COLD SPRING HARBOR LABORATORY

ANNUAL REPORT 1994

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Cold Spring Harbor Laboratory
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PRESIDENT'S REPORT

There has long been much controversy as to the relative contributions of nature versus nurture to our potentialities as human beings. To what extent are our successes or failures in dealing with given tasks due to the exact set of genes that we inherit from our parents as opposed to the nurture provided, say, by our families, our teachers, and the work ethic into which we are first introduced in early life? As an adolescent growing up in Chicago, I used to have friendly disagreements with my mother, who came down strongly on the nature side. She wanted to believe that it was the genes she inherited from her Scottish-born father that made possible her later success in rising above the boarding-house atmosphere in which she was raised after his premature death.

I knew then enough of the basic facts of genetics to know that human genetics was still a very soft science. Human geneticists were obviously unable to make genetic crosses in the way that their peers working with flies or corn so efficiently did. Although some human attributes such as mental stability seemed to have real genetic components, many different genes had to be involved since no obvious Mendelian rules of inheritance could be ascertained by analysis of extended families in which mental illness appeared. Whether there was any strong genetic component to intelligence was even more unclear. Since as a boy I never did super well on the tests said to measure intelligence, I wanted my later nurture within universities to be the major factor determining my success in later life.

In the absence of real facts, past strong views about which human traits have a genetic basis usually reflected culturally derived prejudices. Not surprisingly, many families with long histories of financial and intellectual triumphs wanted to owe their successes to their genes. Correspondingly, they ascribed the failure of other families to prosper to their genetic inadequacies. Francis Galton, the upper-class Englishman and cousin of Charles Darwin, was a very serious proponent of this viewpoint. He originated the term eugenics, the supposed science of improving the qualities of a species or breed by the careful selection of parents. From his viewpoint, human beings were the products of a long evolutionary selection for the intelligence and emotional stability needed for the social societies of modern man. This late nineteenth century way of thinking, frequently referred to as Social Darwinism, became even more popular with the 1900 rediscovery of Mendel's laws which gave the world the gene as the fundamental unit of heredity. Until evolutionists could think in terms of genes, they had no explanation for the multitude of new biological features that emerged during the evolution of an original primitive form of life into the extraordinary variety of life forms existing on the earth today.

Charles Davenport, who directed the Biological Laboratory (now Cold Spring Harbor Laboratory) from soon after its inception until his retirement in 1934 at the age of 68, became a strong proponent of Social Darwinism and used his position as director to enthusiastically promote eugenics. Using money he obtained from Mrs. E.H. Harriman, he founded in 1910 a satellite endowed operation, the Eugenics Record Office, which had its own buildings on land above the Laboratory on Stewart Lane. Its attempts to show genetic origins for widespread human traits initially met with wide approval among the then small band of Amer-

ican geneticists. But it soon became clear that the eugenicists' main experimental approach, the identification of multi-generational human families in which specific traits passed from one generation to another, was inadequate to prove genetic causations for most human conditions. Davenport correctly hypothesized that Huntington's disease was caused by a dominant gene that gave each member of the subsequent generation a 50% risk, but he went far beyond his data, for example, in his wartime postulating of the existence of a dominant thalassophilia gene that predisposed its bearers to lives as naval officers. Equally suspect, and of much more consequence in its social implication, was his belief that those families marked by alcoholism, criminality, and poverty were likely to be genetically destined to produce more children with these same afflictions. Already by 1930, the hope that eugenics could be a science as opposed to a social philosophy had largely vanished in the United States, particularly since the great Stock Market Crash and subsequent Great Depression had made Wall Street, not bad genes, the more compelling villain behind our nation's social problems.

Tragically, the situation in Germany proved to be just the opposite, with the eugenics movement gaining ever greater prominence when the Nazis assumed power in 1933. Most leading German geneticists continued to view eugenics in a positive way and welcomed the rise to power of Adolph Hitler, whose radical ideas had been reinforced by his reading in 1923 of *The Principles of Human Heredity and Rare Hygiene* by the then well-respected German geneticists Bauer, Fisher, and Lenz. Although Lenz initially deplored the anti-Semitism of the Nazis, he saw the central question before society to be the quality of our genetic endowment, which was to him one hundred times more important than disputes over the relative merit of capitalism and socialism. Key to much Nazi thinking was its proclamation of the genetic superiority of the Aryan race, thereby giving to such people the right to dominate the world. Anthropology quickly became a major Nazi science, with its role being to identify inferior non-Germans such as the Gypsies, Slavs, and, in particular, the "anti-Christ" Jews, whom they saw as the principal enemy of German culture. To make their supposed German superiority even more perfect, the Nazis turned also to their psychiatric community for assistance with sterilization policies aimed at preventing the Aryan inhabitants of their mental institutions from having further children.

