with some fascinating biology and a topic of medical importance that is also highly controversial. There are great hopes that transplants of organs from animals might help overcome the acute shortage of human organs for transplantation. However, these hopes are tempered by fear that such transplants might lead to the transmission of viruses from animals to human beings. This workshop, organized by John Coffin (Tufts University), Mark Hanson (Hastings Center), Frederick Murphy (University of California, Davis), and Robin Weiss (Institute of Cancer Research), brought virologists from the academic and biotechnology worlds together with individuals concerned about public health issues of xenotransplantation. They reviewed what is known of the biological basis of the transmission of viruses from one species to another and of the consequences of such cross-species infections. These reviews formed the basis for a discussion of the public policy and ethical issues raised by uncertainties in understanding the risks in the application of xenotransplantation. The meeting was funded by the William Stamps Farish Fund and by the Hastings Center, which is dedicated to considering the societal and ethical issues relating to modern biomedical research. The involvement of the Hastings Center added a particularly valuable element to the meeting.

Viruses featured largely in the meeting on *Microbial Targets for Small Molecules*, organized by Peter Howley (Harvard Medical School), Arnold Levine (Rockefeller University), and Gregory Verdine (Harvard University) and funded by the Laboratory's Corporate Sponsor Program. Participants came from

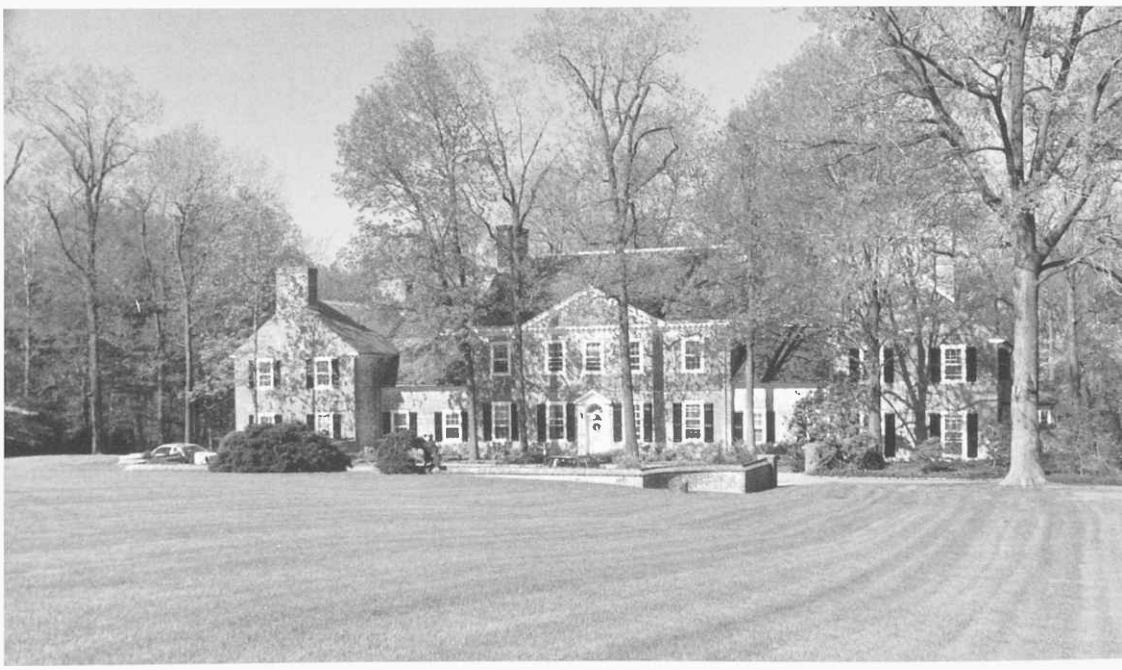
diverse backgrounds and included chemists, structural biologists, cell biologists, and microbiologists. The meeting provided an opportunity for the participants to meet for an informal exchange of ideas and information relevant to potential novel drug targets for bacteria, viruses, or fungi, with a specific emphasis on the development of new strategies for identifying novel small-molecule inhibitors.

Infectious agents of quite a different kind were the focus of the meeting *Physical and Structural Chemistry of Prion Protein and Prion-like Phenomena*. Organized by Byron Caughey (NIAID, NIH) and Kurt Wuthrich (Institut für Molekularbiologie und Biophysik, Zurich), participants discussed the self-propagating protein-protein interactions that result in the accumulation of abnormal conformers and/or polymers of a host protein. These produce pathogenic conditions such as scrapie in sheep, bovine spongiform encephalopathy (BSE) in cows, and Creutzfeld-Jacob disease in human beings. However, there is considerable controversy over the mechanisms by which a protein conformational change can be imposed by an abnormal form of a protein on a normal form. The aim of the meeting was to consider the latest insights into the structural basis of prion-like phenomena and other disease states that involve the insidious spread of aberrant protein.

When thinking about infectious diseases, our tendency is to concentrate on the infectious agent, but it is clear that the host plays an important part in determining whether an infection is established. *Host Pathogens Interactions*, organized by Barry R. Bloom (Harvard University), Sally Blower (University of California, San Francisco), and Nelson B. Freimer (University of California, San Francisco), was funded by the Corporate Sponsor Program. It tried to redress the concentration on infectious agents by reviewing what is known about the role of host genetic variation in relation to susceptibility to infection. The goal of the meeting was to examine interactions between host and pathogen that are influenced by genetics from the perspective of evolutionary biology—in particular, from population genetics and infectious-disease-modeling approaches.

Biological Studies

This group of meetings dealt with what might be called basic research, although even here the research is not far from application.



Robertson House also provides housing accommodations at Banbury Center.

Much research on the regulation of gene expression has concentrated on the sets of proteins—transcription factors—that bind to transcription start sites. Less attention has been paid to the role played by structural changes in the DNA molecule. *The Role of DNA Topology, Conformation and Associated Factors in Gene Expression*, organized by David Levens (National Cancer Institute) and Lucia Rothman-Denes (University of Chicago) and funded by the Corporate Sponsor Program, explored the extent to which topological changes in a DNA molecule act directly on the transcription machinery. Participants reported research on the physics of strained DNA, the topology of DNA molecules—single-stranded regions, hairpins, bends, super coils—and how these changes are brought about by topoisomerases and the transcription apparatus.

Pigmentation is a fascinating biological phenomenon and one that has been the subject of recent advances. The meeting on the *Biology of Pigmentation* organized by Greg Barsh (Stanford University School of Medicine) and Dorothy C. Bennett (St. George's Hospital Medical School, United Kingdom) reviewed a number of longstanding questions in pigmentary cell biology, development, genetics, and evolution in relation to these advances. Participants discussed research on model organisms and systems that may lead to a deeper understanding of human eye, hair, and skin color and of the genetic and evolutionary forces that have contributed to the tremendous variation in human pigmentary phenotypes.

Studies of cell death in plants have been somewhat overshadowed by research on apoptosis in animal cells, especially in relation to cancer. But cell death occurs during many processes of normal plant growth and development, and its regulation is especially important for organisms where dead cells cannot be removed—their cell walls remain. Cell death occurs in response, for example, to pathogens as well as through the developmental regulation of genes whose activities lead to death. Jeffrey Dangl and Alan Jones (University of North Carolina) and Christopher J. Lamb (The University of Edinburgh) invited cell biologists, genetics, and plant pathologists to Banbury for a meeting on *Cell Death in Plants: Functions and Mechanisms*. Participants discussed recent progress toward understanding why cell death occurs in plants and whether common mechanisms lead to cell death.

Neuroscience

Neuroscience plays an increasingly large part in the research of the Laboratory, and this interest is reflected in the increasing number of Banbury Center meetings in this area. *The Functional Organization of the Thalamus and the Cortex and Their Interactions* organized by Paul R. Adams (State University of New York, Stony Brook), Christof Koch (California Institute of Technology), Karel Svoboda (Cold Spring Harbor Laboratory), and S. Murray Sherman (State University of New York, Stony Brook) was one such meeting. Many new advances have occurred in this area, and this meeting, funded by The Swartz Foundation, provided a forum for bringing together experts to discuss issues of emerging importance. Participants included an interesting mixture of experimentalists and theoreticians whose research spanned different levels of description (synaptic, cellular, network, and system).

One of the seemingly inescapable consequences of growing older is the loss of memory, a natural process but one that may be deeply worrisome, especially if it is thought to be the onset of Alzheimer's disease. *Old Memories*, organized by John D. Gabrieli (Stanford University), Michela Gallagher (Johns Hopkins University), and Tim Tully (Cold Spring Harbor Laboratory), was funded by the John Hartford Foundation to review the effects of aging on working memory, long-term memory storage, and recall. In particular, participants examined the question of what constitutes normal versus abnormal age-related memory loss, and how the former is distinguished from the onset of Alzheimer's disease and other disorders that affect memory. Participants also reviewed whether there is any evidence that age-related memory loss is heritable and what therapies exist or might be developed to restore memory in old age.

Science and Public Health Policy

Banbury Center has a long history of meetings that examine issues of public health policy in relation to biomedical research. Examples include the series of early meetings on risk assessment and environmental carcinogenesis. But none has the potential impact of the meeting on *Vaccines for Developing*

Economies, Who Will Pay?, organized by Philip Russell (Albert B. Sabin Vaccine Institute) and Stanley Lemon (University of Texas Medical Branch, Galveston), with Jeffrey Sachs (Center for International Development, Harvard University) and Peter Hotez (Yale University) as co-chairs. The meeting was funded by the Albert B. Sabin Vaccine Institute, Inc., which was established to promote the development and use of vaccines. Participants included scientists, economists, vaccine producers, representatives of international bodies such as the World Health Organization and the World Bank, and individuals from foundations. They came to Banbury to deliberate on how to provide vaccines to those most in need of them.

The Executives' Meeting

We are extremely grateful to Sandy Warner and David Deming of J.P. Morgan, who each year make possible a wonderful meeting. This year, we returned to genomics, the topic of the 1986 meeting, the first in this series. Then, the usefulness of embarking on a human genome project was still being hotly debated, in marked contrast to the 1999 meeting, *Genes and Genomes: Sequences to Proteins*. Now Leroy Hood, who spoke at the first meeting in 1986, was able to review the extraordinary advances that have led to the sequencing of the complete genomes of bacteria, yeast, and the nematode worm *Caenorhabditis elegans*. Richard Gibbs described what it is like to do large-scale sequencing, and David Botstein (who also spoke at the 1986 meeting), Gerald Rubin, John Todd, and Michael Bevan described how genome sequences are being used. The meeting closed with a fascinating and illuminating presentation by Maria Freiere on the patenting of DNA sequences.

Basic Issues of Science

For the third year, the Federal Judicial Center and Cold Spring Harbor Laboratory combined to provide federal and state judges with some insights into the way scientific research is carried out and into the ways that scientists think. The presentations at the meeting ranged from the history of eugenics and experimental biology, through human genetics and its societal implications, to environmental hazards and risk assessment. It was a pleasure to have Rich Roberts come back to Cold Spring Harbor Laboratory to recount his experiences as a scientific expert witness taking part in criminal and patent cases.

Eugenics on the Web

This project, funded by the National Human Genome Research Institute, is progressing very well. On two occasions during 1999, our Editorial Advisory Board came to Banbury Center to review what we had done, offer suggestions, and work at improving the site by writing essays and captions. We are well on target for completing the project on time, and the final meeting of this funding period will take place in January of 2000.

Acknowledgments

It would be impossible for Banbury Center to function at this level of activity without the help of many people: Bea Toliver and Ellie Sidorenko ensure that meetings run smoothly, Katya Davey looks after Robertson House, and Chris McEvoy and Andy Sauer maintain the Banbury grounds. All work very hard to keep the Center running. Outside the Center, special mention must be made of other units of the Laboratory that contributed significantly to the success of our program: The Meetings Office helped with late requests for extra accommodations, the AV team handled the increasingly complex computerized slide and overhead projectors, Blackford responded to late changes in catering requests, and Housekeeping coped with rapid changes between meetings.

Jan Witkowski

MEETINGS

Natural History of Plexiform Neurofibromas in NF-1

February 6–9

FUNDED BY **U.S. Army Medical Research, with additional support from the National Neurofibromatosis Foundation**

ARRANGED BY **B.R. Korf,** Children's Hospital, Boston, Massachusetts

SESSION 1: Overview

B.R. Korf, Children's Hospital, Boston, Massachusetts: Overview of project.
W. Slattery, House Ear Institute, Los Angeles, California: Overview of NF2 project.
S. Huson, The Churchill Hospital, Oxford, United Kingdom: Patterns of plexiform neurofibromas in different anatomical locations.
B.R. Korf, Children's Hospital, Boston, Massachusetts: Subject acquisition protocols.
B.R. Korf, Children's Hospital, Boston, Massachusetts: Reimbursement and administration.

SESSION 2: MRI Protocol

D. Jaramillo, Children's Hospital, Boston, Massachusetts: MRI of peripheral plexiform neurofibromas (including protocol).
T. Young Poussaint, Children's Hospital, Boston, Massachusetts: MRI of cranial and spinal plexiform neurofibromas (including protocol).
J. Tsuruda, University of Utah, Salt Lake City: MR neurography and advanced image processing.
J.B. Zimmerman, WorldCare, Inc., Cambridge, Massachusetts: WorldCare MRI protocol.
J. DiCanzio, Children's Hospital, Boston, Massachusetts: Statistical analysis of radiological data.

SESSION 3: Clinical and Pathological Data

J.M. Friedman, University of British Columbia, Vancouver, Canada: Clinical database.
B.R. Korf, Children's Hospital, Boston, Massachusetts: Patient questionnaire.
D. Wolfe, Mt. Sinai Medical Center, New York: Histopathologic correlates of growth in plexiform neurofibroma.
D. Wolfe, Mt. Sinai Medical Center, New York, and B.R. Korf, Children's Hospital, Boston, Massachusetts: Pathology review facility.

SESSION 4: Tissue Bank and Cell Biology: Studies of Cell Biology

L. Rutkowski, Abramson Center, Philadelphia, Pennsylvania: Understanding biological defects in neurofibroma-derived Schwann cells. Part 1: Resolving obstacles.
N. Ratner, University of Cincinnati College of Medicine, Ohio: Neurofibroma-derived Schwann cells are invasive and show high Ras-GTP.
D. Viskochil, University of Utah, Salt Lake City: Somatic DNA alterations in peripheral nerve sheath tumors.
D.H. Gutmann, Washington University School of Medicine, St. Louis, Missouri: Administration of tissue bank and mechanisms.

SESSION 5: Logistical Issues

B.R. Korf: Children's Hospital, Boston, Massachusetts: Time Line; Publication of Policy; Finance; Consent.

J. Tsuruda, T. Poussaint, D. Jaramillo, J. Zimmerman

What Are Stem Cells? From Embryo to Adult Tissues

February 21–24

FUNDED BY **Osiris Therapeutics, Inc., and SyStemix, Inc.**

ARRANGED BY **D.R. Marshak,** Osiris Therapeutics, Inc., Baltimore, Maryland
R.G. Scollay, SyStemix, Inc., Palo Alto, California
I.L. Weissman, Stanford University School of Medicine, California

SESSION 1
Chairperson: I.L. Weissman, Stanford University School of Medicine, California

R.L. Gardner, University of Oxford, United Kingdom: Stem cells in the preimplantation mouse conceptus.

A.L. Spradling, Howard Hughes Medical Institute, Carnegie Institution of Washington, Baltimore, Maryland: Regulation of *Drosophila* germ line stem cells by short-range extracellular signals.

M.T. Fuller, Stanford University School of Medicine, California: Genetic control of stem cell self-renewal, proliferation, and differentiation in the male germ line.

B.L.M. Hogan, Howard Hughes Medical Institute, Vanderbilt University Medical Center, Nashville, Tennessee: Primordial germ cells.

J.A. Thomson, University of Wisconsin, Madison: Human embryonic stem cells.

General Discussion—Nomenclature
Moderator: I.L. Weissman, Stanford University School of Medicine, California:

SESSION 2
Chairperson: R.G. Scollay, SyStemix, Inc., Palo Alto, California

I.L. Weissman, Stanford University School of Medicine, California: Hematopoietic stem cells.

I.R. Lemischka, Princeton University, New Jersey: The molecular biology of hematopoietic stem cells and their microenvironment; Do unique gene expression patterns reflect unique biological properties?

P.J. Quesenberry, University of Massachusetts Medical School, Worcester: Phenotype of hematopoietic stem cells.

R. Gardner, D. Anderson, M. Fuller

L.I. Zon, Howard Hughes Medical Institute, Children's Hospital, Boston, Massachusetts: Genes that specify hemangioblasts during vertebrate development.
J.M. Isner, St. Elizabeth's Medical Center of Boston, Massachusetts: Mobilization of bone-marrow derived progenitor cells and incorporation into neovascular foci.
H.M. Blau, Stanford University School of Medicine, California: Stem cells—Angioblasts.

SESSION 3
Chairperson: R.L. Gardner, University of Oxford, United Kingdom

P.G. Robey, National Institute of Dental and Craniofacial Research, NIH, Bethesda, Maryland: The biological significance of marrow stromal in health and disease.
D.R. Marshak, Osiris Therapeutics, Inc., Baltimore, Maryland: Mesenchymal stem cells.
R. Cancedda, Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy: Bone marrow stromal cells from healthy donors and from bone-marrow-transplant recipients.
A.B. Moseley, Osiris Therapeutics, Inc., Baltimore, Maryland: Therapeutic implications for MSCs in transplantation.
D.J. Prockop, MCP Hahnemann University, Philadelphia, Pennsylvania: Marrow stromal cells as vectors for diseases of the central nervous system.

SESSION 4
Chairperson: D.R. Marshak, Osiris Therapeutics, Inc., Baltimore, Maryland

D.J. Anderson, Howard Hughes Medical Institute, California Institute of Technology, Pasadena: Neural stem cells in the peripheral nervous system.
F.H. Gage, The Salk Institute, La Jolla, California: Multipotent stem cells from the adult central nervous system.
R. McKay, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Maryland: A CNS stem cell is as good as the neurons it makes.
C.Q. Doe, University of Oregon, Eugene: Neural stem cell division in *Drosophila*.
Y.-N. Jan, Howard Hughes Medical Institute, University of California, San Francisco: The control of neuronal progenitor cell fate.

SESSION 5
Chairperson: B.L.M. Hogan, Howard Hughes Medical Institute, Vanderbilt University Medical Center, Nashville, Tennessee

F.M. Watt, Imperial Cancer Research Fund, London, United Kingdom: Role of cell adhesion in regulating stem cell rate in human epidermis.
M. Grompe, Oregon Health Sciences University, Portland: Therapeutic liver repopulation: Are stem cells needed?
N. Sarvetnick, The Scripps Research Institute, La Jolla, California: Pancreas growth and regeneration.
D.E. Harrison, Jackson Laboratory, Bar Harbor, Maine: Genetic regulation of hemopoietic stem cell aging.
H.R. Bode, University of California, Irvine: Evolution of stem cells.

Comparative Plant Genomics

February 28–March 3

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **B. Burr,** Brookhaven National Laboratory, Upton, New York
J. Doebley, University of Minnesota, St. Paul
R. Martienssen, Cold Spring Harbor Laboratory

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
B. Burr, Brookhaven National Laboratory, Upton, New York
J. Doebley, University of Minnesota, St. Paul
R. Martienssen, Cold Spring Harbor Laboratory

SESSION 1: Sequence Polymorphism and Evolutionary Change
Chairperson: J. Messing, Rutgers University, Piscataway, New Jersey

M.T. Clegg, University of California, Riverside: Dynamics of plant gene family evolution: Duplication and divergence in flavonoid biosynthesis genes.

J. Doebley, University of Minnesota, St. Paul: Insights into maize evolution from the analysis of nucleotide diversity in *teosinte branched 1*.

S. Tingey, DuPont Company, Wilmington, Delaware: EST programs for gene discovery in maize, soybean, wheat, and rice.

O. Savolainen, University of California, Davis: From *Arabidopsis* to pine trees: Genetics of natural populations.

SESSION 2: Syntenic Relationships I
Chairperson: J. Doebley, University of Minnesota, St. Paul

R. Schmidt, Max-Delbruck-Laboratorium, Köln, Germany: Comparative genome analysis in cruciferous plants.

G. Moore, John Innes Centre, Norwich, United Kingdom: To pair or not to pair: The *Ph1* locus 40 years on.

W.R. McCombie, Cold Spring Harbor Laboratory: Sequence analysis of the rice and *Arabidopsis* genomes.

R. Martienssen, Cold Spring Harbor Laboratory: Stripping repeated DNA from the maize genomic sequence.

S. Tingey, D. Zamir, J. Messing, X. Wong

SESSION 3: Syntenic Relationships II
Chairperson: R. Martienssen, Cold Spring Harbor Laboratory

L.D. Stein, Cold Spring Harbor Laboratory: Comparative genomics of animals, mapping, and sequencing.
J. Messing, Rutgers University, Piscataway, New Jersey: Cereal genomics to study chromosome expansion.
P. San Miguel, Purdue University, West Lafayette, Indiana: Plant genome colinearity as modeled by *Adh1* and *sh2/a1* regions in maize, sorghum, rice, and *Arabidopsis*.
R. Tarchini, DuPont Genomics Group, Newark, Delaware: Genome structure and organization around the *Adh1/Adh2* region of rice and rice-maize microsynteny.

SESSION 4: Common Pathways
Chairperson: M.T. Clegg, University of California, Riverside

R.J. Schmidt, University of California, San Diego: *Arabidopsis* to maize.
J.B. Nasrallah, Cornell University, Ithaca, New York: Evolution of mating systems in the Brassicaceae.
T.C. Osborn, University of Wisconsin, Madison: Comparison of *Brassica* and *Arabidopsis* flowering time genes.
B. Burr, Brookhaven National Laboratory, Upton, New York: Genes controlling leaf trichome development may be involved in cotton fiber formation.

SESSION 5: Useful Genes From Wild Relatives
Chairperson: S. Tingey, DuPont Company, Wilmington, Delaware

T. Mitchell-Olds, Max-Planck Institute for Chemical Ecology, Jena, Germany: Wild relatives of *Arabidopsis:* Phylogenetic relationships and comparative genomics.
S.J. Knapp, Oregon State University, Corvallis: Genomics in newly domesticated, neglected, and underutilized oilseed crops.
L.M. Pollak, U.S. Department of Agriculture–Agricultural Research Service, Iowa State University, Ames: Improving corn germplasm for yield and value-added traits by introgression of genes from exotic varieties and *Tripsacum dactyloides*.
D. Zamir, Hebrew University of Jerusalem, Rehovot, Israel: Tomato introgression lines: Applications for synteny, evolution, and QTl mapping.

Meeting participants take a coffee break

Role of DNA Topology, Conformation, and Associated Factors in Gene Expression

March 14–17

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **D.L. Levens,** National Cancer Institute, NIH, Bethesda, Maryland
L.B. Rothman-Denes, University of Chicago, Illinois

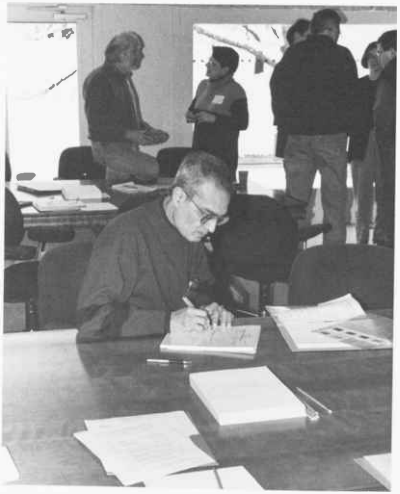
Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
D.L. Levens, National Cancer Institute, NIH, Bethesda, Maryland

SESSION 1: Conformational/Topological Changes at Transcription Sites
Chairperson: N. Hernandez, Cold Spring Harbor Laboratory

M. Timmers, Utrecht University, The Netherlands: Promoter opening and transcription initiation by RNA polymerase II.
J.A. Goodrich, University of Colorado, Boulder: Promoter escape by DNA polymerase II on negatively supercoiled DNA.
M. Meisterernst, Ludwig-Maximilians-Universitat München, Germany: Structure-function analysis of PCY—A transcription cofactor with unique DNA-binding properties.
L.F. Liu, UMDNJ-Robert Wood Johnson Medical School, Piscataway, New Jersey: Suppression of R-loops by DNA topoisomerases.
S. Adhya, National Cancer Institute, NIH, Bethesda, Maryland: DNA strand separation during isomerization: Rate limiting steps.

SESSION 2: Propagation of Stress to Remote Sites
Chairperson: S. Adhya, National Cancer Institute, Bethesda, Maryland

J.F. Marko, University of Illinois at Chicago: Altered states of DNA.
P. Droge, University of Cologne, Germany: The role of DNA topology, conformation, and associated factors in gene expression.
G.W. Hatfield, University of California, Irvine, College of Medicine: DNA supercoiling-dependent, protein-mediated activation of transcription from the $ilvp_6$ promoter of *Escherichia coli*.
H.-Y. Wu, Wayne State University School of Medicine, Detroit, Michigan: *Leu0*, a new transcription regulator.
R.R. Sinden, Texas A&M University, Houston: Transcriptional state of the mouse mammary tumor virus promoter can affect topological domain size in vivo.
M. Dunaway, University of California, Berkeley: Insulators, enhancers, and the DNA path.