Within 6 months of the Nazi takeover, the law for the prevention of progeny with hereditary defects was put into force, allowing the compulsory sterilization of individuals with congenital mental defects, schizophrenia, manic depression psychoses, hereditary epilepsy, and severe alcoholism. By 1937, sterilization was extended to all German "colored" children. Only two more years passed before the decision was made at the start of World War II to replace such sterilization efforts with a psychiatrist-assisted euthanasia program that dispatched more than 70,000 mental patients to the gas chambers. After the so-designated intractable mental patients had been killed, their gas chambers then became the fate for approximately 30,000 Gypsies, as well as for the much larger, Jewish population of several million.

Not surprisingly, when the war ended and the true extent of the Nazi genocide horrors was revealed, the term eugenics fell into deep disrepute. Past preoccupations with supposedly genetically based racial differences were replaced by social policies aimed at more equalizing the potential for economic and social advancement of the world's different populations by educational, nutritional, and medical policies.

Human genetics as a science, not as a social movement, of course continued to exist after World War II, but it lagged behind other aspects of genetics due to

the obvious inability to do human genetic crosses. Except for recessive diseases due to mutations on the X chromosome (e.g., Duchenne muscular dystrophy), the chromosomal location of virtually all genetic diseases remained unknown until the middle 1980s, when the extraordinary powers of recombinant DNA technology became directed toward human genetics. Here, the key insight was the realization in the late 1970s by David Botstein and Walter Bodmer independently that naturally occurring DNA polymorphic markers could be followed through extended multigenerational families to see which markers segregate together, thus revealing their location on the same human chromosome. Genetic analysis could thus be done without new genetic crosses by collecting blood samples from multigenerational families and using the DNA isolated from such cells to follow the segregational patterns of large numbers of different cloned polymorphic markers.

Using this trick, teams led by Helen Donis-Keller outside Boston and Ray White in Salt Lake City assembled the first human genetic maps; both teams used DNA obtained from the Center for Human Polymorphisms (CEPH) established in Paris by Jean Dausset. With such maps in hand, a number of important genetic diseases could quickly be assigned to specific chromosomal locations. Huntington's disease was located on chromosome 4 and cystic fibrosis was assigned to chromosome 7. The way then became open for the actual cloning (isolation) of the disease genes themselves, with the cystic fibrosis gene being cloned in 1989. The cloning of the Huntington's disease gene was more difficult, and was not achieved until early in 1993.

As soon as a gene becomes cloned, the amino acid sequence of its protein product also becomes known, information that often provides important clues to its function and that frequently eventually leads to more effective treatment of the respective disease. In the late 1980s, we nonetheless worried that the very large size of the human genome (3×10^9 base pairs distributed over the 24 different human DNA molecules [chromosomes]) meant even then that the future mapping and actual cloning of most human genetic disease genes would move very slowly; that is, unless an essentially worldwide, international project on the human genome was initiated, with the eventual aim of sequencing the entire human genome. Initially, there was fear that such a Human Genome Project would be prohibitively expensive, but these fears were unfounded, and by 1990 the United States component Human Genome Project officially started with the goal of identifying through DNA sequencing all the anticipated 100,000 human genes by 2005 at a total cost of three billion dollars.

By now we have good reason to believe that the Human Genome Project will achieve its intellectual objective within the desired time frame and so start providing the genetic information that will totally revolutionize our capacity for studying biology and medicine. The human genome is our ultimate blueprint, providing the instructions for the normal development and functioning of the human body. That we are human beings and not chimpanzees, for example, is not due in any sense to our nurturing but to our nature, that is, our genes. Yet in both species, sets of some 100,000 genes carry out roughly the same biochemical tasks. But the five million years of evolution that have separated us from the chimps have led to significant divergencies as to the exact times at which some genes function and the rates at which they produce their respective protein products. Successive, still to be worked out, changes in certain key genes, for example, have led to the retention of many juvenile chimp features into adult human life. The shape of our adult brain is that of the baby chimp brain, and our general lack of body hair resembles the situation in baby apes.

As the Human Genome Project thus moves toward completion and the func-

tions of more and more of our human genes are revealed, we will increasingly have the power to understand the essential genetic features that make us human. Moreover, we will be able to understand at the molecular level the ever-increasing number of human diseases such as diabetes, cancer, and Alzheimer's which arise in part because of imperfections in our genetic instructions.

At the same time, the new human genetics at last gives us the power to begin to assess the relative importance of nature (genes) versus nurture (both social and physical) in controlling human behavior. For example, to what extent do genes cause individuals to become alcoholic as opposed to either poverty or the lack of light during the long nights of winter? Are the schizophrenic inhabitants of mental hospitals more the victims of genes inherited from their parents or have they become psychotic in reaction to the brutality and unloving nurture of all too much of modern society? Equally at question is to what extent is the capacity to perform well on the so-called intelligence tests determined by genes as opposed to the nurture of the home and schooling environment of the respective students. How we answer these questions has immediate impact on how we as human societies respond to the personal tragedies of alcoholism, mental illness, and the inability to learn. These issues have always generated more controversy than questions of how to deal with diseases that appear indifferent to the boundaries of class and economic well being.

Recently, for example, much controversy erupted about the use of government monies to fund a conference in Maryland concerned with the possible genetic factors in violent behavior. Those who opposed the meeting argued that its real purpose was to reinforce the harmful idea that genes might be the primary factor leading to criminal behavior as opposed to inequalities in the distribution of wealth and that any such meetings would lead to more discrimination against members of low economic classes. That the meeting had been structured to contain participants who would strongly argue against genetic determination for most criminal behavior unfortunately did not prevent the director of the National Institutes of Health from effectively blocking it by stopping governmental funding. In canceling the conference, however, the impression was given that political correctness, not scientific judgment, was behind the decision, leading many to give much more credence to genetic causality of criminal behavior than the facts themselves warrant.

In an attempt to negate this unfortunate impression, our Laboratory was soon after urged, in particular by sympathizers to the economically disadvantaged, to hold a more general meeting on the Genetics of Human Behavior. Thus, after much planning by both sides of the apparent nature and nurture chasm, we held this meeting in early March of this year (1995) using funds given to us by the William Stamps Farish Fund. Initially, we expected much vigorous debate between the human behavioral geneticists and their nurture-oriented critics, but virtually all the real arguments were among the behavioral geneticists themselves. Effectively unassailable were the conclusions coming from observations on identical versus fraternal twins that genes play a significant role in predisposing individuals to most behavioral traits, including alcoholism, manic depressive diseases, and schizophrenia, as well as seriously influencing an individual's performance on intelligence tests. At the same time, strong influences of nurture were seen in every behavioral trait studied. Thus, for example, performance on I.Q. tests is influenced by the quality of schools we attend and by the home environment in which we are raised. That the scientific facts now seem so firm in most nature versus nurture arguments does not mean, however, that they will soon be

generally accepted by the worlds of the disadvantaged and those who want to protect them. Those individuals who push environmental causations exclusively for human behavior still can see only harm coming from the increasing role genetics might play in understanding the origins of human inequality.

I suspect, however, that if we avoid using science to find the molecular evidence underlying our abilities to learn and think rationally, we effectively will be condemning those who cannot easily learn or behave to remain forever disadvantaged within modern society. If we could use genetic analysis to help work out the biochemical pathways underlying memory and clear thinking, for example, we might be able to find pharmaceutical compounds to improve these most needed human attributes. Thus, those who want to protect the mentally ill or the slow learner may not get what they strive for if they portray them exclusively as victims of their environment. We might like to think otherwise, but only by reducing the differences in abilities among human beings will we ever have a society in which we can effectively view all individuals as truly equal. Obviously, we shall never be truly able to reach this ideal, for it would be naive to believe that we shall find the pharmaceutical means to fight effectively against all those genes that do not function as we would like. On the other hand, if we were to totally unravel how failures in gene functioning lead, for example, to manic depressive disease, it would be most surprising if we cannot use this knowledge positively to help control this pernicious human affliction.

The high ground for us as scientists is to use all the means at our disposal to understand the human body and the human nature that comes out of it in order to ultimately find the patterns for those who need and want assistance in improving life's lot wherever possible.