S. Adhya

SESSION 3: Stress-induced Conformational Changes
Chairperson: A. Rich, Massachusetts Institute of Technology, Cambridge

P. Nelson, University of Pennsylvania, Philadelphia: Torsional directed walks, entropic elasticity, and DNA twist stiffness.
S.M. Mirkin, University of Illinois at Chicago: DNA structures generated by transcription.
L.B. Rothman-Denes, University of Chicago, Illinois: DNA structural transitions and single-stranded DNA binding proteins in transcription activation.
C.T. McMurray, Mayo Clinic, Rochester, Minnesota: Duplex to cruciform switching in the enkephalin enhancer controls expression of the human pronekephalin gene.
P. Sassone-Corsi, CNRS, Illkirch-Strasbourg, France: Transcriptional repression by DAX-1 via binding to hairpin structures.

SESSION 4: Role of Single-stranded DNA-binding Transcription Factors
Chairperson: D. Reinberg, Howard Hughes Medical Institute, UMDNJ-Robert Wood Johnson Medical School, Piscataway, New Jersey

D.L. Levens, National Cancer Institute, NIH, Bethesda, Maryland: Regulating c-*myc* expression with the FUSE binding protein.
P.D. Gardner, University of Texas Health Science Center, San Antonio: Transcriptional regulation of neuronal nicotinic acetylcholine receptors by single-stranded DNA-binding proteins.
J. Ting, University of North Carolina, Chapel Hill: Regulation of a topologically constrained promoter (class II MHC) by gene-specific coactivator, double-stranded, and single-stranded DNA-binding proteins.
L.D. Kohn, National Institutes of Health, Bethesda, Maryland: Regulation of TSH receptor and MHC gene expression by single-stranded binding proteins and Sox-4: Relevance to autoimmunity.

SESSION 5: Diverse Roles for Stressed-DNA and Associated Factors
Chairperson: L.F. Liu, UMDNJ-Robert Wood Johnson Medical School, Piscataway, New Jersey

C.J. Benham, Mt. Sinai School of Medicine, New York: Structural transitions in superhelical DNA.
A. Rich and T. Schwartz, Massachusetts Institute of Technology, Cambridge: Structural basis of the interaction between Z-DNA and the mRNA editing enzyme dsRNA adenosine deaminase.
E.M. Johnson, Mt. Sinai School of Medicine, New York: Activation of initiation of DNA replication at the JC viral origin by the HIV-1 protein TAT, dependent on cellular sequence-specific single-stranded DNA-binding protein, purα.
A.P. Wolffe, National Institute of Child Health and Human Development, NIH, Bethesda, Maryland: Chromatin, DNA topology, and transcription.
T. Kohwi-Shigematsu, Lawrence Berkeley Laboratory, California: Base-unpairing regions (BURs): Their roles in higher-order chromatin structure, gene regulation, and apoptosis.

Biology of Pigmentation

March 21–23

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **G. Barsh,** Stanford University School of Medicine, California
D.C. Bennett, St. George's Hospital Medical School, London, United Kingdom

Introduction and Goals of the Conference:
G. Barsh, Stanford University School of Medicine, California
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

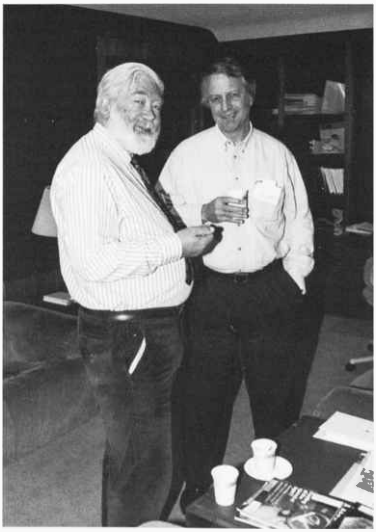
SESSION 1: Biochemistry of Melanins and Melanogenesis
Chairperson: V.J. Hearing, National Institutes of Health, Bethesda, Maryland

S.J. Orlow, New York University School of Medicine: Melanins, cells, and tissues: From molecules to phenotypes.
P.A. Riley, Windeyer Institute of Medical Science, London, United Kingdom: The biochemistry of melanogenesis: The generation and significance of DOPA (3,4-dihydroxyphenylalamine) in phase I melanogenesis.
T.P. Dooley, Altruis, LLC & IntegriDerm, Vestavia Hills, Alabama: Pharmacologic inhibitors of pigmentation.

SESSION 2: Cell Biology of Pigmentation
Chairperson: S.J. Orlow, New York University School of Medicine, New York

R.A. Spritz, University of Colorado Health Sciences Center, Denver: Genetics and functional analysis of Hermansky-Pudlak syndrome.
M.H. Brilliant, University of Arizona Health Sciences Center, Tucson: Aberrant pH of melanosomes in pink-eyed dilution (p) mutant melanocytes.
M. Robinson, University of Cambridge, United Kingdom: The role of the AP-3 complex in the trafficking of proteins to melanosomes.
S. Hoening, University of Gottingen, Germany: Sorting of melanosomal and lysosomal membrane proteins: Implications for the biogenesis of melanosomes.
J. Hammer, National Heart, Lung, and Blood Institute, NIH, Bethesda, Maryland: Myosin V, microtubular motors, and melanosome dynamics in murine melanocytes.
R. Yip, National Cancer Institute–Frederick Cancer Research Center, Maryland: Positional cloning of the mouse coat color mutation ashen.

General Discussion
Moderator: Seth J. Orlow, New York University School of Medicine, New York



P. Riley, V. Hearing

SESSION 3: Extrapigmentary Functions of the Melanocortin System
Chairperson: M.E. Hadley, University of Arizona, Tucson

S. MacNeil, Northern General Hospital, Sheffield, United Kingdom: MSH, oxidative stress, immunomodulation, and (only when all else fails) pigmentation.
J. Tatro, New England Medical Center, Boston, Massachusetts: The CNS melanocortin system in the coordinated response to microbial toxins.
R.A. Adan, Utrecht University, The Netherlands: Roles of the melanocortin system in nerve regeneration, stress, and grooming behavior.

General Discussion:
Moderator: M.E. Hadley, University of Arizona, Tucson

SESSION 4: Development
Chairperson: C.R. Goding, Marie Curie Research Institute, Surrey, United Kingdom

H. Arnheiter, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Maryland: The role of microphthalmia in eye and neural crest development.
D.C. Bennett, St. George's Hospital Medical School, London, United Kingdom: Immortalization and molecular characterization of melanoblasts and pluripotent neural crest-like cells.
M.K. Shin, Princeton University, New Jersey: Determining the spatial/temporal requirement for endothelin receptor B (Ednrb) by a tetracycline-inducible system.
I.J. Jackson,Western General Hospital, Edinburgh, United Kingdom: Survival, proliferation, and migration of melanoblasts in vivo and in organ culture.
H. Yoshida, Kyoto University, Japan: Role of Steel factor in survival of melanocytes in different environments.
D.M. Parichy, Washington University School of Medicine, St. Louis, Missouri: Evolutionary genetics of *Danio* pigment pattern development.

SESSION 5: Cell Signaling
Chairperson: D.C. Bennett, St. George's Hospital Medical School, London, United Kingdom

G. Barsh, Howard Hughes Medical Institute, Stanford University School of Medicine, California: Identification and characterization of the *mahogany* gene.
V. Hearing, National Institutes of Health, Bethesda, Maryland: Biochemical and molecular regulation of melanin biosynthesis.
G. Imokawa, Tokyo Women's Medical University, Japan: Intracellular signaling mechanisms leading to the synergistic effect on melanocyte proliferation of cultured human melanocytes between endothelin-1 and stem cell factor.
E.R. Price, Dana-Farber Cancer Institute, Boston, Massachusetts: Microphthalmia: Central regulator in melanocyte development.
R. Ballotti, Institut National de la Sante et de la Recherche Medicale, Nice, France: Role of microphthalmia-associated transcription factor (MITF) in the regulation of melanogenic enzyme expression.
E.E. Medrano, Baylor College of Medicine, Houston, Texas: Regulation of MITF protein levels by association with the ubiquitin-conjugating enzyme hUBC9.
C.R. Goding, Marie Curie Research Institute, Surrey, United Kingdom: Transcription regulation in melanocytes.

General Discussion:
Moderator: D.C. Bennett, St. George's Hospital Medical School, London, United Kingdom

SESSION 6: Genetics
Chairperson: G. Barsh, Howard Hughes Medical Institute, Stanford University School of Medicine, California

A.H. Robins, University of Cape Town, South Africa: The evolution of human skin color.
A. Chakravarti, Case Western Reserve University, Cleveland, Ohio: Human genetic variation in pigmentation genes.
R.N. Kelsh, University of Bath, United Kingdom: Zebrafish pigmentation mutations, colorless embryos, and a model for human Waardenburg-Shah syndrome.
J. L. Rees, University of Newcastle Medical School, Newcastle-upon-Tyne, United Kingdom: Genetics of the human MC1-R: Genotype and phenotype.
R.A. Sturm, University of Queensland, Australia: Genetic analysis of human pigmentation gene polymorphisms in twins.
W.J. Pavan, National Human Genome Research Institute, NIH, Bethesda, Maryland: A combined informatic expression array approach to dissect the transcriptional regulation of melanocyte development/function.

General Discussion:
Moderator: G. Barsh, Howard Hughes Medical Institute, Stanford University School of Medicine, California

Functional Organization of Thalamus and Cortex and Their Interactions

April 5–9

FUNDED BY **The Swartz Fund for Computational Neuroscience**

ARRANGED BY **P.R. Adams,** State University of New York, Stony Brook
K. Svoboda, Cold Spring Harbor Laboratory
S.M. Sherman, State University of New York, Stony Brook

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
P.R. Adams, State University of New York, Stony Brook
K. Svoboda, Cold Spring Harbor Laboratory
S.M. Sherman, State University of New York, Stony Brook

SESSION 1: Neurons and Synapses
Chairperson and Discussion Leader: D.L. Ferster, Northwestern University, Evanston, Illinois

B.W. Connors, Brown University, Providence, Rhode Island: An electrically coupled network of interneurons mediates feed-forward inhibition from thalamus to cortex.

A. Destexhe, Laval University, Quebec, Canada: Modeling dendritic integration in thalamic and neocortical neurons in vivo.

Y. Amitai, Ben Gurion University, Beer-Sheva, Israel: Thalamocortical and intracortical pathways in the barrel cortex: Distinctive synaptic properties and functional organization.

A.M. Thomson, Royal Free Hospital School of Medicine, London, United Kingdom: Synaptic specialization and frequency filtering (in cortical circuits).

K. Cox, K.-P. Hoffmann

SESSION 2: Thalamocortical I
Chairperson and Discussion Leader: R.M. Shapley, New York University, New York

R C. Reid, Harvard Medical School, Boston, Massachusetts: Thalamocortical relations in the visual system: The role of synchronous input.
E.G. Jones, University of California, Davis: The core and matrix of thalamic organization.
M.J. Berry, Harvard University, Cambridge, Massachusetts: Anticipation of moving stimuli by the retina.
P R. Adams, State University of New York, Stony Brook: Correlation measurement and plasticity control by layer 6 cells.
T. Sejnowski, The Salk Institute for Biological Studies, San Diego, California: Predictive learning of temporal sequences in recurrent neocortical circuits.

SESSION 3: Thalamocortical II
Chairperson and Discussion Leader: T. Sejnowski, The Salk Institute for Biological Studies, San Diego, California

D.L. Ferster, Northwestern University, Evanston, Illinois: The role of neuronal conductance and threshold in orientation selectivity.
K.D. Miller, University of California, San Francisco: Circuitry underlying orientation and temporal frequency selecting in cat visual cortex.
D.A. McCormick, Yale University School of Medicine, New Haven, Connecticut: Dynamic properties of thalamocortical interactions in normal and abnormal (epileptic) function.
T. Elliott, University of Nottingham, United Kingdom: A neurotrophic model of synaptic competition in the developing visual cortex.

SESSION 4: Cortical Circuitry
Chairperson and Discussion Leader: K.D. Miller, University of California, San Francisco

L. Borg-Graham, IAF CNRS, Gif-Sur-Yvette, France: Silent shunting—It's not nothing: Synaptic dynamics in visual cortex.
E.M. Callaway, The Salk Institute for Biological Studies, La Jolla, California: Local circuits in visual cortex.
R.M. Shapley, New York University, New York: The dynamics of visual responses in macaque striate cortex.
M. Nicolelis, Duke University Medical Center, Durham, North Carolina: Thalamocortical interactions in the somatosensory system.
A. Pouget, University of Rochester, New York: Information transfer in population codes: Implications for theories of cortical computation.

SESSION 5: Cortico-cortico and Cortico-thalamo-cortical
Chairpersons and Discussion Leaders: P.R. Adams and S.M. Sherman, State University of New York, Stony Brook

J. Bullier, Faculte de Medecine de Rangueil, Toulouse, France: Role of feedback connections in the visual cortex—Spatial and temporal aspects.
R.W. Guillery, University of Wisconsin School of Medicine, Madison: Corticothalamic pathways: Classification and organization.
H.S. Seung, Massachusetts Institute of Technology, Cambridge: Cellular and circuit mechanisms of short-term memory.
D.H. Ballard, University of Rochester, New York: Synchronous models in predictive coding.

SESSION 6: Cognitive
Chairperson and Discussion Leader: H.S. Seung, Massachusetts Institute of Technology, Cambridge

J.L. McClelland, Carnegie-Mellon University, Pittsburgh, Pennsylvania: Complementary learning systems, Hebbian learning, and human amnesia.
J.L. Gallant, University of California, Berkeley: The function of the nonclassical receptive field in natural vision.
K.-P. Hoffmann, Ruhr-University, Bochum, Germany: Synchronization of neuronal activity in extrastriate cortical areas during movement perception.
S. Treue, University of Tübingen, Germany: The role of attention in visual information processing.

Molecular Neurobiological Mechanisms in Schizophrenia: Seeking a Synthesis

April 11–14

FUNDED BY **The Charles A. Dana Foundation, with additional support from The Nathan S. Kline Institute for Psychiatric Research and Merck KGaA**

ARRANGED BY **A.B. Pardee,** Dana-Farber Cancer Institute, Boston, Massachusetts
A.B. Goodman, The Nathan S. Kline Institute for Psychiatric Research, Orangeburg, New York

Introduction and Goals of Conference:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
A.B. Pardee, Dana-Farber Cancer Institute, Boston, Massachusetts

SESSION 1: Fundamental Questions
Chairperson: A.B. Pardee, Dana-Farber Cancer Institute, Boston, Massachusetts

N.C. Andreasen, University of Iowa Hospitals and Clinics, Iowa City: Schizophrenia: Fundamental questions.
J.F. Nash, Princeton University, New Jersey: Case history.

SESSION 2: Characteristics, Genetics, Animal Models
Chairperson: R. Cancro, The Nathan S. Kline Institute for Psychiatric Research, Orangeburg, New York: Opening remarks

L. Ingraham, J. Nash

L.J. Ingraham, George Washington University, Washington, D.C.: Clinical and subclinical correlates of schizophrenia.
P.S. Holzman, Harvard University, Belmont, Massachusetts: Schizophrenia and area MT.
P. McGuffin, The Maudsley Institute of Psychiatry, London, United Kingdom: Gene mapping and positional cloning.
J.L. Kennedy, Clarke Institute for Psychiatry, Toronto, Canada: Neurodevelopment genes and unstable DNA in schizophrenia.
R.E. Straub, Virginia Commonwealth University, Richmond: Linkage results in schizophrenia—Disappointing only to those with unjustified expectations?
D.R. Weinberger, NIMH Neurosciences Center at St. Elizabeth's Hospital, Washington, D.C.: Schizophrenia, candidate genes, and candidate phenotypes.

A. Pardee, J. Kennedy, M. Tsuang

SESSION 3: Brain Structure, General Brain Models
Chairperson: F.M. Benes, McLean Hospital, Belmont, Massachusetts:
Opening Remarks

F.M. Benes, McLean Hospital, Belmont, Massachusetts: The role of stress and dopamine-GABA interactions in the development of schizophrenia.

P.S. Goldman-Rakic, Yale University School of Medicine, New Haven, Connecticut: The prefrontal model of schizophrenia.

S. Akbarian, Whitehead Institute, Massachusetts Institute of Technology, Cambridge: Altered gene expression in prefrontal cortex of schizophrenics—A perspective from postmortem studies.

A.B. Goodman, The Nathan S. Kline Institute for Psychiatric Research, Orangeburg, New York: Chromosomal colocalization (CC) at genetic loci linked to schizophrenia.

A.-S. La Mantia, University of North Carolina at Chapel Hill: Induction, retinoids, and the initial development of the forebrain.

B.T. Woods, Central Texas Veterans Health Care System, Temple: Discord in the developmental symphony: Can persistently active genetically determined abnormalities of apoptosis or neuritic pruning explain schizophrenia?

SESSION 4: Neuromolecular Biology
Chairperson: J.E. Dowling, Harvard University, Cambridge, Massachusetts:
Opening remarks

J.W. Olney, Washington University School of Medicine, St. Louis, Missouri: NMDA receptor hypofunction model of schizophrenia.

J.T. Coyle, Massachusetts General Hospital, Boston: NMDA receptor hypofunction and the pathophysiology of schizophrenia—Clinical evidence.

B.S. McEwen, Rockefeller University, New York: Stress, sex, and the hippocampus: From animal models to clinical application.

U.C. Drager, E. Kennedy Shriver Center, Waltham, Massachusetts: Retinaldehyde dehydrogenases create patterns of retinoic acid in the central nervous system.

G. Eichele, Baylor College of Medicine, Houston, Texas: Gene expression analysis in the brain: A meaningful approach to mental disorders?

P. Greengard, Rockefeller Institute, New York: Beyond the dopamine receptor: The DARPP-32/protein phosphatase 1 cascade.

SESSION 5: Molecular Signaling and Transcription
Chairperson: J. Maddox, London, United Kingdom: Opening remarks

E.J. Nestler, Yale University, New Haven, Connecticut: Regulation of gene expression in striatum: ΔFosB: A molecular mediator of long-term neural plasticity.

L.L. Iversen, University of Oxford, United Kingdom: Neuropharmacology— Dopamine and 5-hydroxytryptomine.

K.S. Kristensson, Karolinska Institut, Stockholm, Sweden: Targeting of viruses and African trypanosomes in the nervous system.

E. Borrelli, CNRS-INSERM, Strasbourg, France: Role of dopa-mine receptors in schizophrenia and other neuropathologies.

T. Perlmann, Karolinska Institut, Stockholm, Sweden: Role of NURR1 in developing and mature dopamine cells.

B.M. Spiegelman, Dana-Farber Cancer Institute, Boston, Massachusetts: Control of oxidative metabolism and thermogenesis through a regulated transcriptional coactivator.

SESSION 6: Treatment Strategies
Chairperson: A.B. Pardee, Dana-Farber Cancer Institute, Boston, Massachusetts: Opening remarks

M.T. Tsuang, Harvard Institute of Psychiatric Epidemiology and Genetics, Boston, Massachusetts: "Schizotaxia": Prevention strategies for schizophrenia.

T.H. McGlashan, Yale University School of Medicine, New Haven, Connecticut: Can current treatments prevent the dementing process of dementia praecox?

R.A. Heyman, Ligand Pharmaceuticals, Inc., San Diego, California: A pharmacological dissection of RXR signaling pathways with rexinoids.

G. Bartoszyk, Merck KGaA, Darmstadt, Germany: Classification of atypical neuroleptics: Catalepsy versus anticatalepsy.

Y. Ohno, Sumitomo Pharmaceuticals Research Center, Osaka, Japan: Recent advances in antipsychotic development: The role of 5-HT_2 receptor antagonism in schizophrenia treatment.

C. Mondadori, Hoechst Marion Roussel, Bridgewater, New Jersey: The difficulty of selecting the right target.

General Discussion
Chairperson: J.D. Watson, Cold Spring Harbor Laboratory

Image Archive on the American Eugenics Movement Editorial Advisory Panel Workshop

April 15–17

FUNDED BY **National Human Genome Research Institute, NIH**

ARRANGED BY **D. Micklos,** DNA Learning Center, Cold Spring Harbor Laboratory
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1

J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory: Introduction.
D. Micklos, DNA Learning Center, Cold Spring Harbor Laboratory: New panel members, workshop objectives, site narrative structure.
S. Lauter, DNA Learning Center, Cold Spring Harbor Laboratory: Introduction to site interface.

Computer Work I: Site Interface.
Computer Critique I: Site Interface.

Discussion of Theme Essays I
G.E. Allen, Washington University, St. Louis, Missouri: Social origins of the movement.
E.A. Carlson, State University of New York, Stony Brook: Scientific origins of the movement.
S. Selden, University of Maryland, College Park: Eugenics popularization.

SESSION 2

Introduction to Site Editing Shell
M. Christensen, DNA Learning Center, Cold Spring Harbor Laboratory: Caption writing/editing, series, emerging style.

Computer Work II: Captioning.
Computer Critique II: Captioning.

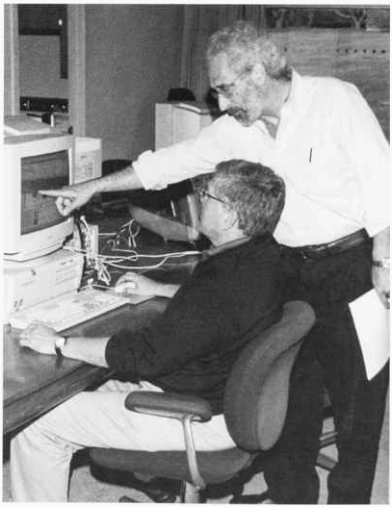
Discussion of Theme Essays II

P. Lombardo, University of Virginia, Charlottesville:
Eugenics and social policy I: Anti-miscegenation;
Eugenics and social policy II: Reproductive restriction and sterilization;
Eugenics and social policy III: Immigration restriction.
E.A. Carlson, State University of New York, Stony Brook: The end of transformation of eugenics.

SESSION 3

Discussion of Theme Essays III: Review.

Computer Work III: Revisit Site Interface and Editing Shell.
Computer Critique III: Additional Problems and Challenges.
Wrap-up, Future Tasks, and Meetings.



T. Shearer, S. Selden

Clinical and Biological Basis of the Ehlers-Danlos Syndrome

April 18–21

FUNDED BY **Ehlers-Danlos National Foundation and the March of Dimes**

ARRANGED BY **P.H. Byers,** University of Washington School of Medicine, Seattle
P. Tsipouras, University of Connecticut Health Center, Farmington

Introduction and Goals of Conference:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
P. Tsipouras, University of Connecticut Health Center, Farmington

SESSION 1: Molecular Basis of the Ehlers-Danlos Syndrome
Chairperson: P.H. Byers, University of Washington School of Medicine, Seattle

A. De Paepe, University Hospital Ghent, Belgium: Collagen gene mutations in the classical type.
U. Schwarze, University of Washington, Seattle: Collagen V gene mutations in the classical type.
R. Wenstrup, Children's Hospital Research Foundation, Cincinnati, Ohio: Collagen V gene mutations in the classical type.

Discussion: Collagen V gene mutations.

U. Schwarze, University of Washington, Seattle: Collagen III gene mutations in the vascular type.
B. Steinmann, University Children's Hospital, Zurich, Switzerland: Collagen I gene mutations in the arthochalasia type.
A. Nicholls, University of Cambridge, United Kingdom: Collagen I gene mutations in the classical type.

Discussion: Collagen I gene mutations.

A. Colige, Tour de Pathologie, Liege, Belgium: Procollagen *N*-peptidase gene mutations in the dermatosparaxis type.

SESSION 2: Molecular Basis/Pathogenesis
Chairperson: R. Wenstrup, Children's Hospital Research Foundation, Cincinnati, Ohio

L. Ala-Kokko, University of Oulu, Finland: *PLOD* gene.
H.N. Yeowell, Duke University Medical Center, Durham, North Carolina: *PLOD* gene mutations in the kyphoscoliosis type.
I. Hausser, Universitats-Hautklinik, Heidelberg, Germany: Histopathological observations.
P.H. Byers, University of Washington School of Medicine, Seattle: Abnormalities in the intracellular processing of collagen.

SESSION 3: Matrix Biology
Chairperson: D.R. Eyre, University of Washington, Seattle

L.Y. Sakai, Shriners Hospitals for Children, Portland, Oregon: Extracellular matrix molecules I.
R.E. Burgeson, Massachusetts General Hospital/Harvard, Charlestown, Massachusetts: Extracellular matrix molecules II.
D.R. Eyre, University of Washington, Seattle: Collagen interactions.
J. Rosenbloom, University of Pennsylvania, Philadelphia: Elastin and microfibrils.
K. Kadler, University of Manchester School of Biological Sciences, United Kingdom: Effects of mutations on collagen fibrillogenesis.

SESSION 4: Natural History
Chairperson: C.A. Francomano, National Human Genome Research Institute, NIH, Bethesda, Maryland

F.M. Pope, University of Wales College of Medicine, Cardiff, United Kingdom: Overview of the natural history of the Ehlers-Danlos syndrome.
M. Pepin, University of Washington, Seattle: Natural history of the vascular type.
B. Steinmann, University Children's Hospital, Zurich, Switzerland: Natural history of the kyphoscoliosis and arthrochalasia types.
N. Schechter, Saint Francis Hospital and Medical Center, Hartford, Connecticut: Musculoskeletal pain.
R.K. Portenoy, Beth Israel Medical Center, New York: Pain and Ehlers-Danlos syndrome.
M. Geraghty, Johns Hopkins Hospital, Baltimore, Maryland: Physiological and psychological management of individuals affected with Ehlers-Danlos syndrome.
C.A. Francomano, National Human Genome Research Institute, NIH, Bethesda, Maryland: Longitudinal studies.
S. Rodeo, Hospital for Special Surgery, New York: Orthopedic treatment of patients with joint laxity.