June 13, 1995 **James D. Watson**

DIRECTOR'S REPORT

In the annual reports written after the first year of tenure of all the previous Directors of Cold Spring Harbor Laboratory and its two parent organizations, particularly the Biological Laboratory that existed here from 1890 to 1963, the central concern and topic of discussion was the precarious financial status of the Laboratory. Although I do not wish to appear overconfident in the current climate of tremendous national debt and cuts in science budgets, I am happy that I was appointed Director when finances were more secure than in previous eras. Through the efforts of John Cairns, who nurtured the newly reorganized Cold Spring Harbor Laboratory through its first precarious years in the 1960s, and particularly through the extraordinary efforts of Jim Watson during the last 25 years in redirecting the science, securing an endowment, and expanding the facilities, the status of the Laboratory's finances and infrastructure now parallels its outstanding history. We must owe a great debt of gratitude to Jim Watson for the marvelous renaissance the Laboratory has gone through during his years as Director, and we must be equally pleased that he continues his love affair with Cold Spring Harbor as the Laboratory's first President. As President, Jim continues to make his mark in the scientific arena by working for the continued success of the many facets of Cold Spring Harbor Laboratory and for the scientific enterprise in general. It is certainly my privilege and pleasure to work with him.

Many have asked me why I have taken on what appears to be a huge administrative task while still trying to run a laboratory to study the replication of DNA. I believe that this field of research is in the middle of its own renaissance and there is much to do that should be exciting and worthwhile. Moreover, as an active scientist, I can remain close to new developments that are constantly being presented. Thus, my science will remain a high priority. At the same time, however, having the opportunity to contribute to the Laboratory as Director is an equally exciting challenge and a privilege. There are many reasons one takes on what appears from the outside to be a considerable task, but the tremendous support from many people makes the challenge a pleasure.

The laboratory has long enjoyed vigorous and generous support from the local community. This support has in many instances throughout our history ensured the very existence and survival of the institution. It is quite common for members of the local community to take a keen interest in our scientific achievements; to get to know the students, postdoctoral fellows, and the staff; and to favor us with encouragement. Unfailing support also comes from the Laboratory Board of Trustees, perhaps one of the most "hands on" Boards of any like institution. They enthusiastically work to ensure future success at the Laboratory, and their marvelous efforts have certainly given me the confidence to pursue what others may deem to be risky science. This confidence also comes from seeing Jim Watson in action, since he has initiated new areas of research at Cold Spring Harbor against the advice of more conservative scientists. This is what Cold Spring Harbor Laboratory should be about. I also am fortunate to receive help from the newly appointed Assistant Director, Winship Herr, and from a lean administration under Morgan Browne that thankfully minimizes bureaucracy and is remarkably efficient. With all this support, it will be much easier to think about

those critical issues that will be important for our continued strength.

Like so many scientists, I first came to Cold Spring Harbor while a graduate student to present my thesis research at a meeting. The meeting was the 1978 Symposium on DNA replication and recombination, and there I had the opportunity to absorb an entire week of outstanding science, meet the leading scientists in the field, and see firsthand the impact Cold Spring Harbor meetings have on science throughout the world. It was a memorable experience and it made me realize the importance of a strong meetings program.

Science at Cold Spring Harbor began following the establishment of a research station that hosted scientists who then had the luxury of a summer without distraction. These researchers came to this lovely setting to pursue research and interact with others of their ilk. This naturally led to the summer courses and later to the scientific meetings which today are one of the most important and unique features of the Laboratory. Both the larger scientific meetings on the main Laboratory campus and the smaller meetings at the beautiful "biological think tank" at Banbury on the other side of the harbor serve the scientific community as a whole and keep us in touch with the latest developments. This aspect of our enterprise is certainly key to our future as a leading research institution, for it is the meetings program that allows us to see firsthand the best young people and provides the scientists here enormous exposure to the scientific community as a whole. Similarly, our laboratory courses, now expanded successfully beyond the summer months, are key to our future success as an educational institution. Not only do the courses serve the needs of the scientific community as a whole, but they also broaden our intellectual makeup in a unique way. So often good science, both here and elsewhere, has emanated from discussions among participants at our meetings and courses. Important for the continued success of our academic program is the ability to provide sufficient temporary "on-site" housing for our visitors so that they can optimally benefit from the Cold Spring Harbor meeting experience. We have certainly progressed from the days of tents on Blackford lawn, a feature of the meetings not so long ago.