SESSION 5: Management and Future Directions
Chairperson: P. Tsipouras, University of Connecticut Health Center, Farmington

P.G. Danias, Beth Israel Deaconess Medical Center, Boston, Massachusetts: Noninvasive assessment of the elastic properties of modicum size arteries.
B.T. Baxter, University of Nebraska Medical Center, Omaha: Management of arterial aneurysms/rupture in the vascular type.
G.F. Whalen, University of Connecticut Health Center, Farmington: Management of bowel rupture.

Panel Discussion: Future directions.

Functional Genomics: Technology Development and Research Applications

April 25–28

FUNDED BY **Merck Genome Research Institute and National Cancer Institute**

ARRANGED BY **M.J.F. Austin,** Brigham and Women's Hospital, Boston, Massachusetts
R.L. Strausberg, National Cancer Institute, NIH, Bethesda, Maryland

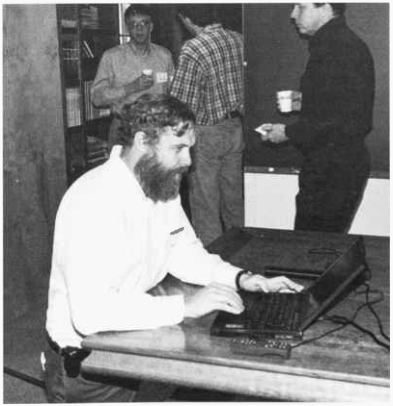
Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
M.J.F. Austin, Brigham and Women's Hospital, Boston Massachusetts
R.L. Strausberg, National Cancer Institute, NIH, Bethesda, Maryland

SESSION 1
Chairperson: M. Boguski, National Library of Medicine, NIH, Bethesda, Maryland

J. Pollack, Howard Hughes Medical Institute, Stanford University Medical School, California: Microarray analysis of gene expression and DNA copy number in breast cancer.
V.G. Cheung, Children's Hospital of Philadelphia, Pennsylvania: Whole-genome microarray— A tool for gene mapping.
D. Pinkel, University of California, San Francisco: Issues in DNA copy number analysis by array CGH.
D. Nickerson, University of Washington, Seattle: Genome sequence variation.
D.C. Muddiman, Virginia Commonwealth University, Richmond: Rapid and accurate characterization of VNTRs, SNPs, and DNA-drug interactions through development of electrospray lionization Fourier transform ion cyclotron resonance mass spectrometry.

SESSION 2
Chairperson: G.M. Church, Harvard Medical School, Cambridge, Massachusetts

K. Gunsalus, Rutgers University, Piscataway, New Jersey: Structure-based functional genomics.
J. Skolnick, The Scripps Clinic Research Institute, La Jolla, California: Structure-based approaches to the prediction of protein function.
M. Yaffe, Harvard Institute of Medicine, Boston, Massachusetts: Placing proteins into pathways: An interactive World Wide Web protein signature motif database using peptide library information.
P. Andrews, University of Michigan, Ann Arbor: Approaches to high-throughput proteome analysis and visualization.
R. Aebersold, University of Washington, Seattle: The proteome: Analysis and utility.
P.H. Uetz, University of Washington, Seattle: Large-scale two-hybrid analysis of the yeast proteome.



G. Church

SESSION 3
Chairperson: D. Nickerson, University of Washington, Seattle

E.A. Golemis, Fox Chase Cancer Center, Philadelphia, Pennsylvania: Interaction technology interactions with organismal biology.
M. Vidal, Massachusetts General Hospital Cancer Center, Charlestown: The *C. elegans* protein interaction map project.
G.M. Church, Harvard Medical School, Cambridge, Massachusetts: Integrating measurements, motifs, and models for comprehensive molecular quantitations of cell populations.
J. Green, National Cancer Institute, NIH, Bethesda, Maryland: Insights from the C3(1)/Tag transgenic models of prostate and mammary cancer.

SESSION 4
Chairperson: L.M. Staudt, National Cancer Institute, NIH, Bethesda, Maryland

G.A. Churchill, The Jackson Laboratory, Bar Harbor, Maine: Sources of variation in large-scale gene-expression experiments.
G. Eichele, Baylor College of Medicine, Houston, Texas: Methods and instrumentation for gene-expression analysis by in situ hybridization.
X. Gao, University of Houston, Texas: A novel method for on-chip parallel syntheses of molecular microarrays using photogenerated reagents.
H.R. Garner, University of Texas Southwestern Medical Center, Dallas: Hardware and software for array-based expression profiling and re-sequencing.
U. Alon, Princeton University, New Jersey: Analysis of gene expression patterns.
S.R. Gullans, Brigham and Women's Hospital, Boston, Massachusetts: Profiling gene expression using DNA microarrays.

SESSION 5
Chairpersons: R.L. Strausberg, National Cancer Institute, NIH, Bethesda, Maryland and **M.J. F. Austin,** Brigham and Women's Hospital, Boston, Massachusetts

L.M. Staudt, National Cancer Institute, NIH, Bethesda, Maryland: Genomic-scale analysis of gene expression in human lymphomas and leukemias using the Lymphochip cDNA microarray.
M.R. Emmert-Buck, National Cancer Institute, NIH, Bethesda, Maryland: An integrated approach to the analysis of human prostate cancer.
G.J. Riggins, Duke University Medical Center, Durham, North Carolina: The Public CGAP/SAGE database as one model for collaborative expression genomics.
P. Spellman, Stanford University School of Medicine, California: Analysis of genome expression data.
M. Boguski, National Library of Medicine, NIH, Bethesda, Maryland: Reflections.

Strategies for Inactivation of Mutant Genes

May 2–5

FUNDED BY **Amyotrophic Lateral Sclerosis Association and Hereditary Disease Foundation**

ARRANGED BY **R.H. Brown,** Massachusetts General Hospital, Charlestown
E.R. Signer, Massachusetts Institute of Technology, Cambridge
N.S. Wexler, Columbia University, New York

Introduction and Goals of Conference:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
R.H. Brown, Massachusetts General Hospital, Charlestown
E.R. Signer, Massachusetts Institute of Technology, Cambridge
N.S. Wexler, Columbia University, New York

SESSION 1
Chairperson: N.S. Wexler, Columbia University, New York

A. Lieberman, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Maryland: Androgen effects on motor neuron gene expression.
R.H. Brown, Massachusetts General Hospital, Charlestown: Mechanisms of neuronal cell death in SOD1-associated motor neuron disease.
S.O. Zeitlin, College of Physicians & Surgeons, Columbia University, New York: Conditional inactivation of the mouse *HD* gene.
H.J. Federoff, University of Rochester, New York: Somatic gene transfer of cre recombinase to alter brain function.

SESSION 2
Chairperson: E.R. Signer, Massachusetts Institute of Technology, Cambridge

E.B. Kmiec, Jefferson Center for Biomedical Research, Doylestown, Pennsylvania: Targeted mutagenesis using chimeric oligonucleotides: Mechanism of action and applications to functional genomics and gene therapeutics.
C.J. Steer, University of Minnesota Medical School, Minneapolis: The use of RNA/DNA oligonucleotides to rewrite genome sequence.
D.M. Turnbull, University of Newcastle-upon-Tyne, United Kingdom: Inhibition of mitochondrial DNA replication by peptide nucleic acids: Potential in the treatment of human disease.

E. Signer, N. Wexler, J. Witkowski

SESSION 3
Chairperson: A. Tobin, University of California, Los Angeles

P.S. Fishman, University of Maryland School of Medicine, Baltimore: Protein delivery to neurons using bacterial toxin-based vectors.
J.W. Engels, Johann Wolfgang Goethe-Universität, Frankfurt am Main, Germany: Hammerhead ribozymes: Chemotherapy contra gene therapy.
W.W. Hauswirth, University of Florida College of Medicine, Gainesville: Ribozyme approaches to therapy and gene discovery in the retina.

SESSION 4:
Chairperson: R.H. Brown, Massachusetts General Hospital, Charlestown

J.J. Rossi, City of Hope National Medical Center, Duarte, California: Ribozymes: Intracellular strategies for genomic studies and therapeutics.
B. Sullenger, Duke University Medical Center, Durham, North Carolina: Ribozyme-mediated repair of mutant RNAs.
L.-H. Yen, Yale University School of Medicine, New Haven, Connecticut: Sequence-specific cleavage of Huntington mRNA by catalytic nucleotides.

SESSION 5
Chairpersons: E.R. Signer, Massachusetts Institute of Technology, Cambridge, and **A. Tobin,** University of California, Los Angeles

N. Muzyczka, University of Florida, Gainesville: Use of viral vectors to study gene function in the central nervous system.
S. Kochanek, University of Cologne, Germany: Gene transfer with "gutless" adenoviral vectors.
D.J. Fink, University of Pittsburgh School of Medicine, Pennsylvania: Novel applications of genomic herpes vectors.

Coffee break between meeting sessions.

Xenotransplantation: A Scientific Basis for Risk Assessment

May 9–12

FUNDED BY **The Hastings Center and William Stamps Farish Fund**

ARRANGED BY **J.M. Coffin,** Tufts University School of Medicine, Boston, Massachusetts
M. Hanson, The Hastings Center, Garrison, New York
F. Murphy, University of California, Davis
R.A. Weiss, University of London, United Kingdom

Welcome and Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
J.M. Coffin, Tufts University School of Medicine, Boston, Massachusetts
M. Hanson, The Hastings Center, Garrison, New York
R.A. Weiss, Institute of Cancer Research, London, United Kingdom

SESSION 1
Chairperson: R.A. Weiss, Institute of Cancer Research, London, United Kingdom

J.L. Platt, Mayo Clinic, Rochester, Minnesota: Potential applications and challenges of xenotransplantation.

H.Y. Vanderpool, University of Texas Medical Branch, Galveston: Risk assessments in the 1996 reports of the Institute of Medicine (USA) and the Nuffield Council on Bioethics (UK).

C.R. McCarthy, Georgetown University, Richmond, Virginia: Risk benefit from the perspective of the IRB, the IACUC, and the subject.

D.R. Salomon, The Scripps Research Institute, La Jolla, California: Clinical trials in xenotransplantation— Why a moratorium based on risk concerns is bad medicine, bad science.

N. Daniels, Tufts University, Medford, Massachusetts: Public accountability for risks and the rationale for a moratorium.

S. Donnelley, F. Bach, N. Daniels

SESSION 2
Chairperson: N. Daniels, Tufts University, Medford, Massachusetts

D.K.C. Cooper, Massachusetts General Hospital, Charlestown: Efforts to induce tolerance in the pig-to-baboon model.
M.A. Michaels, Children's Hospital of Pittsburgh, Pennsylvania: Cytomegalovirus infections after xenotransplantation.
D.E. Onions, Q-One Biotech Ltd., Glasgow, United Kingdom: Control of virological risks associated with porcine xenotransplantation.
C. Patience, BioTransplant Inc., Charlestown, Massachusetts: The biology of PERV in an inbred herd of miniature swine.

SESSION 3
Chairperson: M.A. Martin, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland

S.S. Morse, Joseph L. Mailman School of Health, Columbia University, New York: Strategies for assessing and reducing the infectious disease risk.
M.J. Buchmeier, The Scripps Research Institute, La Jolla, California: Biology and pathogenesis of rodent-borne emerging viruses.
S.J. O'Brien, National Cancer Institute–Frederick Cancer Research Center, Maryland: Endogenous retroviral genomes: Relics of ancient plaques.
J.S. Allan, Southwest Foundation for Biomedical Research, San Antonio, Texas: Simian viruses in baboons: Risks to humans in the transplant setting.
C.R. Parrish, Cornell University, Ithaca, New York: Host range adaptation and evolution.

SESSION 4
Chairperson: J. Stoye, National Institute for Medical Research, London, United Kingdom

E. Fleissner, Columbia University, New York: Retrovirus-host interactions: Why are germ line viruses xenotropic, except in the laboratory?
W. Heneine, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia: Surveillance of xenogeneic retroviral infections in xenograft recipients.
C. Wilson, Food and Drug Administration, Bethesda, Maryland: Studies in cross-species infectivity of porcine endogenous retrovirus: Implications for pig-to-human xenotransplantation.
G. Langford, Imutran Ltd., Cambridge, United Kingdom: Analysis of samples from primates transplanted with hDAF transgenic organs for evidence of cross species transmission of PoERVs.

SESSION 5
Chairperson: M. Hanson, The Hastings Center, Garrison, New York

J.A. Bradbury, Pacific Northwest National Laboratory, Washington, D.C.: Addressing public concerns about risk: Some insights from U.S. Department of Energy and Army programs concerning the disposal of radioactive waste and chemical weapons.
F.H. Bach, Beth Israel-Deaconess, Boston, Massachusetts: Xenotransplantation: Where are we and what needs to be done?
S. Donnelley, The Hastings Center, Garrison, New York: The moral landscape of xenotransplantations.
L. Chapman, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia: Development of U.S. PHS policy on infectious disease issues in xenotransplantation.

Image Archive on the American Eugenics Movement Editorial Advisory Panel Workshop

October 1–3

FUNDED BY **National Human Genome Research Institute**

ARRANGED BY **D. Micklos,** DNA Learning Center, Cold Spring Harbor Laboratory
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1

J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory: Welcome, general instructions and hospitality.
D. Micklos, DNA Learning Center, Cold Spring Harbor Laboratory: Workshop objectives and summary of April meeting transcripts.
S. Lauter, DNA Learning Center, Cold Spring Harbor Laboratory: Features of the modified user interface.

Computer Work I: Site Interface.

Computer Critique I: Site Interface.

Discussion of New Theme Essays.
D. Micklos, DNA Learning Center, Cold Spring Harbor Laboratory: Eugenics research methods.
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory: Traits and conditions studied.
G.E. Allen, Washington University, St. Louis, Missouri: Flaws in eugenics research.

SESSION 2

Caption Style
M. Christensen, DNA Learning Center, Cold Spring Harbor Laboratory: Features of the modified editor interface.

Computer Work II: Captioning and essay illustration

SESSION 3

Computer Work III: Revisit Site Interface and Editing Shell.

Presentation of Revised Work.

Future Tasks and Meetings.

P. Ryan, P. Colbert-Cormier

Physical and Structural Chemistry of Prion Protein and Prion-like Phenomena

October 3–6

FUNDED BY **Cold Spring Harbor Corporate Sponsor Program**

ARRANGED BY **B. Caughey,** Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, NIH, Hamilton, Montana
K. Wuthrich, Institüt für Molekularbiologie und Biophysik, Zurich, Switzerland

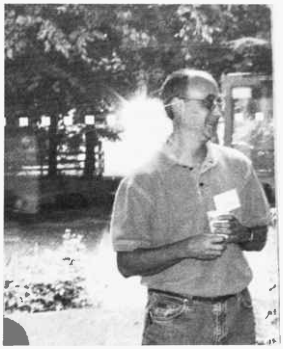
Welcome and Opening Remarks:
J. A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
B. Caughey, Rocky Mountain Laboratories, NIAID, NIH, Hamilton, Montana
K. Wuthrich, Institut für Molekularbiologie und Biophysik, Zurich, Switzerland

SESSION 1: PrP: NMR Structure and Folding
Chairperson: B. Caughey, Rocky Mountain Laboratories, NIAID, NIH, Hamilton, Montana

K. Wuthrich, Institüt für Molekularbiologie und Biophysik, Zurich, Switzerland: Outlook to the molecular structure of PrP in PrP^{Sc}.
H.J. Dyson, The Scripps Research Institute, La Jolla, California: Copper binding to the prion protein.
R. Glockshuber, Institüt für Molekularbiologie und Biophysik, Zurich, Switzerland: Folding of the cellular prion protein.
W.K. Surewicz, Case Western Reserve University, Cleveland, Ohio: Folding intermediates and in vitro aggregation/fibrillization of the recombinant human prior protein.

Roundtable Discussion: Immune responses to PrP and 3-D structures of PrP in vivo and in vitro
Moderator: A. Horwich, Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, Connecticut

Participants:
C. Weissmann, Imperial College School of Medicine at St. Mary's, London, United Kingdom
K. Wuthrich, Institüt für Molekularbiologie und Biophysik, Zurich, Switzerland
J. Collinge, Imperial College School of Medicine at St. Mary's, London, United Kingdom
J. Hope, Institute for Animal Health, Berkshire, United Kingdom
R. Rubenstein, New York State Office of Mental Retardation and Development, Staten Island



W. Surewicz, D. Eisenberg

SESSION 2: Prion Propagating Proteins in Yeast
Chairperson: K. Wuthrich, Institüt für Molekularbiologie und Biophysik, Zurich, Switzerland

R.B. Wickner, National Institutes of Health, Bethesda, Maryland: Generation and propagation of the (*URE3*) prion of *S. cerevisiae:* Ure2p domains, curing and involvement of new factors.
S.L. Lindquist, Howard Hughes Medical Institute, University of Chicago, Illinois: The yeast (PSI^+) factor.
M.D. Ter-Avanesyan, Institute of Experimental Cardiology, Moscow, Russia: Artificial yeast prions.
Y.O. Chernoff, Georgia Institute of Technology, Atlanta: Host proteins influencing formation, propagation, and toxicity of the yeast prion (PSI).
J.S. Weissman, University of California, San Francisco: Mechanism of amyloid formation and propagation: Lessons from a yeast prion.
C.-Y. King, Florida State University, Tallahassee: Nonsense suppression, antisuppression, and strains of the yeast prion (PSI).
K.L. Taylor, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, Maryland: Amyloid-based inheritance of (URE3): Initiation of amyloid formation in vitro by the Ure2p prion domain.

SESSION 3: PrP and Disease I
Chairperson: J. Hope, Institute for Animal Health, Berkshire, United Kingdom

S.B. Prusiner, University of California, San Francisco: Therapeutic approaches to prion diseases.
C. Weissmann, Imperial College School of Medicine at St. Mary's, London, United Kingdom: The role of the lymphoreticular system in experimental mouse scrapie.
J. Collinge, Imperial College School of Medicine at St. Mary's, London, United Kingdom: Molecular studies of prion propagation and strain diversity.
B. Caughey, Rocky Mountain Laboratories, NIAID, NIH, Hamilton, Montana: Interactions between normal and abnormal forms of PrP.
D.A. Harris, Washington University School of Medicine, St. Louis, Missouri: Transgenic models of familial prion disease membrane topology of PrP. Interactions of PrP and copper at the cellular level.

Roundtable Discussion: Infectivity from recombinant PrP and molecular basis of TSE strains
Moderator: D.S. Eisenberg, University of California, Los Angeles

Participants:
S.B. Prusiner, University of California, San Francisco
R. Glockshuber, Institüt für Molekularbiologie und Biophysik, Zurich, Switzerland
J.G. Safar, University of California, San Francisco
B. Caughey, Rocky Mountain Laboratories, NIAID, NIH, Hamilton, Montana
D.A. Harris, Washington University School of Medicine, St. Louis, Missouri

SESSION 4: PrP and Disease II
Chairperson: R.B. Wickner, National Institutes of Health, Bethesda, Maryland

J. Hope, Institute for Animal Health, Berkshire, United Kingdom: Bridging the gap between the chemistry and biology of prion protein.
P.T. Lansbury, Brigham and Women's Hospital, Boston, Massachusetts: Alpha-synuclein fibrillization, Lewy bodies, and Parkinson's disease? What is the connection?
J.G. Safar, University of California, San Francisco: Quantitative traits of prion strains are enciphered in the conformation of the prion protein.
D. Dormont, Commisariat a L'Energie Atomique, Fontenay-aux-Roses, France: Neuronal death induced by PrP peptides.
D.S. Eisenberg, University of California, Los Angeles: A 7-residue fragment of Sup35 that forms on amyloid.
T. Wisniewski, New York University School of Medicine, New York: The conformation of PrP^{Sc} as a determinant of strain properties and as a therapeutic target.
D. Riesner, Institüt für Physikalische Biologie, Dusseldorf, Germany: Intermediates in PrP-refolding: A soluble β-sheeted structure.

SESSION 5: Other Models of Protein Misfolding
Chairperson: C. Weissmann, Imperial College School of Medicine at St. Mary's, London, United Kingdom

J.W. Kelly, The Scripps Research Institute, La Jolla, California: Understanding amyloid disease and developing small-molecule inhibitors of misfolding.
D. Westaway, University of Toronto, Canada: The Doppel gene encodes a novel mammalian prion-like protein.
P. Fraser, University of Toronto, Canada: Assembly and structure of a β amyloid.
R. Wetzel, University of Tennessee Medical Center, Knoxville: Polyglutamine aggregation.

General Discussion

J.P. Morgan & Co., Incorporated/Cold Spring Harbor Laboratory Executive Conference on Genes and Genomes: Sequences to Patents

October 15–17

ARRANGED BY **J.D. Watson,** Cold Spring Harbor Laboratory
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Genes and Genomes

J.D. Watson, Cold Spring Harbor Laboratory: Welcoming remarks.
L. Hood, University of Washington, Seattle: The human genome project: From start to finish.

SESSION 2: Sequencing and Genome Biology

R. Gibbs, Baylor College of Medicine, Houston, Texas: DNA sequencing and the future.
D. Botstein, Stanford University School of Medicine, California: Seeing when genes act: Genome-wide patterns for gene expression.
G. Rubin, Howard Hughes Medical Institute, University of California, Berkeley: Biological annotation of *Drosophila* genome sequence.

SESSION 3: Cold Spring Harbor Laboratory Research

B. Stillman, Cold Spring Harbor Laboratory: Introduction—Genomics at Cold Spring Harbor Laboratory.
L. Stein, Cold Spring Harbor Laboratory: Managing sequence data.
M. Wigler, Cold Spring Harbor Laboratory: Using DNA arrays.

SESSION 4: Applications and Patents

M. Bevan, John Innes Centre, Colney, Norwich, United Kingdom: Sequencing a weed genome.
J. Todd, Cambridge Institute for Medical Research, United Kingdom: The evolution, causes, prevention, and economics of inflammation.
M. Freire, Office of Technology Transfer, NIH, Bethesda, Maryland: Commercial exploitation of genomic sequences.
J.D. Watson, Cold Spring Harbor Laboratory: Discussion and closing remarks.

B. Stillman, J. Todd, C. Harley, J. Watson

Cell Death in Plants: Functions and Mechanisms

October 17–20

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **J. Dangl,** University of North Carolina, Chapel Hill
A. Jones, University of North Carolina, Chapel Hill
C.J. Lamb, John Innes Centre, Norwich, United Kingdom

SESSION 1: Is Developmental PCD in Plants a Cell Recycling Program?
Chairperson: J. Dangl, University of North Carolina, Chapel Hill

M. Hengartner, Cold Spring Harbor Laboratory: Programmed cell death in *C. elegans*.
A. Bleecker, University of Wisconsin, Madison: The role of ethylene in senescence and abscission.
H. Thomas, Institute of Grasslands and Environmental Research, Aberystwyth, Wales, United Kingdom: Creating, mapping, cloning stay-greens (mutants with defective mesophyll cell senescence).
R. Amasino, University of Wisconsin, Madison: Regulation of gene expression during developmental senescence.
V. Buchanan-Wollaston, Horticulture Research International, Warwick, United Kingdom: Links between pathogen responses and leaf senescence in *Arabidopsis*.
R.L. Jones, University of California, Berkeley: Blue light, reactive oxygen, and hormonal control of plant (cereal aleurone) cell death.

SESSION 2: What Is the Role of PCD in Cellular Proliferation and Differentiation?
Chairperson: R. Amasino, University of Wisconsin, Madison

A. Jones, University of North Carolina, Chapel Hill: Regulation of programmed cell death by a secreted protease during terminal differentiation of tracheary elements.
K. Roberts, John Innes Centre, Norwich, United Kingdom: Cell death as a consequence of cell differentiation.
R. Whetten, North Carolina State University, Raleigh: Programmed cell death in pine xylem formation—Signals and mechanisms.
M.C. Drew, Texas A&M University, College Station: Ethylene-dependent programmed cell death in aerenchyma formation in roots.

J. Greenberg, J. Dangl, B. Staskawicz

SESSION 3: How Do *R* Gene Products Initiate HR?
Chairperson: R. Amasino, University of Wisconsin, Madison

D.C. Baulcombe, John Innes Centre, Norwich, United Kingdom: Autoactivation of cell death based on mutant forms of the *Rx* gene from potato.

B. Staskawicz, University of California, Berkeley: Bacterial effector proteins specifying plant cell death and disease resistance.

SESSION 4: Are ROIs Signals, Executioners or Both?
Chairperson: R. Whetten, North Carolina State University, Raleigh

C.J. Lamb, John Innes Centre, Norwich, United Kingdom: Hypersensitive cell-death cues and mechanisms.

D.F. Klessig, Waksman Institute, Rutgers University, Piscataway, New Jersey: NO- and SA-mediated signaling in plant disease resistance.

K. Shimamoto, Nara Institute of Science and Technology, Ikoma, Japan: Rac signaling in cell death and disease resistance of rice.

G. Johal, Pioneer Hi-Bred International, Inc., Johnston, Iowa: Cell death in maize disease lesion mimic mutants.

K.R. Davis, Ohio State University, Columbus: Ozone as a tool for probing programmed cell death in plants.

SESSION 5: How Does Negative Regulation Effect HR?
Chairperson: M. Grant, University of London, Ashford, United Kingdom

J. Dangl, University of North Carolina, Chapel Hill: Negative regulation in disease resistance.

P. Schulze-Lefert, John Innes Centre, Norwich, United Kingdom: A possible role for SCF complexes in the signaling of *R*-gene-triggered hypersensitive cell death.

SESSION 6: Where Do the Early Branchpoints of Pathogen Recognition Lead?
Chairperson: P. Schulze-Lefert, John Innes Centre, Norwich, United Kingdom

J.D.G. Jones, John Innes Centre, Norwich, United Kingdom: Possible roles for CDPKs and NADPH oxidase homologs in plant defense.

D. Scheel, Institute of Plant Biochemistry, Halle, Germany: Calcium and reactive oxygen species in plant defense signaling.

M. Grant, University of London, Ashford, United Kingdom: The role of intracellular calcium increase in hypersensitive cell death.

SESSION 7: What Are The Cellular Rearrangements That Follow Infection?
Chairperson: J.D.G. Jones, John Innes Centre, Norwich, United Kingdom

M.C. Heath, University of Toronto, Canada: Commonality of cell-death induction and executive in different forms of the hypersensitive response.

E. Schmelzer, Max-Planck Institute für Zuchtungsforschtung, Köln, Germany: Hypersensitive cell death upon fungae infection.

E. Lam, Rutgers University, New Brunswick, New Jersey: Caspase involvement in HR cell death.

SESSION 8: How Do Pathogens Usurp Cell Death Pathways?
Chairperson: J.D.G. Jones, John Innes Centre, Norwich, United Kingdom

F.M. Ausubel, Massachusetts General Hospital, Boston: Use of fumonisin B1 to study PCD in *Arabidopsis thaliana*.

A. Levine, The Hebrew University of Jerusalem, Israel: Cell death in plant interaction with necrotrophs.

J.T. Greenberg, University of Chicago, Illinois: Are cell death and cell growth coupled in plants?

The Art of Judging: Perspectives of Science

October 26–29

FUNDED BY **The Federal Judicial Center, Judiciary Leadership Development Council, and Cold Spring Harbor Laboratory**

ARRANGED BY **J.G. Apple,** The Federal Judicial Center, Washington, D.C.
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1

G.E. Allen, Washington University, St. Louis, Missouri: Eugenics: Past, present, and future.

SESSION 2

J. Maienschein, Arizona State University, Tempe: From Darwin to Dolly: Developments in the biological sciences in the 20th century.
L.M. Silver, Princeton University, New Jersey: Cloning: The biological and social implications of a new science.

SESSION 3

J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory: Human genetics.
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory: Human Genome Project.

SESSION 4

R.G. Crystal, Cornell University Medical College, New York, New York: Gene therapy.

SESSION 5

P. Reilly, Eunice Kennedy Shriver Center, Waltham, Massachusetts: Social implications of genetic research.
M.-E. Ruvolo, Harvard University, Cambridge, Massachusetts: Human origins.

SESSION 6

R. Shapiro, New York University, New York: Origins of life.
M.A. Gallo, UMDNJ-Robert Wood Johnson Medical School, Piscataway, New Jersey: Toxicology, the environment, and risk assessment.

SESSION 7

R.J. Roberts, New England BioLabs, Inc., Beverly, Massachusetts: The process of science vs. science in the courtroom.

Banbury Conference Center

The Molecular Neurobiology of ATM

November 7–10

FUNDED BY **A-T Children's Project**

ARRANGED BY **S.J. Elledge,** Baylor College of Medicine, Houston, Texas
N. Heintz, Rockefeller University, New York
E.M. Johnson, Washington University Medical School, St. Louis, Missouri

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
B. Margus, A-T Children's Project, Deerfield Beach, Florida

SESSION 1: Can We Identify Key Issues That Should Be Covered In This Meeting?

B. Margus, A-T Children's Project, Deerfield Beach, Florida
S.J. Elledge, Baylor College of Medicine, Houston, Texas

SESSION 2: Review of A-T the Disease
Chairperson and Discussion Leader: H.M. Lederman, Johns Hopkins Hospital, Baltimore, Maryland
Overview: T. Crawford, Johns Hopkins Hospital, Baltimore, Maryland
Discussion Point(s): What can the clinical picture tell us about missing areas of research?

SESSION 3: The A-T Protein and Interacting Proteins/Complexes
Chairperson and Discussion Leader: S.J. Elledge, Baylor College of Medicine, Houston, Texas
Overview: Y. Shiloh, Tel Aviv University Sackler School of Medicine, Israel
Discussion Point(s): Resolving controversies—Identifying key experiments.
Brief Presentations by Participants:
C.J. Bakkenist, St. Jude Children's Research Hospital, Memphis, Tennessee: Activation of neuronal ATM by growth factors.
M.H.L. Green, University of Brighton, United Kingdom: Ataxia telangiectasia and endogenous mutagens.
S. Harris, University of Connecticut Health Center, Farmington: Suppression of ATM kinase defects by mutational inactivation of a RecQ helicase.
B. Hempstead, New York Hospital–Cornell Medical College, New York: Novel signaling pathways regulating neuronal survival.
D.S. Lawrence, The Albert Einstein College of Medicine, Bronx, New York: Gene expression patterns in A-T cell lines.
E.Y.-H. Lee, University of Texas Health Science Center at San Antonio: Multiple signal transduction pathways mediated by ATM.
J. Qin, Baylor College of Medicine, Houston, Texas: Purification and identification of ATM complexes.
Y. Shiloh, Tel Aviv University Sackler School of Medicine, Israel: ATM-mediated signaling pathways.
R. Vibhakar, University of Iowa, Iowa City: Regulation of the AKT(PKB) kinase by ATM.

SESSION 4: New Insights or Plans Involving Mouse Models of A-T
Chairperson and Discussion Leader: M. Segal, The Weizmann Institute, Rehovot, Israel
Overview: C. Barlow, The Salk Institute for Biological Studies, La Jolla, California
Discussion Point(s): How "authentic" are mouse models?
Brief Presentations by Participants:
P.J. McKinnon, St. Jude Children's Research Hospital, Memphis, Tennessee: ATM-dependent apoptosis in the nervous system.
R. Eilam, The Weizmann Institute of Science, Rehovot, Israel: Nigro-striatal deficits in the ATM mice.
G.S. Rotman, Tel Aviv University, Sackler School of Medicine, Israel: SAT mice—An animal model for increased oxidative stress on the background of ATM deficiency.
M. Segal, The Weizmann Institute, Rehovot, Israel: Dopaminergic deficits in ATM knockout mice.

SESSION 5: Electrophysiology and Ion Channel Defects
Discussion Point(s)

SESSION 6: Progress toward Gene Therapy
Overview: S. Wang, Human Gene Therapy Research Institute, Des Moines, Iowa
Discussion Point(s)
Brief Presentation by Participant:
S. Wang, Human Gene Therapy Research Institute, Des Moines, Iowa: ATM gene delivery and expression by a Heerper amplicon vector.

SESSION 7: Cytokines and A-T
Overview: L.C. Gahring, University of Utah School of Medicine, Salt Lake City
Discussion Point(s)
Brief Presentation by Participant:
L.C. Gahring, University of Utah School of Medicine, Salt Lake City: Exploring the role(s) of cytokines in the brain.

SESSION 8: Primate Models of A-T
Overview: R.L. Sidman, New England Regional Primate Research Center, Southborough, Massachusetts, and **R. Norgren,** University of Nebraska Medical Center, Omaha
Discussion Points(s): Potential and Problems of Using Primate Models
Brief Presentations by Participants:
R. Norgren, University of Nebraska Medical Center, Omaha: Development of a rhesus macaque of ataxia-telangiectasia.
R.L. Sidman, New England Regional Primate Research Center, Southborough, Massachusetts: Initial steps toward a cerebellar Purkinje neuron degeneration model in monkeys by injection of onconase.

SESSION 9: Stem Cells, Neural Implantation, and A-T
Overview: E.Y. Snyder, Children's Hospital, Boston, Massachusetts
Discussion Point(s)
Brief Presentation by Participant:
D.A. Steindler, University of Tennessee Center Health Science, Memphis: Adult neural stem cells: Molecular cell biology and clinical applications.

SESSION 10: Oxidative Stress and A-T
Chairperson and Discussion Leader: E.M. Johnson, Washington University Medical School, St. Louis, Missouri
Overview: G.S. Rotman, Tel Aviv University, Sackler School of Medicine, Israel
Discussion Point(s)
Brief Presentations by Participants:
C. Barlow, The Salk Institute for Biological Studies, La Jolla, California: Oxidative stress and lysosomal accumulation in ATM-deficient mouse brain.
A. Barzilai, Tel Aviv University, Israel: Oxidative stress as a possible cause for neurodegeneration in A-T.
L.L. Dugan, Washington University School of Medicine, St. Louis, Missouri: Developmental mechanisms underlying increased free radical load in brain relevant to A-T.
H.M. Geller, UMDNJ-Robert Wood Johnson Medical School, Piscataway, New Jersey: Molecular mechanisms of neuronal apoptosis.
M.F. Lavin, Queensland Institute of Medical Research, Brisbane, Australia: Peroxisomal ATM and oxidative stress.

SESSION 11: Closing Discussion—Identifying Key Issues and Critical Experiments
Brief Comments:
S.J. Elledge, Baylor College of Medicine, Houston, Texas
E.M. Johnson, Washington University Medical School, St. Louis, Missouri
Y. Shiloh, Tel Aviv University Sackler School of Medicine, Israel
C. Barlow, The Salk Institute for Biological Studies, San Diego, California

Old Memories

November 14–17

FUNDED BY **The John A. Hartford Foundation, Inc.**

ARRANGED BY **J.D. Gabrieli,** Stanford University, California
M. Gallagher, Johns Hopkins University, Baltimore, Maryland
T. Tully, Cold Spring Harbor Laboratory

SESSION 1

T. Tully, Cold Spring Harbor Laboratory: CREB, Hartford, and old age.
J.R. Burke, Duke University Medical Center, Durham, North Carolina: The natural history of mild cognitive impairment in the elderly.
B. Johansson, University College of Health Sciences, Jonkoping, Sweden: Individual differences in episodic memory.
D. Carmelli, SRI International, Menlo Park, California: Brain structure and cognitive function in men remain heritable in the seventh and eighth decades of life.
R.E. Cabeza, University of Alberta, Canada: Functional neuroimaging of cognitive aging.

SESSION 2

P.R. Rapp, Mt. Sinai School of Medicine, New York: Strategies for exploring cognitive and neurobiological aging in the monkey.
D.C. Park, University of Michigan, Ann Arbor: Visuo-spatial and verbal working memory across the lifespan: Integrating brain data with behavioral data.
K.F. Berman, National Institute of Mental Health, NIH, Bethesda, Maryland: Context-dependent, neural system-specific neurophysiological concomitants of aging: Mapping PET correlates during cognitive activation.
C.L. Grady, Rotman Research Institute, Baycrest Centre for Geriatric Care, Toronto, Canada: Neuroimaging studies of aging and memory.
L.L. Light, Pitzer College, Claremont, California: Some aspects of episodic priming in young and older adults.
R. West, University of Notre Dame, Indiana: Is working memory more variable in older than younger adults?

SESSION 3

J.D. Gabrieli, Stanford University, California: Age-associated changes in brain activation during memory performance.
F.I.M. Craik, University of Toronto, Canada: Age-related changes in encoding and retrieval processes in human memory.
N. Raz, Weizmann Institute of Science, Rehovot, Israel: Differential age-related changes in the brain and their role in age-related changes in memory and executive function.
C.M. Hulette, Duke University Medical Center, Durham, North Carolina: Neuropathological changes associated with normal aging.
S.A. Small, Columbia University, New York: Regional analyses of the hippocampal formation in aging and Alzheimer's disease.

SESSION 4

M. D'Esposito, Hospital of the University of Pennsylvania, Philadelphia: Isolating the neural mechanisms of age-related changes in human working memory using event-related functional MRI.
H. Tanila, University of Kuopio, Finland: Memory encoding in old and young rat hippocampus.
C. Mondadori, Hoechst Marion Roussel, Bridgewater, New Jersey: The dynamics of long-term memory and drug-induced memory.
Y. Stern, Columbia University, The Sergievsky Center, New York: Cognitive and imaging studies of memory in aging.
M. Gallagher, Johns Hopkins University, Baltimore, Maryland: New data in old models: Effects of aging on hippocampus in rats with cognitive impairment.

SESSION 5

A.J. Silva, University of California, Los Angeles: Age-dependent cognitive decline: A tale of unwelcome channels, lazy synapses, and blasé neurons.
P. Chapman, University of Wales, Cardiff, United Kingdom: Animal models of Alzheimer's disease: The role of aging in behavioral and physiological pathology.
R.N. Rosenberg, University of Texas Southwestern Medical Center at Dallas: How is Alzheimer's disease first detected?
A. Wingfield, Brandeis University, Waltham, Massachusetts: Age-related speech-speed preferences.
C.R. Green, Mt. Sinai School of Medicine, New York: Memory training for healthy adults: Where do we go from here?

Microbial Targets for Small Molecules

November 28–December 1

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **P. Howley,** Harvard Medical School, Boston, Massachusetts
A.J. Levine, Rockefeller University, New York
G.L. Verdine, Harvard University, Cambridge, Massachusetts

Introductory Remarks and Welcome:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Viral Structure Targets
Chairperson: A.J. Levine, Rockefeller University, New York

D.C. Wiley, Howard Hughes Medical Institute, Harvard University, Cambridge, Massachusetts: The structure of an HIV-1-specific cell entry inhibitor in complex with the HIV-1 gp41 trimeric core.
P.S. Kim, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: HIV entry and its inhibition.
J.J. Skehel, National Institute for Medical Research, London, United Kingdom: Influenza HA in virus entry.
J.J. Hogle, Harvard Medical School, Boston, Massachusetts: Structurally biased combinatorial design of antivirals.
R.A. Lamb, Howard Hughes Medical Institute, Northwestern University, Evanston, Illinois: Influenza virus M2 ion channel and paramyxoviral fusion-protein structure.

SESSION 2: Bacterial Targets
Chairperson: J.E. Davies, TerraGen Discovery Inc., Vancouver, Canada

W.R. Jacobs, Howard Hughes Medical Institute, Albert Einstein College of Medicine, Bronx, New York: Lipid metabolism: A life and death struggle for tubercle bacillus.
C.T. Walsh, Harvard Medical School, Boston, Massachusetts: Enzymes conferring resistance to vancomycin.
T. Muir, Rockefeller University, New York: Peptide inhibitors of virulence in *Staphylococcus aureus*.
A. Tomasz, Rockefeller University, New York: New targets against multiresistant clones of *Staphylococcus aureus* and *Enterococcus faecium:* Clues from molecular epidemiology and from mechanisms of resistance.
D. Williams, University of Cambridge, United Kingdom: Restoring the affinity of vancomycin-group antibiotics against resistant bacteria without modification of the binding site.

J. Davies, P. Howley, A. Tomasz

SESSION 3: Molecular Targets I
Chairperson: P.M. Howley, Harvard Medical School, Boston, Massachusetts

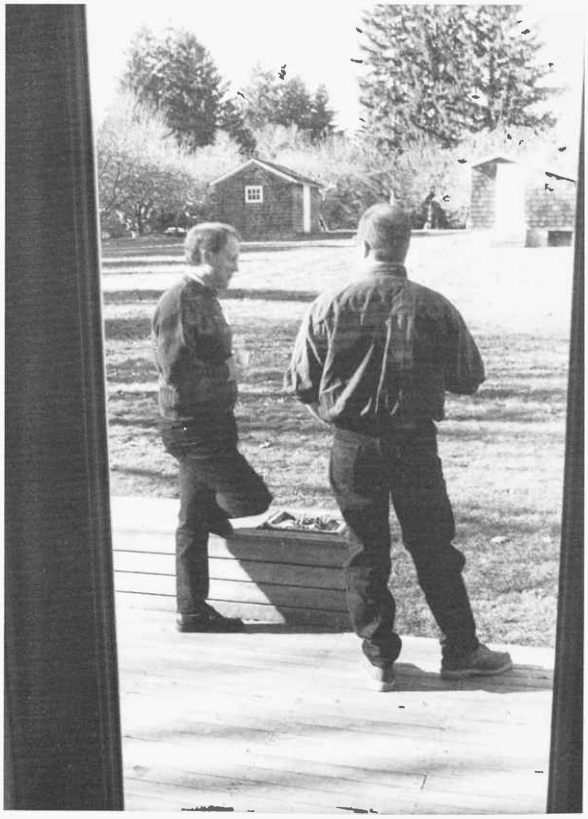
A.J. Levine, Rockefeller University, New York: Oligonucleotide array analysis.
H.L. Ploegh, Harvard Medical School, Boston, Massachusetts: New tools to study proteolytic pathways.
G.L. Verdine, Harvard University, Cambridge, Massachusetts: Chemical biology approaches to macromolecular targeting and functional analysis.
D. Cane, Universite Louis Pasteur, Strasbourg, France: Enzymatic and molecular strategies for the biosynthesis of unnatural natural products.
E. Kieff, Brigham and Women's Hospital, Brookline, Massachusetts: Molecular targets for preventing or treating herpesvirus-related cancers.

SESSION 4: Fungal and Bacterial Targets
Chairperson: C.T. Walsh, Harvard Medical School, Cambridge, Massachusetts

J.E. Davies, TerraGen Discovery, Inc., Vancouver, Canada: Mining molecular diversity from microbes.
J.C. Clardy, Cornell University, Ithaca, New York: Dihydroorotate dehydrogenase as a bacterial target.
E. Elion, Harvard Medical School, Boston, Massachusetts: A signal transduction rescue system for cell wall damage in *Saccharomyces cerevisiae*.
E.K. O'Shea, University of California, San Francisco: Regulation of nuclear transport.
T.J. Silhavy, Princeton University, New Jersey: Two component regulatory systems: The sensory organs of bacteria.

SESSION 5: Molecular Targets II
Chairperson: G.L. Verdine, Harvard University, Cambridge, Massachusetts

D.E. Kahne, Princeton University, New Jersey: New targets for glycopeptide antibiotics: How to overcome resistance.
J.M. Berger, University of California, Berkeley: Mechanisms of drug activity in type II topoisomerases.
C.R. Raetz, Duke University Medical Center, Durham, North Carolina: Enzymes of lipid A biosynthesis: Targets for the design of new antibiotics.
P.M. Howley, Harvard Medical School, Boston, Massachusetts: Harnessing the ubiquitination machinery to degrade specific cellular proteins.
S. Hecht, University of Virginia, Charlottesville: RNA as a therapeutic target.



Participants continue discussing the meeting outdoors.

Vaccines for Developing Economies: Who Will Pay?

December 5–7

FUNDED BY **Albert B. Sabin Vaccine Institute, Inc.**

ARRANGED BY **P. Russell,** Albert B.Sabin Vaccine Institute, Inc., Potomac, Maryland
S. Lemon, University of Texas Medical Branch, Galveston

CO-CHAIRPERSONS **J. Sachs,** Harvard University, Cambridge, Massachusetts
P. Hotez, Yale University, New Haven, Connecticut

Introduction:
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
H.R. Shepherd, Albert B. Sabin Vaccine Institute, Inc., New Canaan, Connecticut

SESSION 1: Setting the Stage: The Imbalance of Global Science
Chairperson: D.T. Jamison, University of California, Los Angles

D.T. Jamison, University of California Los Angeles: What harms and kills the world's children: Implications for science and technology.
M. Miller, World Health Organization, Geneva, Switzerland: Economic and epidemiological factors encouraging adoption of vaccines into national vaccine programs.
A. Mahmoud, Merck and Company, Inc., Whitehouse Station, New Jersey: Market pressures on academic biotechnological advances.
W. Vandersmissen, SmithKline Beecham BioServices, Rixensart, Belgium: Market pressures on industry.
G.W. Perkin, Global Health Program, Bill and Melinda Gates Foundation, Seattle, Washington: The new role of foundations.
T. Godal, UNICEF, Geneva, Switzerland: The GAVI perspective.
K.W. Bernard, National Security Council, Washington, D.C.: International Public Health—The evolving U.S. government policy on vaccines.

SESSION 2: Case Studies In Orphan Vaccines
Chairperson: P.K. Russell, Albert B.Sabin Vaccine Institute, Inc., Potomac, Maryland

B. Schwartz, Centers for Disease Control and Prevention, Atlanta, Georgia: Case studies in orphan vaccines.
P.K. Russell, Albert B. Sabin Vaccine Institute, Inc., Potomac, Maryland: Malaria vaccines.
P. Hotez, Yale University School of Medicine, New Haven, Connecticut: Helminth vaccines.
R.E. Shope, University of Texas Medical Branch, Galveston: Dengue vaccines.
S. Berkley, International AIDS Vaccine Initiative, New York: HIV vaccines.

SESSION 3: Mobilization of Science and Technology for Developing Countries

A. Attaran, The Malaria Project, Vancouver, Canada: Globalizing intellectual property.

SESSION 4: The Millennium Vaccine Fund: Impact of a "Promised Market"
Chairperson: G.T. Keusch, Fogarty International Center, National Institutes of Health, Bethesda, Maryland

M. Kremer, Harvard University, Cambridge, Massachusetts: Overview of incentive issues.
A. Batson, The World Bank, Washington, D.C.: Results of World Bank study.
J.D. Sachs, Harvard University, Cambridge, Massachusetts: Millennium Fund proposal.

L. Eros, R. Skolnik, A. Mahmoud

Host-Pathogen Interactions

December 12–15

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program, with additional support from Glaxo Wellcome Inc.**

ARRANGED BY **B.R. Bloom,** Harvard University, Boston, Massachusetts
S. Blower, University of California, San Francisco
N.B. Freimer, University of California, San Francisco

SESSION 1: Approaches for Studying Host-Pathogen Interactions
Chairperson: N.B. Freimer, University of California, San Francisco

J.M. McNicholl, Centers for Disease Control and Prevention, Atlanta, Georgia: Host genes and infectious disease: A public health perspective.
N.B. Freimer, University of California, San Francisco: An overview of genetic-mapping approaches.
C.M. Fraser, The Institute for Genomic Research, Rockville, Maryland: Genomics of microorganisms.
B. Rannala, State University of New York, Stony Brook: Phylogenetic methods to reconstruct the evolutionary history of a virulent pathogen.
D. Kirschner, University of Michigan Medical School, Ann Arbor: Understanding host-pathogen interactions using modeling.

SESSION 2: Pathogen Evolution
Chairperson: S. Blower, University of California, San Francisco

J.L. Gerberding, Centers for Disease Control and Prevention, Atlanta, Georgia: Antibiotic resistant bacteria: A public health perspective.
M. Achtman, Max-Planck Institute for Molecular Genetics, Berlin, Germany: Population genetics of *N. meningitidis, H. pylori,* and *Y. pestis*.
G. Myers, Los Alamos National Laboratory, New Mexico: Sexually transmitted disease pathogens: A database approach.
J.W. Kazura, Case Western Reserve University, Cleveland, Ohio: Polymorphisms for *Plasmodium vivax* malaria: Simplicity and complexity.

D. Kirschner, C. Fraser

SESSION 3: Host Evolution
Chairperson and Introduction: N.B. Freimer, University of California, San Francisco

M. Carrington, National Cancer Institute–Frederick Cancer Research and Development Center, Maryland: Host susceptibility to HIV.
P. Demant, The Netherlands Cancer Institute, Amsterdam: Genetic dissection of disease susceptibility in the mouse.
P. Gros, McGill University, Montreal, Canada: Role of NRAMP genes in macrophage function and divalent cation transport.

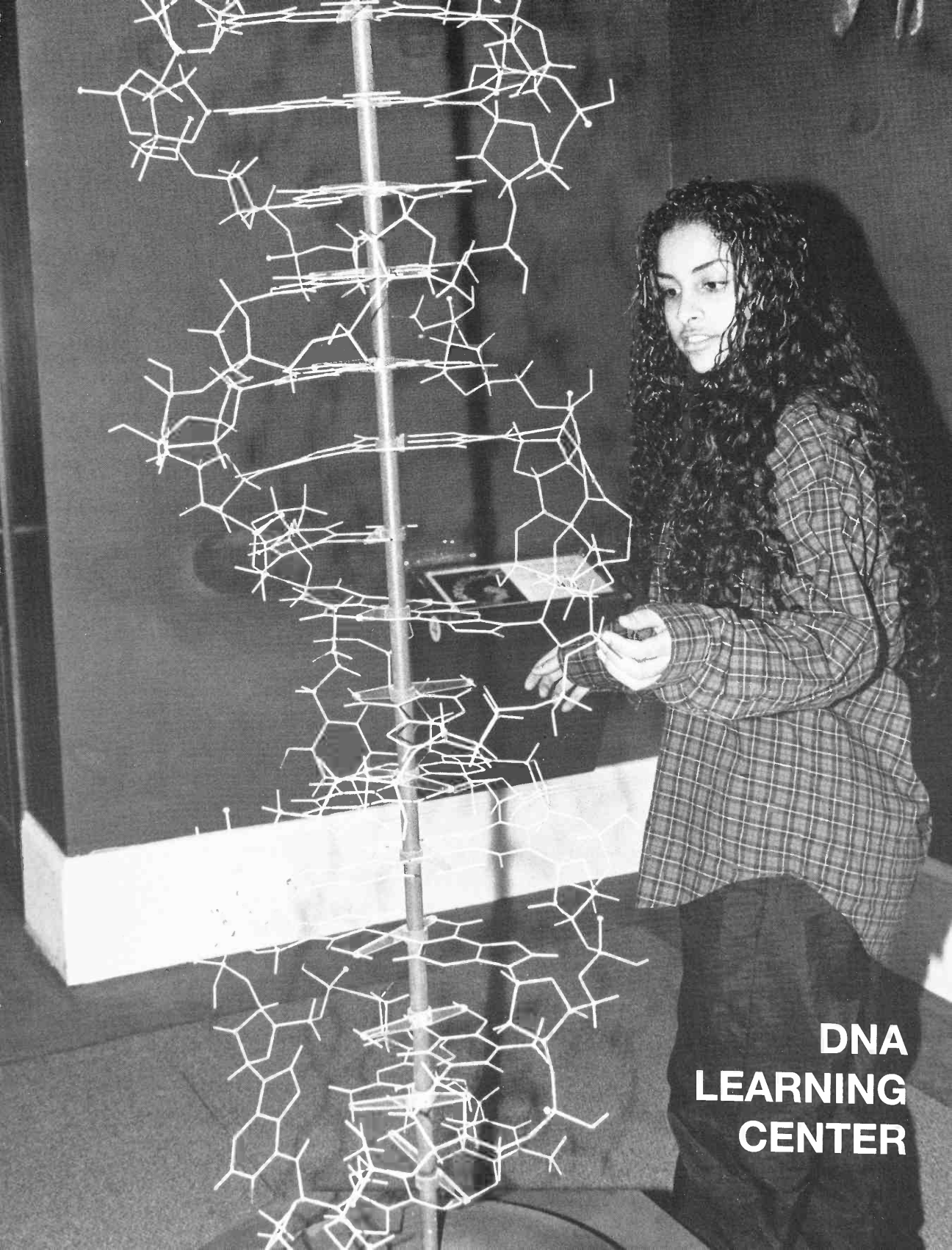
SESSION 4: Tuberculosis as a Model for Host-Pathogen Interactions
Chairperson: B.R. Bloom, Harvard University, Boston, Massachusetts

B.R. Bloom, Harvard University, Boston, Massachusetts: Introduction to TB.
J.M. Musser, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, NIH, Hamilton, Montana: Molecular population genetic analysis of antibiotic resistance and antigen genes in *M. tuberculosis*.
D.B. Young, Imperial College School of Medicine, London, United Kingdom: Trafficking of mycobacteria and mycobacterial antigens in infected macrophages.
I. Kramnik, Harvard School of Public Health, Boston, Massachusetts: Genes regulating host resistance to virulent mycobacteria.
A.V.S. Hill, University of Oxford, United Kingdom: Genome-wide screening for TB susceptibility genes.
R.D. Fleischmann, The Institute for Genomic Research, Rockville, Maryland: The genome of *M. tuberculosis*.
S. Blower, University of California, San Francisco: Epidemic models of TB.

SESSION 5: Co-Evolution and Future Directions
Chairperson: S. Blower, University of California, San Francisco

J.T. Williams, Southwest Foundation for Biomedical Research, San Antonio, Texas: Statistical genetic analysis of host-pathogen interaction.
M.J. Wade, Indiana University, Bloomington: Evolution of host maternal effects in response to pathogens affecting offspring.
D.R. Taylor, University of Virginia, Charlottesville: The evolution of infectious mitochondrial mutants in plants.
S. Gupta, University of Oxford, United Kingdom: The effects of immune selection on pathogen population structure.
M.J. Roossinck, The Noble Foundation, Ardmore, Oklahoma: The role of the host in the evolution of RNA viruses.
T. Lenormand, University of British Columbia, Vancouver, Canada: Long-term resistance management in vectors: The mosquito case.

Meeting participants outside the conference center.


DNA
LEARNING
CENTER

DNA LEARNING CENTER

ADMINISTRATION

David Micklos
Judy Cumella-Korabik
Nancy Daidola
Vin Torti

INSTRUCTION

Scott Bronson
Patricia Maskiell
Amanda McBrien
Martha Mullally
Danielle Sixsmith

MULTIMEDIA

Susan Lauter
Shirley Chan
Chun-hua Yang
Susan Conova

To many, 1999 will be remembered as the year of the Internet. As Internet stocks and initial public offerings soared, so did the number of visitors to our World Wide Web (WWW) site, *Gene Almanac*. Like other established WWW sites—and people who bought technology stocks early on—we were able to ride the crest of Internet interest in 1999. More than 380,000 people visited *Gene Almanac*, requesting 14.5 million documents from our Web server—a threefold increase over 1998. Visitation peaked in October, when our server logged 68,221 visitors, 177,863 page views, and 2.5 million document requests. This growth was fueled primarily by the release of our animated online genetics primer, *DNA from the Beginning (DNAFTB)*, which by year's end accounted for the majority of pages viewed at our site.

Unlike conventional textbooks, which are organized around chapters, *DNAFTB* is organized around key concepts. To date, we have released sections on 24 concepts in classical and molecular genetics. Each contains an animation that presents key experiments as done by the scientists, a gallery with seldom-seen archival photos, video interviews, biographies, and links to other Web sites of interest. The site received positive reviews in the NetWatch column of *Science* (http://www.science.com), *HMS Beagle* (http://www.biomednet.com/hmsbeagle), and *Oncology Times*.

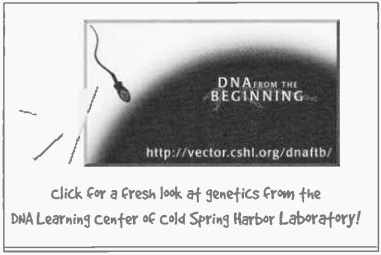
Late in the year, we passed another milestone when Carolina Biological Supply Company (CBS) became the first sponsor of *Gene Almanac*. CBS is one of the nation's oldest and most respected suppliers of science products for the education market. Since 1987, the DNA Learning Center (DNALC) has collaborated with CBS to develop the *DNA Science* laboratory text and more than ten molecular genetics experimental kits. We were surprised to find that other major science centers have yet to seek sponsorships for their WWW sites.

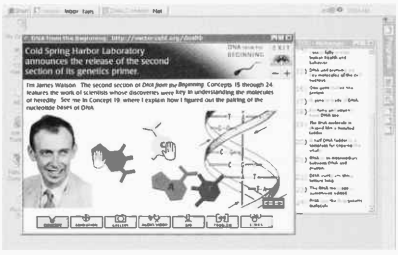
This logo first appeared on the DNALC Web site in November.

We continued to expand our server infrastructure to accommodate the exponential increase in visitation. Frequent crashes showed that our Microsoft NT server and database system could not efficiently handle some of our complex scripts and bioinformatics routines. At the same time, experiments revealed that this system could not be scaled up to the heavy loads we predict over the next year. To remedy this situation, in November, we switched over to a Linux server running an open-source operating system, Web server, and database server. This combination has proved remarkably stable and has not experienced a single crash yet. Using open-source software has the added bonus of reducing our license costs to near zero, making future system expansions more affordable.

At year's end we had a portfolio of $2.66 million in the following projects involving Internet science education, making the DNALC one of the largest providers of multimedia learning materials for biology education:

- *DNAFTB*, funded by an $820,000 grant from the Josiah Macy, Jr., Foundation, is the worlds first online, animated genetics text. An encyclopedia of genetic disorders will be added soon.
- *VectorNet*, funded by a $500,000 grant from the Howard Hughes Medical Institute (HHMI), is a mobile computer laboratory designed to provide state-of-the-art bioinformatics to New York City students and teachers across the nation.





Word was spread about *DNA from the Beginning* with two postcard mailings to 12,000 biology educators around the nation. The mailings were timed to coincide with the release of the first section of concepts in March, and the second section in September.

- *Digital Image Archive on the American Eugenics Movement*, funded by a $405,000 grant from the National Human Genome Research Center, will allow students to explore materials from several archives that have never before been released for public use.
- *A Partnership to Develop Advanced Technology Units on Genomic Biology*, funded by a $600,000 grant from the National Science Foundation (NSF), is a nationwide program for high school and college faculty, which includes extensive use of Internet tools for analyzing genes.
- *The Science and Issues of Human DNA Polymorphisms*, funded by a $335,000 grant from the Department of Energy (DOE) Human Genome Project, is a nationwide training program for high school biology teachers, which includes custom, online databases of student "DNA fingerprints."

We Anxiously Await Construction of an Expanded Facility

As our virtual visitation continued its exponential growth, our real-world visitation has plateaued at about 30,000. Although we increased student lab instruction by nearly one third during the last 2 years, our teaching lab facilities are now completely saturated. Labs are fully booked year-round, and our staff teaches as many elementary students in schools as at the DNALC.

To make matters worse, the doubling of our staff during the past 2 years has made the basement offices cramped as well as dreary. At one point in 1999, 12 staff members shared 1000 square feet of office space. This situation was eased slightly at the expense of converting the Barbara McClintock exhibit into an administrative office. As the year progressed, we realized that we had "hit the wall" of growth and creativity in our current facility. At times it seemed that the deterioration of our work environment even threatened our high level of innovation. Therefore, news that the Town of Huntington approved our building permit for a 9000-square-foot *Biomedia* addition ended the year on a hopeful note. It now seems certain that several years of dreaming and planning will come to fruition. Construction will begin in spring 2000, with completion of the new facility scheduled for summer 2001. The prospect of upstairs offices, an additional teaching lab, and state-of-the-art facilities for multimedia production tempered the news that ten staff members will be relocated during construction. In the upcoming year, the instructional group will make its home in the small exhibit gallery on the main floor, and the multimedia group will move to a temporary office (a.k.a. a trailer) on the main CSHL campus.

Centerbrook Architects and Planners of Essex, Connecticut, have designed the new facility. This firm has been responsible for the eclectic mix of beautiful buildings and additions constructed at CSHL in the past 30 years. Their handiwork is also seen in the initial renovation of the DNALC, including the striking multimedia auditorium, or "multitorium." In 1998, the American Institute of Architects honored Centerbrook as architectural firm of the year.

True to form, principal architect Jim Childress has crafted a two-story brick addition that merges easily with the existing Georgian revival structure. Cunningly, the main level extends into the hill to the rear of the current building, allowing the second story to "emerge" on grade at the crest of the hill. Thus, seen from the back, the addition appears to be a single-story structure—in keeping with the residential feel of our neighborhood. The centerpiece of the addition, a hexagonal computer laboratory, is a 21st century reprise of the classical architecture that inspired the original building. A new exhibit on the Human Genome Project—including a *Visible Sequencing Lab* in which DNALC staff will produce DNA sequences from samples submitted by biology classes from around the country—will occupy expanded galleries. We cannot wait to inhabit our new spaces.

Developing the *VectorNet* Mobile Bioinformatics Laboratory

In the summer, we received a 4-year grant from the HHMI Precollege Science Education Initiative for Medical Research Institutes, effectively renewing a previous 5-year award. The new program will introduce high school students and teachers to the use of modern networked computing in genomic biology. Of 150 applicants and 35 awardees, the DNALC was one of only two institutions to receive the maximum award of $500,000.

The focal point of the program is *VectorNet*, a stand-alone, portable computer laboratory that demonstrates state-of-the-art server and networking technology. Ten laptop computers are linked to a central server via a wireless network. The central server mirrors the entire DNALC Web site, including bioinformatics tools and GenBank data sets. A presenter laptop with video projector allows the instructor to demonstrate use of the programs. This "bioinformatics laboratory in a box" can be shipped and set up anywhere, providing full bioinformatic resources without the need for an Internet connection.

VectorNet incorporates insight from 3 years' experience conducting bioinformatics training at sites in Great Britain and around the United States. We have found that even in the best of dedicated computer laboratories, any number of problems can halt work, including Internet and local network unreliability, local security provisions, nonuniform hardware setups, and software misconfigurations. By the same token, we have found that even the best of students and teachers have difficulty staying on task when they have the ability to check E-mail or sports scores. Therefore, we concluded that the best bioinformatics training experience is ensured by providing one's own network and all needed resources *offline*. This offers all the power of distributed computing without the distractions of an open Internet connection.

During the academic year, *VectorNet* will be placed in one public high school in each of the five boroughs of New York City. This local component of the program, *New York City Genes*, will allow large numbers of minority students in metropolitan New York to participate in an authentic analog of the Human Genome Project. The five schools are culturally and economically diverse: Stuyvesant High School, Manhattan; Jamaica High School, Queens; J.F. Kennedy High School, Bronx; Port Richmond High School, Staten Island; and Brooklyn Technical High School, Brooklyn. Each has a working DNA laboratory where students can generate DNA-polymorphism data, which can be used as a starting point in bioinformatics explorations. During a several-week loan period, *VectorNet* will be set up in the science wing of each high school, allowing students to move seamlessly among classroom theory and discussion, wet labs on DNA polymorphisms, and online computer analyses. *New York City Genes* is supported by an extensive collaboration with the Gateway to Higher Education Program of the Mount Sinai School of Medicine and New York City Public Schools. The Gateway Program prepares minority and low-income students to graduate from high school and go on to high achievements in college programs in medicine, science, and technology.

During the summer, *VectorNet* will be used in teacher-training workshops at locations around the country. The *Vector Bioinformatics Workshop* will target lead biology teachers, most of whom already incorporate hands-on DNA laboratories into their classes. Our goal is for teacher participants to gain an intuitive command of key principles of genomic biology and bioinformatics, accelerate the development of technology environments in their own schools, incorporate case studies and workshop units into their teaching, and help catalyze instructional change at the local and regional levels. Workshops will be hosted by members of the Association of Independent Research Institutions (AIRI), of which Cold

Spring Harbor Laboratory is a member. Hosts for the summers of 2000 and 2001 will include the Foundation for Blood Research, Fred Hutchinson Cancer Research Institute, Oklahoma Medical Research Institute, Salk Institute, and Trudeau Institute.

Eugenics Archive Readied for Release

By year's end, we had substantially finished our 2-year project to develop an online *Digital Image Archive on the American Eugenics Movement*. Eugenics was an effort to apply Mendel's laws to breed "better" human beings. Eugenicists encouraged people with "good" genetic stock to reproduce and discouraged people with "bad" stock. They wrongly assumed that single genes explained complex behaviors and mental illnesses that we now know involve many genes. Eugenicists sought an exclusively genetic explanation of human development, neglecting the important contributions of the environment. The American movement began in about 1910 and reached its peak in the 1920s, when eugenics permeated many facets of public and private life. Flawed eugenics data were the basis for social legislation to separate racial and ethnic groups, restrict immigration from southern and eastern Europe, and sterilize people considered "genetically unfit." After enduring years of criticism, the American movement collapsed in 1940, as the horrid results of the Nazi eugenics program were revealed.

Digital Image Archive on the American Eugenics Movement, which will open to the public early in 2000, gives students, teachers, scholars, and the interested public an extraordinary window into a

The *Eugenics Archive* home page (*left*) includes links to "virtual exhibits" that provide context for the 1200 images in the *Archive*. The image window (*above*) provides basic information about the image, a link to a higher resolution "detail" image, access to source archives to use permissions, and a printer-formatted page.

"hidden" chapter of history. We hope that the opportunity to revisit this period will stimulate people to think critically about our current involvement in human genetics. The site contains more than 1200 images drawn from four major scholarly archives: the American Philosophical Society Library, the Rockefeller University Archive Center, the Truman State University Archives, and the Cold Spring Harbor Laboratory Research Archives. By providing access to the eugenicists' own words and "data," we hope to challenge visitors to assume the role of historian-researcher. By focusing primarily on visual documents, we hope to engage young people and others who would not normally access a scholarly collection.

The *Eugenics Archive* makes no attempt to lead users to a "correct" interpretation of the materials. However, the site assists users in understanding the historical, social, political, and ethical context in which the American eugenics movement developed, flourished, and finally collapsed. Context is built into the *Archive* on two levels. First, users are encouraged to enter the site through a series of 11 virtual exhibits that introduce the key events, people, and social conditions that contributed to the development of eugenics. Second, all images are sorted into more than 20 topic areas. Browsing by topic or searching by keyword returns a set of related images with extended captions. The topic captions are designed to help the user understand relationships among images and the relationship of the image to the eugenics movement and society. Both levels were developed in collaboration with several leading historians of eugenics. At each level, users are reminded that the vast majority of what is presented as scientific "fact" by eugenicists was fundamentally flawed and has been discredited by modern research standards. In November, the *Archive* was the subject of a feature article in the popular online magazine *Salon* (http://www.salon.com/tech/feature/1999/11/17/eugenics/index.html).

We believe that the *Eugenics Archive* can serve as a model for other online projects on the history and social interpretation of modern science. At the start of the project, none of the participating archives had policies governing the release of their materials over the Internet, and this project marks the first large-scale release of items via the Internet by a second party. We developed guidelines for online publication, educational "fair use" of documents, and privacy protections that can guide other projects dealing with the release of sensitive documents via the Internet. These policies were developed by consensus during 6 days of workshop sessions with our 15-member advisory panel:

Garland E. Allen, Washington University, St. Louis, Missouri
Elof Carlson, State University of New York, Stony Brook
Patricia Colbert-Cormier, Lafayette High School, Louisiana
Nancy L. Fisher, Regence Blue Cross, Seattle, Washington
Henry Friedlander, City University of New York
Daniel J. Kevles, California Institute of Technology, Pasadena
Philip Kitcher, University of California, San Diego
Martin L. Levitt, American Philosophical Society, Philadephia, Pennsylvania
Paul Lombardo, University of Virginia, Charlottesville
Nancy Press, Oregon Health Sciences University, Portland
Philip R. Reilly, Shriver Center for Mental Retardation, Inc., Waltham, Massachusetts
Pat Ryan, Carolina Biological Supply Company, Burlington, North Carolina
Marsha Saxton, World Institute on Disability, San Francisco, California
Steven Selden, University of Maryland, College Park
G. Terry Sharrer, National Museum of American History, Washington, D.C.

Instructional Program Continues to Grow

A record 10,150 students, 30% of them minorities, conducted experiments during field trips to the DNALC facility. Middle school labs—typically DNA extraction, microscope observation, or "green gene" engineering—take 1 hour. High-school labs—DNA restriction analysis, bacterial transformation, and human DNA polymorphisms—take between 2 1/2 and 3 1/2 hours. We also had a busy summer, instructing 444 students at 17 workshops at the DNALC and at sites in the New York metropolitan area. Five workshops for minority students, supported by the HHMI, were conducted at Central Islip High School (Suffolk County), Intermediate School #59 (Queens), Brooklyn Technical High School (Queens),

and J.F. Kennedy High School (Bronx). In addition, 400 students attended the 15th annual *Great Moments in DNA Science* honors seminars:

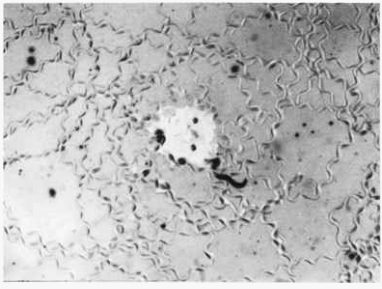
Sequence analysis of complex genomes, Dick McCombie, CSHL
Molecular studies on HIV Nef, an essential viral protein, Michael Greenburg, CSHL
Biology's gold rush: Mining genes from the Human Genome Project, John Kruper, DNALC

Programs funded by the DOE, NSF, and Gateway to Higher Education reached 227 teachers at sites around the nation. During the academic year, the DOE workshop, *The Science and Issues of Human DNA Polymorphisms*, was conducted at California Lutheran University (Thousand Oaks), Fred Hutchinson Cancer Research Center (Seattle, Washington), and Laney Community College (Oakland, California). The NSF workshop, *Genomic Biology*, was conducted at New Hampshire Technical Community College (Portsmouth), Madison Area Technical College (Wisconsin), University of Maryland School of Medicine (Baltimore), and University of Texas (Austin). *Gateway Summer Institutes* for middle and high school faculty were conducted at Stuyvesant High School (Manhattan).

The middle school program, *Genetics as a Model for Whole Learning (GMWL)*, continued to expand, reaching 10,500 students from 60 elementary and middle schools. Participants include 19 districts and five private schools in Nassau, Suffolk, and Queens Counties. Under the *GMWL* program, fifth and sixth grade students typically perform labs in school, such as constructing cell models, observing examples of cells under compound microscopes, constructing DNA models, and observing mutated fruit flies with stereomicroscopes. In addition, students perform DNA extractions and bacterial transformations during a field trip to the DNALC. A chemistry lab sequence for advanced students includes experiments on molecular modeling, enzyme structure and function, practical enzymology, and bioreactors. These efforts were aided by a complete redesign of our instructional materials to make them more children friendly.

Two labs usually reserved for high school students—genetically engineering bacteria to glow green and making a personal DNA fingerprint—were successfully piloted with advanced middle school children. In collaboration with Mike Hengartner's lab, we also piloted *Caenorhabditis elegans* as a model for observing mutations in living organisms.

The *GMWL* program received a significant endorsement when we signed a 3-year, $100,000 contract with the New York City Board of Education to provide genetics instruction for Community School District 29 in southeast Queens. Located near Kennedy Airport, School District 29 serves 25,000 students in grades K through 8—of whom 95% are minorities, 67% are eligible for lunch assistance, and 31% have limited English proficiency. The new contract is the culmination of a collaboration begun in 1996, which has involved a total of 5100 fifth through seventh grade students. In addition to teacher training, the *GMWL* also includes parent workshops and a recognition dinner to familiarize district administrators and school board members with the program. We hope that the District 29 agreement becomes a model for other large-scale projects in New York City.



Middle-school students looked at *Caenorhabditis elegans* mutations as part of a pilot program. The *C. elegans* worm is the dark squiggle in the center of the image, and the longer lines are *C. elegans* tracks. This image was provided by Daniel Hoeppner of Cold Spring Harbor Laboratory.

Genomic Laboratory Development

During the year, we continued to develop new educational experiments and improve existing ones. As in the past, our objective is to identify research methods that can be modified for use in educational settings. We strive to identify "icon" labs, which illustrate key techniques and integrate key biological concepts. Recently, we have concentrated on labs that have a strong bioinformatics component and allow students to share data via the Internet. Typically, we test and popularize new labs through grant-funded workshops, and then develop a ready-to-use kit to be distributed by the Carolina Biological Supply Company.

Through grants from the HHMI, NSF, and DOE, we developed educational polymerase chain reaction (PCR) kits that use a 20-minute sample prep and three-part chemistry based on freeze-dried core reagents. These highly reproducible experiments allow students to analyze two types of chromosomal DNA polymorphisms—an *Alu* insertion and a VNTR repeat. These DNA variations offer an excellent starting point for discussions of disease diagnosis, forensic biology, identity testing, and the ethical implications of this technology.

In parallel with human-based labs, we developed a new lab for introducing plant genomics. Here, we have focused on *Arabidopsis thaliana*, a member of the mustard family that is considered the simplest model system for flowering plants. The lab allows students to visually compare wild-type and mutant plants, and then to relate the mutant phenotype to a PCR genotype showing an insertion polymorphism. In this case, a dwarf, curly-leaf phenotype is due to the insertion of the *Ds* transposon at the *Clf-2* locus. This system aptly illustrates how the first transposon system, discovered in the 1950s by CSHL scientist Barbara McClintock, has now been transformed into a research reagent that provides a relatively straightforward method for cloning genes of interest. As a defective transposon, *Ds* is similar to *Alu*—these ubiquitous "junk" DNA sequences can also stimulate students to consider the possible role of transposons as evolutionary agents. In developing this system for education, we relied heavily on research collaborators at the main Laboratory campus. Jean-Philipe Vielle-Calzada provided us with an amazingly rapid and efficient DNA prep from leaf material that does not require organic extraction, Rob Martienssen provided us with *Clf-2* stocks, and farm manager Tim Mulligan provided greenhouse space and taught us how to culture *Arabidopsis*.

Especially exciting has been the success of our new *Sequencing Service*, which allows students and teachers to analyze differences in their own DNA sequences. First, a highly variable region of the mitochondrial genome, the control region, is amplified via PCR. The amplified samples are mailed to the DNALC, where a high school intern performs the final DNA sequencing reaction. The mitochondrial sequences are then sent to the CSHL Genome Sequencing Center, where they are loaded onto a gel for analysis. Finally, the sequence data are posted at the *Sequence Server* facility at our WWW site (http://vector.cshl.org/sequences/). An easy to use interface allows users to launch database searches, sequence alignments, and phylogenetic analyses from a centralized workspace. With these tools, students can use their own DNA data to explore human population genetics and test theories of human evolution. Using this protocol, we have processed and posted more than 1300 sequence samples. We also piloted a mitochondrial sequencing field trip, in which students amplified and electrophoretically visualized their mitochondrial DNA during a 3-hour visit to the DNALC. Several days after being processed, their sequence data were posted on the Internet. We hope to introduce this as a standard lab field trip in academic year 2000–2001.

While we will continue to focus effort on the human mitochondrial control region, we intend to develop other educational sequencing projects that allow students to collect, analyze, and share new data. A pilot project on mitochondrial typing of trout, initiated by several of our high school interns, embodies several features that may make it attractive to students and teachers in many parts of the country: (1) Trout and other members of the salmon family are widely distributed in freshwater and saltwater throughout the United States. (2) Commercial and sport fisheries are of considerable economic importance in most regions of the country. (3) Conservation of wild populations and hybridization between species are important biological problems. (4) Tissue samples are readily available from fishermen, conservation departments, and supermarkets. (5) Primer sequences are conserved in most, if not all, species.

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Corporate Advisory Board

During the past several years, we have benefited greatly from the help of our Corporate Advisory Board (CAB), a group of business leaders from large and small companies around Long Island. Each year, this group takes on the challenge of raising 15% of the DNALC's annual operating expenses from local business sources. As has become its fashion, the CAB again exceeded its goal in 1999, raising a total of $256,000. Most of this went to support the activities of the DNALC, but some also supported the *Partners for the Future* Program at the main Laboratory campus. The CAB contribution has been a major factor in achieving a balanced budget for the past 2 years, including full payment of depreciation.

The 1999 board was ably led by Jack Leahy, of Citibank, who has been Chairman of the Board for the past 4 years. Key to the year's success was the 6th annual golf tournament, chaired by Eddie Chernoff and cochaired by John Kean. The tournament, which was hosted by special guest Deborah Norville, drew approximately 200 players and netted $145,000. The year was topped off by an additional $111,000 from the winter Annual Fund Drive.

Operating behind the scenes—organizing meetings, making calls, and nudging board members to follow up on leads—was Julie Sutherland, Development Officer of Corporate and Foundation Relations. We owe her special thanks for a job done so well. Julie succeeded brilliantly, despite having a very tough act to follow when she assumed this liaison position from Laura Hundt 2 years ago. During Laura's tenure, the CAB had also exceeded its yearly goals.

Staff and Interns

During the past 2 years, the management of the DNALC has become increasingly decentralized, allowing for greater collaboration among staff members. Under this arrangement, we have created a tier of senior "coordinators," who share responsibility for key management functions. This is in line with the educational movement to site-based management, where teacher teams are more directly responsible for institutional policy and change.

The first element of this decentralization occurred in 1998, when the DNALC high school and middle school education groups were merged into a single group. Since then, this team of five people has been co-led by Educational Coordinators Patricia Maskiell, who is the primary liaison with school clients, and Scott Bronson, who is primarily responsible for laboratory development. The education group was strengthened in 1999 with the recruitment of lab instructor Danielle Sixsmith. Before she joined the DNALC, she made use of her degrees in biology and science education as a biology teacher at Martin Van Buren High School in Queens. In addition to her strong instructional background, Danielle has completed an internship at ImClone, a Manhattan-based biotechnology company.

The departure of *BioMedia* group leader John Kruper to the Internet startup company unext.com provided an opportunity to apply the same strategy of shared responsibility within the multimedia group. Thus, Shirley Chan was promoted to multimedia coordinator, and Matt Christensen was promoted to technology coordinator. Shirley is responsible for the content and day-to-day operation of the WWW sites. Her earlier work as science writer set the style and tone for *DNA from the Beginning*. Matt is responsible for the DNALC's computer backbone and functionality of the WWW sites. With expertise in Perl programming, he has developed many unique features at our WWW sites, including online editing interfaces that can be reached from any computer.

When part-time animator Gisella Walter left for a position in Manhattan's "silicon alley," we were fortunate to hire Chun-hua Yang as a full-time replacement. After completing undergraduate work in her native Taiwan, Chun-hua received two master's degrees—one in computer graphics from C.W. Post and one in design technology from Parsons School of Design. She brings a new world view and a fresh look to our WWW site. The resynthesized *Biomedia* group was rounded out with the recruitment of science writer Susan Conova. While doing doctoral research in marine zoology, Susan began writing about research topics for the newsletter of the Duke University Marine Laboratory. Just before she joined the DNALC, she completed an American Association for the Advancement of Science writing fellowship at ABCNEWS.com. The *Biomedia* group was further assisted by two summer interns, funded through a grant from the Josiah Macy, Jr., Foundation. Writing intern Ryan Luce recently completed his doctorate in chemistry at the University of Washington, and media design intern Maria Gililova, of Syracuse University, returned for a winter internship. Maria was primarily responsible for the fun caricatures of each staff member found in the "About" section of our WWW site.

The core administrative group, headed by Program Coordinator Judy Cumella-Korabik, was rounded out with the addition of secretary Nancy Daidola. Nancy obtained excellent administrative experience in several departments at Grumman Aerospace and most recently was a real estate agent with Coldwell Banker Sammis.

New employees in 1999:

From the left, Nancy Daidola, Secretary; Chun-hua Yang, Multimedia Designer; Danielle Sixsmith, Laboratory Instructor; and Susan Conova, Science Writer.

As the year ended, we received news that the Laboratory had recruited Vin Torti as a full-time development officer dedicated to the DNALC. In addition to managing the CAB, Vin will raise funds for the construction of the *Biomedia* addition, as well as the substantial operating increase it will engender. Vin joined the Laboratory at the beginning of the new year, after having spent 3 years as development officer for Xaverian High School in Brooklyn. We were thrilled to find that Vin's background includes 15 years of teaching, so he has a true appreciation for our work. Vin will report to Development Director Rick Cosnotti, with whom he shares a background in theology.

Several researchers from the main CSHL campus provided part-time instruction for high school lab field trips: Joan Alexander (Wigler lab), Thomas Volpe (Futcher lab), Michelle McDonough (Van Aelst lab), and James Tong (Zhong lab). We bid farewell to Joel Stern, who left for graduate studies at Columbia University, and to Vivek Mittal, who assumed responsibility for the microarray program in Michael Wigler's lab.

College students and interns from high schools in Nassau and Suffolk Counties provided key support for the instructional and multimedia staff. Jermel Watkins, of the New York Institute of Technology, extended his record service to 5 years by providing regular help during the academic year while Mera Goldman returned from Barnard College to help with summer workshops. Veteran high school interns Ken Mizuno (Cold Spring Harbor) and Yan Haung (Harborfields) assumed leadership roles as three of their colleagues left for their first year of college: Rebecca Yee (Huntington) to Wellesley, Karin Glaizer (Portledge) to Vassar, Hana Mizuno (Cold Spring Harbor) to the Massachusetts Institute of Technology, and Gerry DeGregoris (Chaminade) to Notre Dame. In the fall, we welcomed newcomers Daniel Goldberg (Half Hollow Hills East), Janice Lee (Oyster Bay), Adam Frange (Wantagh), Greg Bautista (Chaminade), Caroline Lau (Syosset), and Rebecca Shoer (Syosset). The *Biomedia* group was assisted by a brother-sister team from Syosset High School—Tracy Mak who came to work when brother Stephen began his freshman year at Cornell.

In addition to prepping for labs and editing multimedia files, many interns conduct independent research projects. In many cases, these projects contribute to the development of new student field trips, student and teacher workshops, and kits distributed by Carolina Biological Supply Company. Ken Mizuno was awarded high honors in the Long Island Science Congress for his work on mitochondrial DNA sequence analysis of Long Island brown trout (*Salmo trutta*), and Yan Huang performed a similar study of local rainbow trout (*Onchorhynchus mykiss*). This work laid the foundation for a collaboration with students from Curtis High School in Tacoma, Washington, who used our protocols and bioinformatics tools to study wild populations of coho and chinook salmon. Laura Roche (Cold Spring Harbor) was one of only six Long Island students selected for the Laboratory's *Partners for the Future* Program. Under this program, Laura joins Michael Hengartner's lab, which is investigating the developmental genetics of the nematode worm *C. elegans*.

1999 WORKSHOPS, MEETINGS, AND COLLABORATIONS

January 9–11	Department of Energy ELSI Workshop, *The Science and Issues of Human DNA Polymorphisms*, California Lutheran University, Thousand Oaks
January 12–16	Department of Energy Contractor-Grantee Meeting, Oakland, California
January 21–22	National Human Genome Research Institute ELSI Project, *Eugenics Image Archive*, collection visit to American Philosophical Society Library, Philadelphia, Pennsylvania
January 26	National Human Genome Research Institute ELSI Project, *Eugenics Image Archive*, and *DNAFTB* collection visit to Rockefeller University Archive Center, Tarrytown, New York
January 28–29	Site visits to DNA Lab Schools, New York, New York
March 8–10	National Human Genome Research Institute ELSI Project, *Eugenics Image Archive*, collection visit to the Harry Laughlin Archives, Truman State University, Kirksville, Missouri
March 26–28	Department of Energy ELSI Workshop, *The Science and Issues of Human DNA Polymorphisms*, Fred Hutchinson Cancer Research Center, Seattle, Washington
March 30	*DNA from the Beginning* interview, Frank Stahl, University of Oregon, Eugene
April 3	Laboratory for Rampart and Sierra High Schools, Colorado Springs, Colorado
April 5	Site visit by Marie-Luce Vignais, Institut de Génétique Moléculaire, Montpellier, France
April 13	*DNA from the Beginning* interview, Joshua Lederberg, Rockefeller University, New York, New York
April 15–17	National Human Genome Research Institute ELSI Project, *Eugenics Image Archive*, Editorial Advisory Panel Meeting, Banbury Center
April 20	Site visit by Donna DeSoto, Cablevision
April 26	*DNA from the Beginning* interview, Marshall Nirenberg, National Institutes of Health, Bethesda, Maryland
April 27	*Great Moments in DNA Science* Honors Students Seminar, CSHL
April 28	*DNA from the Beginning* interview, Paul Zamecnik, Harvard University, Boston, Massachusetts
May 4	*DNA from the Beginning* interview, Phillip Sharp, Massachusetts Institute of Technology, Boston *Great Moments in DNA Science* Honors Students Seminar, CSHL
May 5	*DNA from the Beginning* interview, Richard Roberts, New England Biolabs, Beverly, Massachusetts
May 6	*DNA from the Beginning* interview, Matthew Meselson, Harvard University, Boston, Massachusetts
May 7	*DNA from the Beginning* interview, Mahlon Hoagland, Thetford, Vermont
May 11	*Great Moments in DNA Science* Honors Students Seminar, CSHL
May 24–27	National Science Foundation ATE Project, *Genomic Biology*, Editorial Advisory Board Meeting, DNALC
June 1	Site visit to Long Island Children's Museum, Garden City, New York
June 2	Site visit by Victor Albert, New York Botanical Garden, Bronx
June 3	Site visit by June Osborn, Josiah Macy, Jr., Foundation, New York, New York
June 4	Site visit by Mark Hertle, Howard Hughes Medical Institute, Chevy Chase, Maryland
June 7	National Institutes of Health ELSI Review Panel, Bethesda, Maryland
June 9	National Science Foundation ATE Biotechnology Fellows Workshop, San Francisco, California
June 14–19	National Science Foundation Workshop, *Genomic Biology*, Madison Area Technical College, Wisconsin
June 17	Site visit by Seyed Hasnain, Centre for DNA Fingerprinting and Diagnosis, Hyderbad, India
June 21–26	National Science Foundation Workshop, *Genomic Biology*, University of Texas, Austin
June 28–July 2	*Fun With DNA* Workshop, DNALC *DNA Science* Workshop, DNALC
June 28–July 9	*Genomic Biology and PCR* Minority Workshop, Central Islip High School, New York
July 6–9	*World of Enzymes* Workshop, DNALC *Green Genes* Workshop, DNALC
July 12–16	*Fun With DNA* Workshop, DNALC *Fun With DNA* Minority Workshop, Intermediate School 59, Springfield Gardens, New York *DNA Science* Workshop, DNALC *DNA Science* Minority Workshop, John F. Kennedy High School, Bronx, New York
July 19–23	*Fun With DNA* Workshop, DNALC *Fun With DNA* Minority Workshop, Intermediate School 59, Springfield Gardens, New York

	DNA Science Minority Workshop, Brooklyn Technical High School, New York
July 26–30	*Fun With DNA* Workshop, DNALC
	Genomic Biology and PCR, DNALC
July 29	Site visit by Shozo Enokita, The Institute of Physical and Chemical Research, Saitama, Japan
August 2–6	*World of Enzymes* Workshop, DNALC
August 2–7	National Science Foundation Workshop, *Genomic Biology*, University of Maryland, School of Medicine, Baltimore
August 9–13	*Genetic Horizons* Workshop, DNALC
	DNA Science Workshop, DNALC
August 9–14	National Science Foundation Workshop, *Genomic Biology*, New Hampshire Community Technical College, Portsmouth
August 16–20	*Fun With DNA* Workshop, Section I and II, DNALC
August 23–27	*World of Enzymes* Workshop, DNALC
	DNA Science Workshop, DNALC
August 30–September 3	*Genomic Biology and PCR* Workshop, DNALC
October 1–3	National Human Genome Research Institute ELSI Project, *Eugenics Image Archive*, Editorial Advisory Panel Meeting, Banbury Center
October 7–8	Site visit to Carolina Biological Supply Company, Burlington, North Carolina
October 11–13	Howard Hughes Medical Institute Director's Meeting, Chevy Chase, Maryland
October 20	Tour of DNALC and luncheon for educators from Queens District 29
October 22–23	National Science Foundation ATE Principal Investigator Meeting, Alexandria, Virginia
October 25	Site visit by Fiona Cunningham, Murdoch Institute, Melbourne, Australia
October 28	Site visit and laboratory for participants in Federal Judicial Center workshop, *Basic Issues of Science*
October 28–31	National Association of Biology Teachers Annual Meeting, Fort Worth, Texas
November 4–7	*Science Education Website Developers* Meeting, Eccles Institute of Human Genetics, Salt Lake City, Utah
November 15	Site visit by Robert Vizza, The Dolan Foundation
December 8–5	Department of Energy Workshop, *The Science and Issues of Human DNA Polymorphisms*; Laney College, Oakland, California
December 11	Biology Education Workshop at the Annual American Society for Cell Biology Meeting, Washington, D.C.
December 15	Site visit and seminar by Dr. Carey Phillips, Bowdoin College, Brunswick, Maine
December 30	Site visit by John Watson, European Initiative for Biotechnology Education, Luxembourg

Sites of Major Faculty Workshops 1985–1999

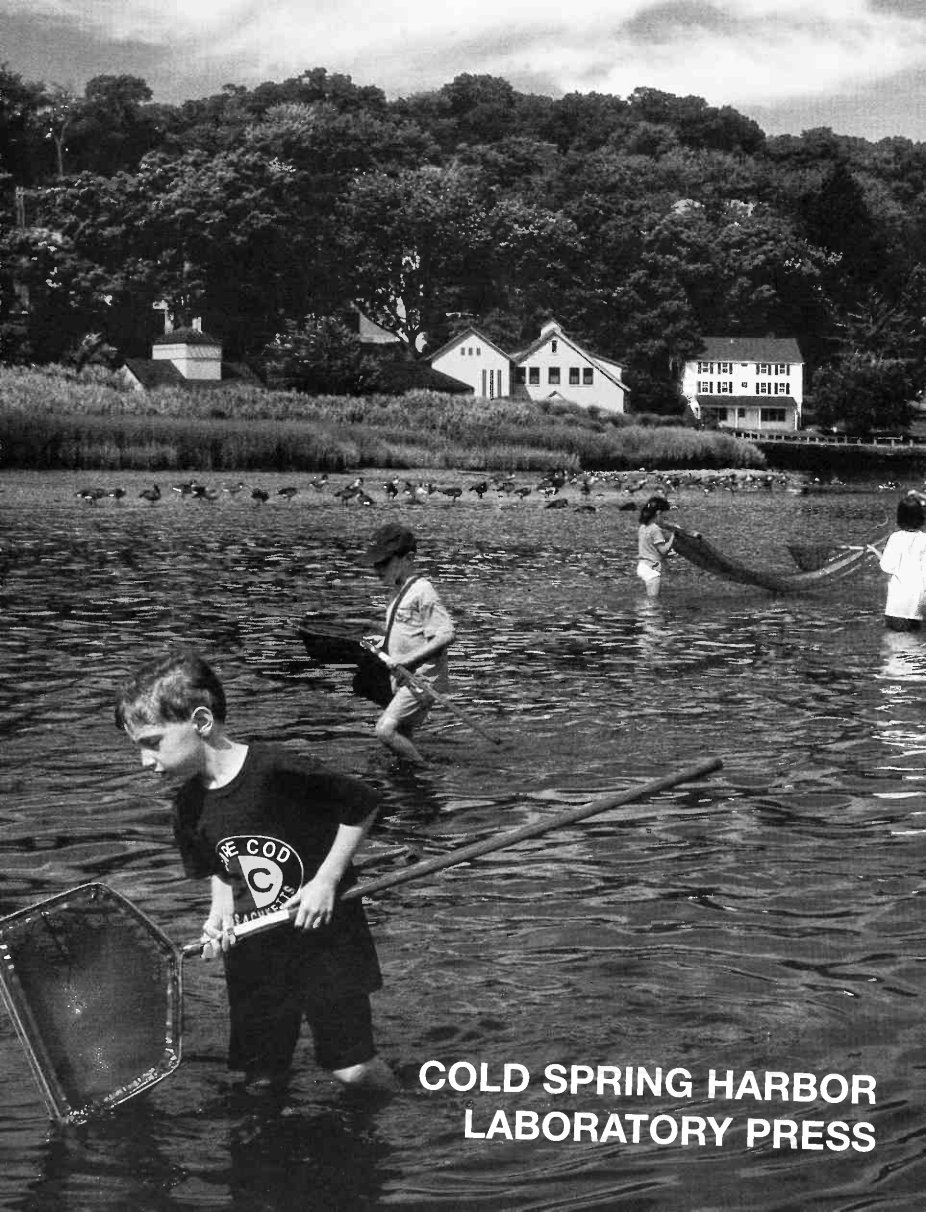
Key: High School **College** *Middle School*

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**
	Cañada College, Redwood City	1997
	Pierce College, Los Angeles	**1998**
	California Lutheran University, Thousand Oaks	1999
	Laney College, Oakland	1999
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**
INDIANA	Butler University, Indianapolis	1987
IDAHO	University of Idaho, Moscow	1994
IOWA	Drake University, Des Moines	1987
KANSAS	University of Kansas, Lawrence	1995
KENTUCKY	Murray State University	1988
	University of Kentucky, Lexington	1992
	Western Kentucky University, Bowling Green	1992
LOUISIANA	Jefferson Parish Public Schools, Harvey	1990
	John McDonogh High School, New Orleans	1993
MAINE	Bates College, Lewiston	1995
MARYLAND	Annapolis Senior High School	1989
	Frederick Cancer Research Center, Frederick	1995
	McDonogh School, Baltimore	1988
	Montgomery County Public Schools	1990–1992
	St. John's College, Annapolis	*1991*
	University of Maryland, School of Medicine, Baltimore	**1999**
MASSACHUSETTS	Beverly High School	1986
	CityLab, Boston University School of Medicine	1997
	Dover-Sherborn High School, Dover	1989
	Randolph High School	1988
	Winsor School, Boston	1987
	Boston University	**1994, 1996**
MICHIGAN	Athens High School, Troy	1989
MISSISSIPPI	Mississippi School for Math and Science, Columbus	1990, 1991
MISSOURI	Washington University, St. Louis	1989
	Washington University, St. Louis	**1997**
NEW HAMPSHIRE	St. Paul's School, Concord	1986, 1987
	New Hampshire Community Technical College, Portsmouth	**1999**
NEVADA	University of Nevada, Reno	1992
NEW YORK	Albany High School	1987
	Bronx High School of Science	1987
	Columbia University, New York	**1993**
	Cold Spring Harbor High School	1985, 1987
	DeWitt Middle School, Ithaca	*1991, 1993*
	DNA Learning Center	1988–1995
	DNA Learning Center	**1990, 1992, 1995**
	DNA Learning Center	*1990–1992*
	Fostertown School, Newburgh	*1991*
	Huntington High School	1986
	Irvington High School	1986
	Junior High School 263, Brooklyn	*1991*
	Lindenhurst Junior High School	*1991*
	Mt. Sinai School of Medicine, New York	1997
	Orchard Park Junior High School	*1991*

	Plainview-Old Bethpage Middle School	*1991*
	State University of New York, Purchase	1989
	State University of New York, Stony Brook	1987–1990
	Titusville Middle School, Poughkeepsie	*1991, 1993*
	Wheatley School, Old Westbury	1985
	U.S. Military Academy, West Point	**1996**
	Stuyvesant High School, New York	1998, 1999
NORTH CAROLINA	North Carolina School of Science, Durham	1987
OHIO	Case Western Reserve University, Cleveland	1990
	Cleveland Clinic	1987
	North Westerville High School	1990
OKLAHOMA	School of Science and Mathematics, Oklahoma City	1994
PENNSYLVANIA	Duquesne University, Pittsburgh	1988
	Germantown Academy	1988
SOUTH CAROLINA	Medical University of South Carolina, Charleston	1988
	University of South Carolina, Columbia	1988
TEXAS	J.J. Pearce High School, Richardson	1990
	Langham Creek High School, Houston	1991
	Taft High School, San Antonio	1991
	Trinity University, San Antonio	**1994**
	University of Texas, Austin	1999
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**
	Fred Hutchinson Cancer Research Center, Seattle	**1999**
WEST VIRGINIA	Bethany College	1989
WISCONSIN	Marquette University, Milwaukee	1986, 1987
	University of Wisconsin, Madison	1988, 1989
	Madison Area Technical College	**1999**
WYOMING	University of Wyoming, Laramie	1991

AUSTRALIA	Walter and Eliza Hall Institute and University of Melbourne	1996
CANADA	Red River Community College, Winnipeg, Manitoba	1989
ITALY	Porto Conte Research and Training Laboratories, Alghero	1993
	International Institute of Genetics and Biophysics, Naples	1996
PANAMA	**University of Panama, Panama City**	**1994**
PUERTO RICO	University of Puerto Rico, Mayaguez	1992
	University of Puerto Rico, Mayaguez	**1992**
	University of Puerto Rico, Rio Piedras	**1993**
	University of Puerto Rico, Rio Piedras	1994
RUSSIA	Shemyakin Institute of Bioorganic Chemistry, Moscow	1991
SWEDEN	Kristineberg Marine Research Station, Fiskebackskil	1995


COLD SPRING HARBOR
LABORATORY PRESS

1999 PUBLICATIONS

Laboratory Manuals

Imaging Neurons: A Laboratory Manual
Rafael Yuste, Fredrick Lanni, and Arthur Konnerth (eds.)

Early Development of Xenopus laevis: *A Laboratory Manual*
Hazel L. Sive, Robert M. Grainger, and Richard M. Harland

CSHL Monograph Series

Prion Biology and Diseases
Stanley B. Prusiner (ed.)

General Books

Mechanisms of Transcription
Cold Spring Harbor Symposia on Quantitative Biology LXIII

Essentials of Glycobiology
Ajit Varki, Richard Cummings, Jeffrey Esko, Hudson Freeze, Gerald Hart, and Jamey Marth

Illuminating Life: Selected Papers from Cold Spring Harbor (1903–1969)
Jan Witkowski

Transcriptional Regulation of Eukaryotes
Michael Carey and Stephen Smale

Cancer Surveys Series

Vol. 33: *Infections and Cancer*
Robert Newton, Valerie Beral, Robin A. Weiss

Journals

Genes & Development (Volume 13, 24 issues)
T. Grodzicker and D. Solter (eds.)

Genome Research (Volume 9, 12 issues)
A. Chakravarti, R. Gibbs, E. Green, R. Myers, M. Boguski, and L. Goodman (eds.)

Learning & Memory (Volume 6, 6 issues)
J.H. Byrne (ed.)

Other

CSHL Annual Report 1998

Banbury Center Annual Report 1998

Administration and Financial Annual Report 1998

Video

Manipulating the Early Embryo of Xenopus laevis: *A Video Guide*
Robert M. Grainger and Hazel Sive

COLD SPRING HARBOR LABORATORY PRESS

In 1999, the Laboratory`s publishing program completed a decade of operation under the banner of Cold Spring Harbor Laboratory Press. Its evolution from an administrative department of the Laboratory into a fully developed operating unit with its own balance sheet and day-to-day financial management has produced a substantial and gratifying growth in size, scope, ambition, and professionalism. And if judged by the quality of the program`s output, its financial performance, and the creation of opportunities for future growth, the past year was one of the most successful yet.

Books for Scientists

Eight new books and a videotape were published for use by scientists at postgraduate level. Laboratory manuals are the core of our program, and our growing list was enhanced by two titles with roots in courses taught at the Laboratory. The scope of *Imaging Neurons* goes far beyond what could be taught during students' two-week stay, encompassing a wide range of technologies that offer a look at living cells in action. The publication of the manual coincided with the completion of the Laboratory's new Marks Building, in which many of the approaches described in the book are being put to use. The second manual, *Early Development of* Xenopus laevis, and its companion videotape are the product of several years' evolution of an important course taught by the authors at the Laboratory. They are invaluable assets for the community of investigators for whom amphibian eggs are the most useful system for studying events in embryogenesis.

Two of the 1999 books provided a structured introduction to subjects with growing cross-disciplinary importance. Nature makes use of carbohydrates in many and varied ways, and glycoproteins have roles as diverse as receptors for pathogens, antifreeze agents for cells, and structural support for tissues. Studying these compounds is technically demanding but there is growing appreciation of their importance in cell biology, structural biology, molecular genetics, and even clinical medicine, as defects in carbohydrate function are linked with disease states. *Essentials of Glycobiology* is therefore a timely book, assembled by a committed group of editors intent on providing the scientific community with an excellent introduction to carbohydrate biology. The book's enthusiastic reviews suggest that they have succeeded. *Transcriptional Regulation in Eukaryotes* is also a crusading work, by experienced teachers of a course at the Laboratory who believe that the regulation of gene expression must be understood by anyone investigating a biological or medical problem in which DNA is implicated. Their book, a combined textbook and laboratory manual, has been very warmly received.

Special mention must also be made of a handsome book initially conceived by James Watson as a gift to all future incoming graduate students at the Laboratory. A collection of outstanding research papers published by scientists at Cold Spring Harbor from 1903 to 1969, *Illuminating Life* is an account of the Laboratory's scientific heritage, made attractive and accessible through a series of masterly commentaries on the papers and their authors by Jan Witkowski, director of the Banbury Center.

The celebrated series of Cold Spring Harbor Laboratory monographs was expanded by the addition of a new title, *Prion Biology and Diseases*. This book is the most comprehensive and authoritative yet published on these fascinating and mysterious infectious proteins and was made possible by the unrelenting energy of the Nobel-prize-winning discoverer of prions, Stanley Prusiner, who not only edited the book but wrote or co-wrote a substantial part of it.

Amid the noisy debate about the value of the Internet as an alternative distribution medium for books, the staff of our Editorial Development Department quietly laid the foundation for its use in a less glamorous but nevertheless fundamental task. FTP sites were used extensively for communication between authors and our far-flung network of talented developmental editors. *Transcriptional Regulation*

in Eukaryotes was the first title to be handled entirely in this manner, with no resort to expensive couriers for manuscript delivery. In addition, we incorporated the use of EndNote® reference management into the development of our books and created databases for information such as cautions and trademarks that recur in many different titles.

Books for Students

In March, Alex Gann joined us as senior editor for textbooks and quickly took the lead in identifying interesting publishing projects and likely authors. To this task, he brings postdoctoral research and writing experience with Laboratory Trustee Mark Ptashne, a stint as an editor at *Nature* magazine, teaching experience at the University of Lancaster, broad scientific interests, and a flair for language. The beginnings of this new and ambitious program are promising, and by year's end several books were under way. The use of the Meier House at the Banbury Center is an invaluable asset to this program, allowing authors of complex publishing projects to get together in comfort without distraction.

Books Online

Although high-quality books remain popular and economically viable, the use of the Internet as a publishing medium is very alluring and can only grow in importance as screen and printing technologies advance. A number of initiatives were taken this year that will bear fruit in 2000 and beyond. Among them was an agreement to partner with the National Center for Biotechnology Information in their Books@NCBI program. This is an experiment in the integration of book content with the invaluable PubMed database of journal bibliographic information. A user doing a keyword search in PubMed will be able to go beyond the journal abstracts to sections from selected books that provide background or reference information on the topic being explored.

The Cold Spring Harbor books to be included are the authoritative monograph *Retroviruses* and the new advanced textbook *Essentials of Glycobiology*. Neither will be available electronically in its entirety, but the usage data available from the NCBI Web site will tell us much about how readers might use such information if whole texts were to be posted online. And although these projects are very early stage, we are already reflecting on how this means of distribution would affect the editorial organization of scholarly books and the mechanics of bookmaking.

Journal Publishing

All three Cold Spring Harbor journals, *Genes & Development*, *Genome Research*, and *Learning & Memory*, had a satisfactory year. Subscription levels were either maintained or actually increased, no small achievement under current market conditions. Although keenly interested in the many experiments with online-only subscriptions being conducted by scientific societies, we elected to continue offering both the print and online editions in a combined subscription package to both individual and institutional subscribers. All three journals were offered substantially more manuscripts than ever before. Advertising sales in the two larger journals jumped by a remarkable 30%. The utility of the online editions continued to be enhanced by the software tools developed by our excellent electronic publishing partner HighWire Press at Stanford University. In the case of *Genome Research*, this included the ability to publish papers online much ahead of their appearance in print.

The impact factor of all three journals, measured by citation analysis, continued to rise. *Genes & Development's* score, 19.07, maintained the journal's rank among the top ten primary research journals in biology.

After 12 years' experience in the creation of successful, high-quality publications, journal publishing is decidedly a core competence of CSHL Press. In that time, we have declined a number of offers of journal-publishing partnerships. However, this year we competed for, and won, a contract to publish the journal *Protein Science* for its owner, the Protein Society. This journal is well established, has a large and productive constituency, and is well positioned for the deluge of new scientific information to come from the era variously called "postsequence biology" or "proteomics." It also has much unfulfilled potential for subscription and advertising sales and the application of electronic publishing techniques. The first issue of the journal published from Cold Spring Harbor will be dated January 2001, and by year's end, we were already working with the Society's editors and officers on the myriad details required to ensure a seamless transition from the journal's previous publisher.

Sales, Marketing, and Distribution

Promotional activities in 1999 encompassed direct mail, meeting exhibits, and advertising in print publications and online. We attended ten professional meetings, displaying our newest books as well as popular backlist titles. The Press's Web site was enhanced to include the covers of all new books as well as excerpts from reviews. Sales from the site continued to rise significantly.

A complete database of information on our titles was created to supply appropriately formatted data to online booksellers such as Amazon.com and Barnesandnoble.com, an increasingly important marketing channel.

A new, more aggressive sales program aimed at major resellers resulted in a substantial increase in orders. New agreements were signed with partners who could provide effective book promotion and distribution in Canada and in China, Taiwan, and Hong Kong. A long-standing agreement for distribution of our books in Japan was renewed. Agreement was reached for the creation of low-cost editions of several titles for exclusive sale in Korea, a first step toward the routine publication of affordable international editions for scientists in less wealthy countries. We have long had difficulty ensuring reliable and effective distribution of Cold Spring Harbor books through bookstores in Europe, and after an intense round of meetings at the Frankfurt Book Fair in October, we closed the year poised to launch an ambitious plan to address the European challenge.

Staff

The staff members of the Press (as of December 1999) are listed elsewhere in this volume. Their hard work, commitment, cordial dealings with the scientific community, and dedication to high standards were essential to the year's achievements. Many of the new initiatives in sales and marketing were the responsibility of Guy Keyes, who added the new role of sales manager to his duties as fulfillment and distribution manager. We also welcomed several new members of staff: Alex Gann as senior textbook editor; Emily Huang as assistant editor for *Genome Research* and *Learning & Memory*; Jennifer Bloch as financial assistant; Mala Mazzullo as production assistant; and Nora McInerny as development assistant. In October, Kaetrin Simpson joined us as developmental editor for the annual *Symposium* volume and *Annual Report*. After her outstanding research career had been cut short by illness, her abundant talent in this new role was quickly evident. Her death in February was a tragedy for her brave and dedicated family and robbed us of a much valued colleague.

After the past decade's growth, we now have a staff of 40 in five locations throughout the United States engaged in a publishing program of increasing size, diversity, and technical complexity. I am more grateful than ever for the leadership and drive for excellence that characterize the senior staff of our organization: Jan Argentine, editorial development manager; Ingrid Benirschke, marketing manager; Marcie Ebenstein, advertising manager; Nancy Hodson, business manager; Guy Keyes, sales and

fulfillment manager; Denise Weiss, production manager; and the editors of our journals, Terri Grodzicker at *Genes & Development* and Laurie Goodman at *Genome Research*. New media technologies are fascinating in their power to create and transform communities and change the way information is distributed and used. But effective publishing relies primarily on the efforts of talented people at every level and the success with which they work together. At Cold Spring Harbor, we are fortunate to have both an outstanding staff and access to remarkable authors and editors, to whose service in the next decade we will continue to bend the best of the new technologies.

John R. Inglis

FINANCE

FINANCIAL STATEMENTS

CONSOLIDATED STATEMENTS OF FINANCIAL POSITION
December 31, 1999 and 1998

Assets:	*1999*	*1998*
Cash and cash equivalents	$ 34,830,286	16,258,527
Investments	203,853,845	158,108,972
Accounts receivable:		
Publications	837,564	782,598
Other	333,310	172,405
Grants receivable	2,759,349	2,384,707
Contributions receivable	4,878,895	2,657,748
Publications inventory	2,138,874	1,923,784
Prepaid expenses and other assets	1,321,313	1,482,543
Investment in employee residences	2,751,580	2,464,260
Land, buildings, and equipment:		
Land and land improvements	12,614,303	12,557,726
Buildings	80,460,206	69,897,882
Furniture, fixtures, and equipment	5,427,259	5,037,823
Laboratory equipment	12,405,968	12,626,629
Library books and periodicals	365,630	365,630
Construction in progress	4,514,827	4,256,539
	115,788,193	104,742,229
Less accumulated depreciation and amortization	(34,481,784)	(32,455,453)
Land, buildings, and equipment, net	81,306,409	72,286,776
Total assets	$ 335,011,425	258,522,320
Liabilities and Net Assets:		
Liabilities:		
Accounts payable and accrued expenses	$ 4,840,651	2,809,788
Notes payable	251,918	276,163
Bonds payable	45,200,000	30,000,000
Deferred revenue	2,878,365	3,358,722
Total liabilities	53,170,934	36,444,673
Net assets:		
Unrestricted		
General operating	14,690,474	10,598,288
Designated by board for:		
Research programs	1,900,000	1,900,000
Capital expenditures	812,186	1,207,889
Endowment	110,214,894	71,296,987
Net investment in plant	33,760,513	42,010,613
Total unrestricted	161,378,067	127,013,777
Temporarily restricted	8,587,520	4,185,424
Permanently restricted	111,874,904	90,878,446
Total net assets	281,840,491	222,077,647
Total liabilities and net assets	$ 335,011,425	258,522,320

CONSOLIDATED STATEMENT OF ACTIVITIES
Year ended December 31, 1999
With comparative totals for the year ended December 31, 1998

	Unrestricted	Temporarily Restricted	Permanently Restricted	1999 Total	1998 Total
Revenue, gains, and other support:					
Public support (contributions and nongovernment grant awards)	$ 8,998,897	8,587,520	6,291,765	23,878,182	22,568,032
Government grant awards	17,658,439	–	–	17,658,439	15,929,141
Indirect cost allowances	11,377,795	–	–	11,377,795	11,230,797
Other revenue:					
Program fees	2,385,687	–	–	2,385,687	2,388,531
Rental income	358,934	–	–	358,934	333,196
Publications sales	6,400,303	–	–	6,400,303	6,341,142
Dining services	2,445,184	–	–	2,445,184	2,444,384
Rooms and apartments	1,865,047	–	–	1,865,047	1,877,794
Royalty and licensing fees	606,691	–	–	606,691	754,705
Gain on sale of investments (net)	3,192,266	–	3,327,199	6,519,465	10,426,775
Investment income (interest and dividends)	6,967,857	–	–	6,967,857	6,053,220
Miscellaneous	146,841	–	–	146,841	124,984
Total other revenue	24,368,810	–	3,327,199	27,696,009	30,744,731
Net assets released from restrictions	4,185,424	(4,185,424)	–	–	–
Total revenue, gains, and other support	66,589,365	4,402,096	9,618,964	80,610,425	80,472,701
Expenses:					
Research	31,454,215	–	–	31,454,215	28,718,081
Meetings and courses	9,153,276	–	–	9,153,276	7,834,447
Publications	6,159,371	–	–	6,159,371	6,219,895
Banbury Center conferences	1,111,921	–	–	1,111,921	877,834
DNA Learning Center programs	1,059,168	–	–	1,059,168	1,034,538
General and administrative	7,767,286	–	–	7,767,286	6,663,226
Dining services	3,117,043	–	–	3,117,043	2,850,714
Total expenses	59,822,280	–	–	59,822,280	54,198,735
Increase (decrease) in net assets	6,767,085	4,402,096	9,618,964	20,788,145	26,273,966
Other changes in net assets:					
Net unrealized gain on fair value of investments	28,195,266	–	11,377,494	39,572,760	1,517,302
Extraordinary item–loss on refinancing of debt	(598,061)	–	–	(598,061)	–
Change in net assets	34,364,290	4,402,096	20,996,458	59,762,844	27,791,268
Net assets at beginning of year	127,013,777	4,185,424	90,878,446	222,077,647	194,286,379
Net assets at end of year	$ 161,378,067	8,587,520	111,874,904	281,840,491	222,077,647

CONSOLIDATED STATEMENTS OF CASH FLOWS
Years ended December 31, 1999 and 1998

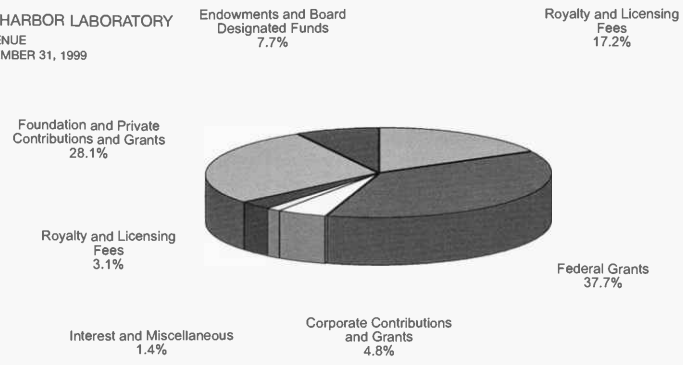
	1999	1998
Cash flows from operating activities:		
Increase in net assets	$ 59,762,844	27,791,268
Adjustments to reconcile increase in net assets to net cash provided by operating activities:		
Depreciation and amortization	3,526,229	3,443,290
Write off deferred bond costs	598,061	–
Net appreciation in fair value of investments	(46,092,225)	(11,944,077)
Contributions restricted for long-term investment	(10,043,571)	(10,762,186)
Changes in assets and liabilities:		
(Increase) decrease in accounts receivable	(215,871)	32,885
(Increase) decrease in grants receivable	(374,642)	654,186
Increase in contributions receivable	(2,221,147)	(950,143)
(Increase) decrease in publications inventory	(215,090)	67,923
Increase in prepaid expenses and other assets	(436,831)	(105,279)
Increase in accounts payable and accrued expenses	2,030,863	210,027
Decrease in deferred revenue	(480,357)	(2,195,315)
Net cash provided by operating activities	5,838,263	6,242,579
Cash flows from investing activities:		
Capital expenditures	(12,545,862)	(11,132,700)
Proceeds from sales and maturities of investments	70,618,250	25,341,828
Purchases of investments	(70,270,898)	(30,933,170)
Net change in investments in employee residences	(287,320)	(194,915)
Net cash used in investing activities	(12,485,830)	(16,918,957)
Cash flows from financing activities:		
Permanently restricted contributions	6,291,765	9,342,949
Contributions restricted for investment in land, buildings, and equipment	3,751,806	1,419,237
Repayment of bonds payable	(27,000,000)	–
Issuance of bonds payable	42,200,000	–
Repayment of notes payable	(24,245)	(369,676)
Net cash provided by financing activities	25,219,326	10,392,510
Net (decrease) increase in cash and cash equivalents	18,571,759	(283,868)
Cash and cash equivalents at beginning of year	16,258,527	16,542,395
Cash and cash equivalents at end of year	$ 34,830,286	16,258,527
Supplemental disclosures:		
Interest paid	$ 1,494,414	1,254,036

COMPARATIVE OPERATING HISTORY 1995–1999
(Dollars in Thousands)

	1995	1996	1997	1998	1999
Revenue:					
Main Lab:					
Grants and contracts	$ 19,653	20,879	22,743	24,025	27,397
Indirect cost reimbursement	8,881	9,704	9,910	11,054	11,207
Other	7,461	7,859	8,472	9,441	9,426
CSHL Press	5,119	4,805	5,238	6,341	6,400
Banbury Center	1,732	1,214	1,495	1,444	1,848
DNA Learning Center	954	754	875	1,334	1,392
Total income	43,800	45,215	48,733	53,639	57,670
Expenses:					
Main Lab:					
Research and training	19,653	20,879	22,743	24,025	27,397
Operation and maintenance of plant	5,266	5,446	5,274	5,549	5,765
General and administrative	3,329	3,438	3,625	3,378	3,844
Other	4,959	5,367	5,759	7,328	7,863
CSHL Press	5,079	5,032	5,320	6,141	6,077
Banbury Center	1,643	1,225	1,437	1,321	1,614
DNA Learning Center	958	781	887	1,228	1,280
Total expenses, excluding depreciation	40,887	42,168	45,045	48,970	53,840
Excess before depreciation, amortization, and release of designated funds	2,913	3,047	3,688	4,669	3,830
Depreciation and amortization	(2,821)	(2,988)	(3,371)	(3,443)	(3,526)
Release (designation) of funds (1)	–	–	–	(750)	–
Net operating excess	$ 92	59	317	476	304

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 imaging, computational neuroscience, and other research programs.

COLD SPRING HARBOR LABORATORY
SOURCES OF REVENUE
YEAR ENDED DECEMBER 31, 1999



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 1999.

GRANTS January 1, 1999–December 31, 1999

COLD SPRING HARBOR LABORATORY

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*1999 Funding•*
FEDERAL GRANTS			
NATIONAL INSTITUTES OF HEALTH			
Program Projects	Dr. Herr	1/92–12/01	$ 4,115,617
	Dr. Stillman	8/90–7/00	2,867,555
Research Support	Dr. Cline	12/95–11/04	459,969
	Dr. Cline	3/98–2/01	274,338
	Dr. Enikolopov	8/99–5/04	315,957*
	Dr. Enikolopov	9/94–11/03	346,363
	Dr. Futcher	4/93–3/01	275,448
	Dr. Futcher	1/91–6/00	57,273
	Dr. Hannon	8/95–5/00	286,286 *
	Dr. Helfman	9/99–8/04	316,950 *
	Dr. Hengartner	5/95–4/00	252,086
	Dr. Herr	8/96–7/00	219,725
	Dr. Hirano	5/96–4/00	223,376
	Dr. Joshua-Tor	8/96–7/00	239,659
	Dr. Krainer	7/89–6/02	425,968
	Dr. Lowe	7/99–6/04	308,741 *
	Dr. Malinow	5/92–4/00	260,426
	Dr. Malinow	4/95–2/03	368,051
	Dr. McCombie	1/99–12/03	432,555 *
	Dr. McCombie	9/99–9/02	1,306,667 *
	Dr. Neuwald	10/98–8/01	334,876
	Dr. Skowronski	4/98–3/03	459,736
	Dr. Stillman	7/91–5/00	535,861
	Dr. Svoboda	12/98–11/03	68,943
	Dr. Tonks	8/91–3/01	515,571
	Dr. Tonks	5/97–4/01	290,564
	Dr. Tully	4/94–3/02	342,191
	Dr. Tully	8/96–6/00	299,938
	Dr. Van Aelst	12/97–11/01	370,787
	Dr. Wigler	9/95–8/00	256,486
	Dr. Wigler	7/98–4/02	887,709
	Dr. Wigler	5/99–4/00	168,000 *
	Dr. Xu	1/98–12/02	294,209
	Dr. Yin	9/96–4/02	345,586
	Dr. M. Zhang	9/97–8/00	364,376
	Dr. Zhong	2/96–1/00	260,402

*New Grants Awarded in 1999
•Includes Direct & Indirect Cost

Grantor	Program/Principal Investigator	Duration of Grant	1999 Funding*
Fellowships	Dr. Groover	5/99–4/01	31,720 *
	Dr. Haas	7/98–6/01	36,700
	Dr. Horiuchi	10/98–9/00	36,700 *
	Dr. Nimchinsky	9/98–8/01	38,368
	Dr. Ruthazer	3/99–2/02	36,700 *
Training Support	Training in Cancer Cell Biology and Tumor Virology	7/94–12/03	279,686
Course Support	Cancer Research Center Workshops	4/92–3/00	276,440
	Neurobiology Short-term Training	5/82–4/01	149,803
	Analysis of Large DNA Molecules	4/91–3/01	56,288
	Computational Genomics	9/91–8/01	42,461
	In Situ Hybridization and Immunocytochemistry	7/98–6/03	67,757
	Automated Genome Sequencing	4/95–3/01	80,299
	Molecular Biology and Development of *Xenopus laevis*	4/96–3/00	10,000
	C. elegans	8/98–6/01	36,1010
Meeting Support	Genome Mapping and Sequencing	4/90–3/02	34,900 *
	Telomeres and Telomerase	3/99–4/00	19,320 *
	Learning and Memory	3/99–2/00	15,525 *
	Tyrosine Phosphorylation and Cell Signaling	4/99–4/00	8,000 *
	Biology of Proteolysis	4/99–4/00	11,000 *
	Neurobiology of *Drosophila*	6/99–5/00	13,000 *
	64th Symposium: Signaling and Gene Expression in the Immune System	6/98 5/01	10,000
	Microbial Pathogenesis and Host Defense	6/99–5/00	2,000 *
	Mechanisms of Eukaryotic Transcription	7/99–6/00	7,000 *
	Eukaryotic mRNA Processing	7/99–7/00	6,000 *
	Programmed Cell Death	8/99–7/00	10,000 *
	Eukaryotic DNA Replication	8/99–8/00	11,000 *
	Making/Using DNA Microarrays	9/99–9/01	49,034 *
NATIONAL SCIENCE FOUNDATION			
Cooperative Agreement	Dr. McCombie	2/99–1/02	2,831,574 *
Research Support	Dr. Cline	5/99–8/03	120,000 *
	Dr. Grossniklaus	8/97–7/00	120,000
	Dr. Helfman	2/99–1/03	105,426 *
	Dr. Jackson	1/98–12/00	100,000
	Dr. Martienssen	11/98–10/99	545.691
	Drs. Nestler/Wigler	4/98–3/00	100,000
Training Support	Undergraduate Research Program	6/91–5/00	50,000
Course Support	Advanced Bacterial Genetics	5/99–4/02	62,879 *
	Arabidopsis Molecular Genetics	6/94–5/00	60,000
	Early Development of *Xenopus laevis*	9/98–8/02	19,311 *
Meeting Support	Biology of Proteolsis	4/99–3/00	6,000 *
	Tyrosine Phosphorylation and Cell Signaling	4/99–3/00	8,000 *
	Learning and Memory	4/99–3/00	9,488 *
	Mechanisms of Eukaryotic mRNA Processing	7/99–6/00	5,000 *

*New Grants Awarded in 1999
*Includes Direct & Indirect Cost

Grantor	Program/Principal Investigator	Duration of Grant	1999 Funding*
	Mechanisms of Eukaryotic Transcription	7/99–6/00	5,000 *
	Eukaryotic DNA Replication	8/99–7/00	6,000 *
	Programmed Cell Death	9/99–8/01	4,000 *
	Neurobiology of *Drosophila*	10/99–9/00	15,010 *
Equipment	A Scanning Electron Microscope for Studies in Developmental Biology	3/99–2/01	129,654 *
DEPARTMENT OF ENERGY			
Research Support	Dr. Martienssen	8/91–2/01	100,000
UNITED STATES DEPARTMENT OF AGRICULTURE			
Research Support	Drs. McCombie/Martienssen	9/97–8/00	124,042
	Dr. Martienssen	9/97–8/00	67,097
	Drs. McCombie/Martienssen	7/98–8/01	90,444
	Dr. Jackson	9/99–8/01	105,000 *
	Dr. Grossniklaus	9/98–8/00	60,000
UNITED STATES DEPARTMENT OF THE ARMY			
Research Support	Dr. Futcher	7/97–6/00	102,628
	Dr. Hannon	9/96–8/00	149,998
	Dr. Helfman	7/99–6/02	117,600 *
	Dr. Hengartner	7/97–6/00	103,120
	Dr. Van Aelst	7/99–6/02	117,600 *
	Dr. Zhong	9/99–8/02	238,901 *
Fellowship Support	Dr. Donovan	7/97–6/00	41,053
	J. Polyakova	7/97–6/00	20,000
	Dr. Samuelson	6/98–5/01	41,053
	Dr. De Stanchina	5/99–6/02	40,920 *
	Dr. Simpson	7/99–6/02	42,000 *
Meeting Support	Microbial Pathogenesis and Host Response	3/99–2/00	10,000 *
MISCELLANEOUS GRANTS			
Research Support			
Alzheimer's Association	Dr. Barria	8/99–7/00	40,000 *
	Dr. Esteban	8/99–7/00	40,000 *
	Dr. Malinow	8/99–7/00	60,000 *
American Cancer Society	Dr. Wigler, Professorship	1986–2012	50,000
	Dr. Wigler, Supply Allowance	1999	10,000
	Dr. Lowe	7/00–6/02	150,000 *
	Dr. Joshua-Tor	7/00–6/02	120,000 *
R. Badgeley Trust	Dr. Hengartner	6/99–5/00	25,000 *
Calbiochem-Novabiochem Corporation	Dr. Krainer	2/96–1/00	60,000
Council for Tobacco Research	Dr. Futcher	1/97–12/99	70,3040
	Dr. Skowronski	7/97–6/00	83,000
Devgen N.V.	Dr. Hengartner	4/98–3/01	200,000

*New Grants Awarded in 1999
†Includes Direct & Indirect Cost

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*1999 Funding†*
Fairchild Martindale Foundation	Advanced Neuroscience Imaging Center Equipment	1999–2000	50,000 *
The Fraxa Foundation	Dr. Svoboda	6/99–6/00	30,000 *
The Lillian Goldman Charitable Trust through The Breast Cancer Research Foundation	Dr. Wigler	1999	200,000
Lita Annenberg Hazen Foundation	Neurobiology Support	12/98–11/00	200,000
The John A. Hartford Foundation, Inc.	Dr. Tully	1/97–12/99	317,706
Human Frontier Science Program	Dr. Hirano	7/98–6/01	55,000
	Dr. Svoboda	9/98–8/01	68,690
	Dr. Tully	8/99–8/00	46,670 *
The Hyde and Watson Foundation	Advanced Neuroscience Imaging Center Equipment	1999–2000	10,000 *
L.I.F.E.	Dr. Wigler	10/98–9/99	48,822 *
Manhassett Women's Coalition Against Breast Cancer	Dr. Wigler	1999	25,000 *
March of Dimes	Dr. Enikolopov	6/98–5/00	66,048
	Dr. Hengartner	6/99–5/00	54,402 *
Edwin and Nancy Marks	Advanced Neuroscience Imaging Center Program and Equipment Costs	1999–2000	1,000,000 *
G. Harold & Leila Y. Mathers Charitable Foundation	Dr. Svoboda	3/99–2/02	290,138 *
Maxfield Foundation	Dr. Lazabnik	1/98–12/99	10,000
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
Merck Genome Research Institute	Dr. M. Zhang	11/97–10/99	90,000
Monsanto, Inc.–Plant Consortium Program	Dr. Martienssen	1/98–12/02	135,000
Neurofibromatosis Foundation	Dr. Van Aelst	1999	40,000 *
	Dr. Zhong	1999	50,000 *
NIH/Sloan Kettering Consortium Agreement	Dr. Wigler	9/95–8/00	589,819
NIH/Sloan Kettering Consortium Agreement	Drs. Tonks/Van Aelst	8/97–5/01	457,484
NIH/Nanoprobes, Inc. Consortium Agreement	Dr. Spector	9/96–8/00	31,100
	Dr. Spector	9/99–2/00	12,180 *
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 *
	Dr. Stein	3/99–2/00	35,576 *
	Dr. Stein	9/99–8/00	134,118 *
N.A.T.O.	Dr. Hannon	1/98–4/99	5,080
Novartis–Plant Consortium Program	Dr. Martienssen	1/98–12/02	135,000
N.S.F./Clemson	Dr. McCombie	10/99–9/02	365,500 *
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	1999	25,000
Seraph Foundation	Dr. Enikolopov	12/98–11/99	27,000 *
	Dr. Lazebnik	12/98–11/99	23,000 *

*New Grants Awarded in 1999
†Includes Direct & Indirect Cost

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*1999 Funding**
Schwarz Foundation	Dr. Svoboda	2/99–1/00	33,440 *
SNP Consortium	Dr. McCombie	11/99–11/00	89,057 *
	Dr. Stein	4/99–9/01	1,176,916 *
Lauri Strauss Leukemia Foundation, Felix Schnyder Memorial Fund	Dr. Tonks	1999	15,000*
St. Giles Foundation	Dr. Wigler	4/98–3/00	257,400
Tularik, Inc.	Dr. Wigler	10/97–10/03	660,000
U.S.D.A./Clemson	Dr. McCombie	10/99–9/02	365,500 *
Westvaco–Plant Consortium Program	Dr. Martienssen	1/98–12/02	135,000
The Whitaker Foundation	Dr. Svoboda	6/98–7/01	52,940
Zeneca, Ltd–Plant Consortium	Dr, Martienssen	7/99–12/02	135,000 *
Fellowships			
Rita Allen Foundation	Dr. Hengartner	9/94–8/99	30,000
	Dr. Lowe	9/99–8/00	50,000 *
American Cancer Society	Dr. Drier	7/99–6/02	30,000 *
Burroughs Wellcome Fund	Dr. Mainen	9/97–8/00	121,000
Cancer Research Fund of the Damon Runyan–Walter Winchell Foundation	Dr. Z. Zhang	6/99/5/02	37,000 *
CSHL Association		1999	193,280 *
Joyce Green Foundation	Dr. Zhong	1/99–12/99	10,000 *
Joseph G. Goldring Foundation	Dr. Stillman	7/99–6/00	60,000 *
Helen Hoffritz Foundation	Dr. Cline	12/97–12/99	30,000
The Ellison Medical Foundation	Dr. Grewal	9/99–8/00	50,000 *
Sidney Kimmel Foundation	Dr. Tansey	7/99–6/01	100,000 *
Esther A. and Joseph Klingenstein Fund, Inc.	Dr. Svoboda	7/98–6/01	40,000
Charles Henry Leach II Foundation	Dr. Enikolopov	1/97–12/99	25,000
Leukemia Society of America Inc.	Dr. Julien	7/99–6/02	33,250 *
	Dr. Kimura	7/97–6/00	36,700
	Dr. Pendergrast	7/98–6/01	39,700
	Dr. Soengas	7/99–6/02	39,700 *
	Dr. Weinreich	7/97–6/00	36,700
NARSAD	Dr. Esteban	7/99–6/00	30,000 *
Life Science Research Foundation	Dr. Vollbrecht	6/99–5/02	40,000 *
Pew Charitable Trust	Dr. Hannon	7/97–6/01	60,000
	Dr. Hirano	7/96–6/00	60,000
	Dr. Svoboda	7/98–6/02	60,000
Searle Scholars Program	Dr. Grossniklaus	7/98–6/01	60,000
Andrew Seligson Memorial Fellowship	Dr. Herbst	1999	35,000
	Dr. MacCullum	1999	35,000
Tularik, Inc.	Fellowships	1/98–12/03	150,000
The V Foundation	Dr. Myers	1999	50,000
Helen Hay Whitney Foundation	Dr. Sabatini	4/99–3/02	32,000 *
	Dr. Sun	1/97–12/99	36,000
Course Support			
Burroughs Wellcome Fund	Genome Informatics	1999	25,000*
Chroma Technology Corporation	Advanced Molecular Cytogenics	1998–1999	2,500
Grass Foundation	Scholarships	1999	15,000

*New Grants Awarded in 1999
·Includes Direct & Indirect Cost

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*1999 Funding**
Howard Hughes Medical Institute	Advanced Neurobiology Courses	1991-1999	275,000
Institute for Biological Recognition & Catalysis, Inc.	Crystallography Workshop	1999	1,000 *
Esther A. and Joseph Klingenstein Fund, Inc.	Advanced Neurobiology Courses	3/98–2/01	60,000
Merck Genome Research Institute	Genome Informatics	1999	20,000 *
Meeting Support			
Alexis Biochemical Corp.	Corporate Contribution Program	1999	1,500 *
Asi/Applied Spectral Imaging	Advanced Molecular Cytogenics	1999	7,000 *
AstraZeneca	Pathogenesis	1999	2,000 *
Biogen, Inc.	Corporate Contribution Program	1999	6,000 *
Bruker Axs, Inc.	Crystallography Workshop	1999	1,000 *
Burroughs Wellcome Fund	Pathogenesis	1999	5,000 *
Chiron Corporation	Molecular Approaches to Vaccine Design	1999	10,000 *
Chroma Technology Corp.	Advanced Molecular Cytogenics	1999	2,500 *
CuraGen Corporation	Corporate Contribution Program	1999	6,000 *
Genome Systems, Inc.	Corporate Contribution Program	1999	6,000 *
Hoffmann-LaRoche, Inc.	Crystallography Workshop	1999	500 *
Intrabiotics Pharmaceuticals	Pathogenesis	1999	1,500 *
NCI-Frederick Cancer Research and Development Center	Advanced Molecular Cytogenics	1999	25,000 *
Nonius	Crystallography Workshop	1999	300 *
Pfizer	Crystallography Workshop	1999	750 *
Pharmacia and Upjohn Company	Pathogenesis	1999	2,000 *
PharMingen	Corporate Contribution Program	1999	6,000 *
Protein Solutions, Inc.	Crystallography Workshop	1999	500 *
Qiagen GMBH	Corporate Contribution Program	1999	3,000 *
Alfred P. Sloan Foundation	Computational Biology Workshop	1999	9,650 *

*New Grants Awarded in 1999
*Includes Direct & Indirect Cost

BANBURY CENTER

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*1999 Funding**
FEDERAL SUPPORT			
The Federal Judicial Center, Judiciary Leadership Development Council	The Basic Issues of Science for Federal and State Judges	1999	$ 18,325 *
NIH–NCI-Frederick Cancer Research and Development Center	Functional Genomics: Technology Development and Research Applications	1999	20,000 *
NIH–National Human Genome Research Institute	Digital Image Archive on the American Eugenic Movement Editorial Advisory Panel Workshops	1999	17,025
U.S. Army Medical Research (through a grant to Children's Hospital)	Natural History of Plexiform Neurofibromas in NF-1	1999	13,567 *
NONFEDERAL SUPPORT			
Meeting Support			
ALS Association	Strategies for Inactivation of Mutant Genes	1999	13,919 *
A-T Children's Project	The Molecular Neurobiology of ATM	1999	30,624 *
The Charles A. Dana Foundation	Molecular Neurobiological Mechanisms in Schizophrenia: Seeking a Synthesis	1999	45,000 *
Ehlers-Danlos National Foundation	The Clinical and Biological Basis of the Ehlers-Danlos Syndrome	1999	25,009 *
The William Stamps Farish Fund	Xenotransplantation: A Scientific Basis for Risk Assessment	1999	21,950
Glaxo Wellcome Inc.	Host Pathogens Interactions	1999	7,200 *
The John A. Hartford Foundation, Inc.	Old Memories	1999	31,639
The Hastings Center	Xenotransplantation: A Scientific Basis for Risk Assessment	1999	9,249 *
Hereditary Disease Foundation	Strategies for Inactivation of Mutant Genes	1999	13,919 *
The Esther A. and Joseph Klingenstein Fund, Inc.	Klingenstein Neuroscience Fellows Conference	1999	44,187 *
Merck & Co., Inc.	Functional Genomics: Technology Development and Research Applications	1999	10,778 *
Merck KGaA	Molecular Neurobiological Mechanisms in Schizophrenia: Seeking a Synthesis	1999	5,000 *
The National Neurofibromatosis Foundation, Inc.	Natural History of Plexiform Neurofibromas in NF-1	1999	11,406 *
Osiris Therapeutics, Inc.	What are Stem Cells? From Embryo to Adult Tissues	1999	23,032 *
Albert B. Sabin Vaccine Institute, Inc.	Vaccines for Developing Economies: Who Will Pay?	1999	30,983 *
SyStemix, Inc.	What are Stem Cells? From Embryo to Adult Tissues	1999	15,000 *
The Swartz Fund for Computational Neuroscience	The Functional Organization of Thalamus and Cortex and Their Interactions	1999	44,187 *

*New Grants Awarded in 1999
*Includes Direct & Indirect Cost

DNA LEARNING CENTER

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*1999 Funding**
FEDERAL GRANTS			
NATIONAL INSTITUTES OF HEALTH ELSI Research Program	Creation of a *Digital Image Archive on the American Eugenics Movement*	3/98–2/00	$181,948
NATIONAL SCIENCE FOUNDATION	*A Partnership to Develop Advanced Technology Units on Genomic Biology*	8/97–7/00	178,915
DEPARTMENT OF ENERGY	*The Science and Issues of Human DNA Polymorphisms: An ELSI Training Program for High School Biology Teachers*	1/97–1/00	85,732
NONFEDERAL GRANTS			
Hearst Foundation	*Genetics as a Model for Whole Learning*	7/98–6/99	27,766
Howard Hughes Medical Institute	Precollege Science Education Initiative for Biomedical Research Institutions	7/94–8/99	82,730
Josiah Macy, Jr. Foundation	*DNA from the Beginning*	10/97–9/00	264,775

The following schools each awarded a grant for *Genetics as a Model for Whole Learning* Program:

East Meadow Union Free School District	$4,025	Lawrence Union Free School District	$ 6,650
East Woods School	730	Locust Valley Central School District	13,650
Elwood Union Free School District	2,850	Northport-East Northport Union Free School District	1,650
Garden City Public School	6,535	NYC Community School District 17	365
Great Neck Union Free School District	4,860	Plainedge Union Free School District	1,075
Green Vale School	3,550	Port Jefferson Union Free School District	250
Harborfields Central School District	8,800	Port Washington Union Free School District	3,600
Half Hollow Hills Central School District	2,875	Old Westbury School of the Holy Child	1,775
Intermediate School 59	350	South Huntington Union Free School District	16,970
Jericho Union Free School District	6,950	St. Dominic Elementary School	3,175
LaSalle Regional	125	Syosset Central School District	23,625

The following schools each awarded a grant for the *Curriculum Study* Program:

Commack Union Free School District	$1,100	Locust Valley Central School District	$1,100
East Meadow Union Free School District	1,100	Long Beach City School District	1,100
East Woods School	1,500	North Shore Central School District	1,100
Friends Academy	1,500	Oceanside Union Free School District	1,500
Garden City Union Free School District	1,100	Plainedge Union Free School District	1,100
Great Neck Union Free School District	1,100	Plainview-Old Bethpage Central School District	1,100
Half Hollow Hills Central School District	2,200	Portledge School	1,100
Harborfields Central School District	2,100	Port Washington Union Free School District	1,100
Herricks Union Free School District	1,100	Ramaz School	1,100
Island Trees Union Free School District	1,100	Roslyn Union Free School District	1,100
Jericho Union Free School District	1,100	Syosset Central School District	1,100
Lawrence Union Free School District	2,200	West Hempstead Union Free School District	1,100
Levittown Union Free School District	1,100		

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 (35.8%) of our annual revenues are derived from Federal grants and contracts, and thus, we rely heavily on support from the private sector: foundations, corporations, and individuals. Contributions from the private sector are tax exempt under the provisions of Section 501(c)(3) of the Internal Revenue Code. In addition, the Laboratory is designated a "public charity" and, therefore, is enabled to receive funds resulting from the termination of "private foundations."

METHODS OF CONTRIBUTING TO COLD SPRING HARBOR LABORATORY

Gifts of Money can be made directly to Cold Spring Harbor Laboratory.

Securities: Stock certificates may be reassigned directly or transferred through your broker. Appreciated securities should be given outright, which will avoid capital gains taxes on the appreciated value. Securities that have decreased in value should be sold, and the proceeds donated. In this way, a donor will receive a deduction for both the loss and the charitable contribution.

Life Insurance: You may designate the Laboratory as the beneficiary of an existing or new policy, or irrevocably assign ownership of the policy. There are estate tax benefits in either case. If ownership is assigned, there is an immediate tax deduction.

Pooled Income Funds: Combine gifts from a number of donors in a pool for attractive investment and tax purposes.

Appreciated Real Estate or Personal Property: Sizable tax benefits can result from such donations; the Laboratory can use some in its program and can sell others.

Charitable Remainder Trusts can be structured to suit the donor's specific desires as to extent, timing, and tax needs.

Bequests: Most wills probably need to be updated. Designating Cold Spring Harbor Laboratory as beneficiary ensures that a bequest will be utilized as specified.

Conversion of Private Foundation to "Public" Status on Termination: This may be done by creating a separate fund within Cold Spring Harbor Laboratory whereby the assets of the private foundation are accounted for as a separate fund and used for the purposes specified by the donor. Alternatively, the private foundation can be established as a supporting organization of Cold Spring Harbor Laboratory.

Matching Gifts: Many employers will match gifts to Cold Spring Harbor Laboratory and/or the Watson School of Biological Sciences. Please check with your employer to augment your gift.

For additional information, please contact the Chief Development Officer, Cold Spring Harbor Laboratory, One Bungtown Road, Post Office Box 100, Cold Spring Harbor, NY 11724, 516-367-8840.

CAPITAL AND PROGRAM CONTRIBUTIONS

January 1, 1999–December 31, 1999

Contributions of $5,000 and above, exclusive of Annual Fund

In 1999, the Laboratory received significant support in the form of capital and program contributions from individuals, foundations, and corporations.

Rita Allen Foundation, Inc.
Arrow Electronics
Rose M. Badgeley Residuary Charitable Trust
Banbury Fund, Inc.
The Breast Cancer Research Foundation
Davenport Family Foundation
Ellison Medical Foundation
Fraxa Research Foundation
Samuel Freeman Charitable Trust
Bernard F. and Alva B. Gimbel Foundation
Goldring Family Foundation
The Grass Foundation
Mr. Henry U. Harris, Jr.
The John A. Hartford Foundation
Mr. Jeffrey C. Hawkins
Lita Annenberg Hazen Foundation
The Helen Hoffritz Foundation
Illinois Neurofibromatosis, Inc.
Sidney Kimmel Foundation for Cancer Research
The Esther A. and Joseph Klingenstein Fund, Inc.
Charles Henry Leach II Foundation
Long Island Foundation for the Elimination of Breast Cancer
Mr. and Mrs. David L. Luke III
Manhasset Women's Coalition Against Breast Cancer
G. Harold & Leila Y. Mathers Charitable Foundation
Marjorie A. and William L. Matheson Charitable Trust
The Maxfield Foundation
Elizabeth McFarland Foundation through the Long Island Foundation for the Elimination of Breast Cancer
Mellam Family Foundation
Neurofibromatosis, Inc., Mass Bay Area
1 in 9: The Long Island Breast Cancer Action Coalition
Oxnard Foundation
Pew Charitable Trust
Michael L. Rankowitz and Sheila Heffron
John R. Reese
Row for a Cure
Schering-Plough Foundation
Mr. and Mrs. Alan Seligson
Robin and Enrique Senior Philanthropic Fund
The Seraph Foundation
Alfred P. Sloan Foundation
St. Giles Foundation
Laurie Strauss Leukemia Foundation
Swartz Foundation
Texas Neurofibromatosis Foundation
Mary G. Turner
Dr. and Mrs. James D. Watson
Westvaco
The Whitaker Foundation
Helen Hay Whitney Foundation

Total	**$5,470,387**

NANCY AND EDWIN MARKS LABORATORY CAPITAL CAMPAIGN

January 1, 1999–December 31, 1999

Contributions of $10,000 and above, exclusive of Annual Fund

The Nancy and Edwin Marks Laboratory, which was dedicated and officially opened in October 1999, employs 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 1999, the Laboratory received significant support from individuals, trusts, and foundations.

The Ira W. DeCamp Foundation
Estate of Lachlan P. Braden
Fairchild Martindale Foundation
Irving A. Hansen Memorial Foundation
The Hyde and Watson Foundation
Mary D. Lindsay
Marks Family Foundation
G. Harold & Leila Y. Mathers Charitable Foundation
William E. and Maude S. Pritchard Charitable Trust
Fannie E. Rippel Foundation
The Starr Foundation
Dr. and Mrs. James D. Watson
The Weezie Foundation

Total	**$4,355,419**

WATSON SCHOOL OF BIOLOGICAL SCIENCES CAPITAL CAMPAIGN

January 1, 1999–December 31, 1999

Contributions of $10,000 and above, exclusive of Annual Fund

The Watson School of Biological Sciences at Cold Spring Harbor Laboratory was established for the purpose of granting the Ph.D. degree. In its first year, the Watson School received 130 applications for the five available student positions. Six students accepted over other high-caliber schools, and classes started in September 1999. In 1999, the laboratory received significant support from individuals,

Core Course in Scientific Exposition and Ethics	Mr. Nicholas C. Forstmann	
Dean's Chair	Lita Annenberg Hazen Charitable Trust Annenberg Foundation	
Faculty Lectureships	Mr. and Mrs. George W. Cutting Mr. and Mrs. Norris Darrell, Jr. Mr. and Mrs. Edward H. Gerry	The Esther A. and Joseph Klingenstein Fund, Inc. Quick & Reilly Group, Inc.
Pall Fund	The Glickenhaus Foundation Mr. and Mrs. Abraham Krasnoff Leydig, Voit & Mayer, LTD.	
Fellowships	European Foundation for the Advancement of Medicine The William Stamps Farish Fund Mr. and Mrs. William R. Miller	
Fund for Innovative Graduate Education	Mr. and Mrs. Leslie C. Quick, Jr.	
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Total **$293,745**

Total Watson School Funds $11,981,776

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The Corporate Sponsor Program continues to be an essential factor in the overall success of the Laboratory's meetings programs, for meetings held in Grace Auditorium on the main Laboratory campus and at Banbury Center. The funding provided by the Program enables us to plan ahead for the following year's meetings, and we are able to use it to select new and unusual topics. However, the wave of mergers in the pharmaceutical and biotechnology industries is severely reducing the pool of companies that we can approach, and we are therefore ever more grateful to the 40 corporate sponsors and affiliates who joined us in 1999 and are listed below.

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The members of the Program receive special privileges in acknowledgment of their contributions. We waive all on-site fees for eight representatives of each company at our meetings. The number of meetings has increased, so that in 1999, no fewer than 17 meetings took place in Grace Auditorium. Three scientists from member corporations may attend meetings at Banbury Center, where attendance is otherwise only by invitation of the organizers. Corporate Sponsors receive gratis copies of Cold Spring Harbor Laboratory Press publications, including the journals *Genes and Development*, *Learning and Memory*, and *Genome Research*. We acknowledge our Sponsors in all relevant publications, including the books of abstracts given to each of the 7000 participants who come to the meetings each year. The names of the sponsoring companies are listed on the poster describing the meetings; this is mailed to approximately 17,000 scientists throughout the world. In addition, the companies are listed on the Cold Spring Harbor Laboratory Web site, on the Meetings and Banbury Center pages.

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Total DNA Learning Center Annual Fund **$256,111**

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President's Report

J.L. Spingarn, D. Ayres, C. Large, L. Schiff

The last year of the century was a very busy one for Cold Spring Harbor Laboratory, with its ever-expanding research and educational programs, not to mention new and planned facilities. Just trying to grasp all this growth and accomplishment tends to energize those of us involved with the "public" arm of the Lab, the Cold Spring Harbor Laboratory Association.

The year 1999 was momentous in many ways, and the membership of the CSHLA responded with a resonant determination to add our own success to the fabulous achievements of the Lab itself.

First, in honor of our late esteemed past president, Vernon L. Merrill, the Association board members funded a special contribution to the Laboratory to be used specifically for breast cancer research.

Our annual meeting, held February 6, commenced with the election of officers and new directors. I am honored to have been elected president and am delighted to have very capable fellow officers: Trudy H. Calabrese, first vice president; John Grace, second vice president; Cathy Cyphers Soref, secretary; and Antoine C. Kemper, Jr., treasurer. Mary Alice Kolodner, Eileen K.S. Pulling, and Lawrence Remmel were elected new directors. Our retiring directors, Dill Ayres, Carol Large, David Clark, Phillip Satow, and Lisa Schiff, gave unstintingly of their expert, devoted service and made substantial contributions to the Association. Even now we miss their sage counsel and leadership, for which we again thank them. Our new directors have already been most worthy successors, and we are delighted they have joined us.

The lecture that followed our annual meeting was given by Edward O. Wilson, the world-famous expert on biodiversity and professor from Harvard University. Professor Wilson's topic was

"Consilience: Science Meets the Arts" and his delightful and easy-to-follow interpretation of the very significant interfacing of science, particularly biology, with the creative accomplishments of mankind (and how to make this work and succeed) was very well received. His audience rightfully thought they had experienced a unique opportunity to understand an important subject from a master. A dinner followed in Blackford Hall, while our young leadership group, the Next Generation Initiative, was treated to a short presentation and dinner at the DNA Learning Center.

On March 7, John Kruper of the DNA Learning Center made a presentation on the virtual-textbook project at an Association-sponsored lecture. It was resoundingly applauded.

A fabulous jazz concert, much of it arranged by the Lab's own Director of Development, Rick Cosnotti, with a group of top jazz musicians from Pittsburgh, was held in Grace Auditorium on April 17. It was a wonderful evening—and the group is coming back on April 15, 2000.

On February 4, Pam and David Banker hosted a lovely and well-attended reception in Manhattan. Both Dr. Watson and Dr. Stillman addressed the attendees.

Our great annual event, the Dorcas Cummings Memorial Lecture, was held on June 6. Irving L. Weissman, Ph.D., gave a fascinating talk on "Repairing the Body: The Promise of Blood and Tissue Stem Cells." Learning more about this fascinating area of research and medical treatment was both very illuminating and encouraging. Its dividends, if successful, could be life-enhancing, and Dr. Weissman made the research very interesting. The lecture was followed by 19 fabulous dinner parties which were enjoyed greatly by all (including visiting scientists and our own scientists and friends). The parties were organized by Cynthia Stebbins and Eileen Pulling—many kudos for a great job. Our thanks to all of you who so generously hosted these dinners:

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On October 12, with the generous sponsorship of U.S. Trust Company, we held the First Annual Biotech Enterprise Luncheon, in New York City. Many distinguished invitees had an opportunity to learn much directly from Dr. Stillman and hedge fund manager Dr. Stuart Weisbrod. Thank you, Larry Remmel, Linda Franciscovich, and U.S. Trust!

Carolyn and Ollie Grace were the perfect couple to host a most enjoyable tribute on October 22 to our most significant supporters at the annual Major Donor Cocktail Party. Carolyn and Ollie, a most sincere thank you!

In addition to the extraordinary success of the marvelous Gala Concert and Dinner on October 5, chiefly orchestrated by Lola Grace, Trustee and Treasurer of the Laboratory, we raised $739,453.35, a new record. We are especially grateful to those individuals who supported both the new Watson School for the Biological Sciences and the Association and made our 1999 Annual Fund Campaign a wonderful success. We thank each and every one of you for your generous interest, time, and support and wish you all a very happy, healthy new year.

January 2000

James L. Spingarn

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