Equally important for our educational program is the DNA Learning Center in the village of Cold Spring Harbor. This advanced teaching laboratory aims to educate the next generation in matters relating to DNA and modern biology. As the use of DNA and genetics has an increasing impact in society, the general public has to be more aware of the huge advantages, and the potential pitfalls, of the technology. This can only come from education at an early age, and therefore the programs at the DNA Learning Center must continue to grow to meet these challenges. A steady source of funding for these programs is essential, but unfortunately, such funding is perhaps harder to obtain than research support.

Nine months after attending the 1978 Symposium, I returned to the Laboratory as a Postdoctoral Fellow to experience another aspect of Cold Spring Harbor. I initially intended to stay for just two years, the duration of my Damon Runyon-Walter Winchell Fellowship, but this is such a marvelous place to do science that I simply could not say no when the opportunity arose to join the staff and stay a little longer. The laboratory then was a powerhouse for innovative research, and I learned a style of science that has served me well over the years.

It is remarkable that throughout the laboratory's entire history, the quality of science accomplished here has been outstanding, and this was particularly so over the last quarter of a century. It is striking that much of this success has come from scientists who were (or are) in the formative stages of their careers. Such young scientists come here and enjoy the luxury of focusing on research

without the burden of distractions so often found at other institutions. Cold Spring Harbor Laboratory should be a place where first-rate science is the priority and where the individual feels emboldened to achieve the best. This is an atmosphere that we must strive to maintain by continuing to offer young scientists the necessary encouragement, resources, and help needed for excellence. Of course a large part of this is to secure sufficient funds so that newly appointed investigators can jump head first into their research projects while they attempt to attract grant support that will sustain them in the long run. In today's climate of intense competition for ever-diminishing federal research funds, it is becoming more difficult to sustain our successful and established investigators, let alone to support new endeavors. But it is these new endeavors and the young investigators that will keep the institution vibrant. Thus, to remain at the forefront of research, we must maintain a healthy balance of outstanding, established investigators and newer bright young people, both of whom continue to break new ground.

This principle should also apply to the entire scientific community as we decide where to allocate funds for peer-reviewed research. The peer-review system that has served this country so well in the past is in danger of hindering scientific progress if review panels fund only the obvious and incremental science and shy away from the bold new approaches that may have a lower probability of success. It is often difficult to convince colleagues on review panels to take a chance on an untested idea or new approach when only one quarter to one tenth of the research grant applications to the National Institutes of Health receive funding. Moreover, it is all too easy to criticize innovative ideas compared to the ordinary and obvious. Although the task of deciding where to allocate limited funds is increasingly difficult, peer reviewers should recognize that new ideas and research directions must be given a chance if our science is to remain vigorous. Senior investigators who have benefited from such support in the past should work to keep these valuable goals alive.

As I look to the future of research at Cold Spring Harbor and to science in general, I am sure that we will not be immune to the financial pressures that have so concerned previous Directors. But at the same time, we can be proud of our past and look to an exciting future with the hope that great science will be self-sustaining.

May 15, 1995 **Bruce Stillman**

HIGHLIGHTS OF THE YEAR

Research Highlights

Our research programs in cancer biology, plant genetics, neurobiology, computational biology, and structural biology all saw significant progress this year. In cancer research, some of the most exciting work came from David Beach's laboratory. In 1993, David's group discovered the first members of a new family of cancer-related genes. Called cyclin-dependent kinase inhibitors, the proteins made by these genes inhibit a major class of proteins, the cyclin-dependent kinases, which are the key regulators of the cell division cycle. The first two of these proteins called $p16^{INK}$ and p21 were the founders of two different families of growth regulators that have grown during the last year. The $p16^{INK}$ gene is altered in a wide variety of human tumors, suggesting that it acts as a tumor suppressor, and a new member of this family has been demonstrated to be induced by an extracellular growth inhibitor called tumor growth factor β. In other work, the Beach laboratory has linked signal transduction through the RAS pathway to control of the cell cycle by cyclin-dependent kinases, via a known regulatory protein called cdc25. In collaboration with the Beach laboratory, Bruce Stillman's group has shown that the p21 cell proliferation inhibitor has a dual role in cell cycle regulation. The p21 protein inhibits DNA replication via its interaction with proliferating cell nuclear antigen (PCNA), an essential protein required for DNA replication and DNA repair, but interestingly, it still allows DNA repair. The gene encoding the p21 protein is activated by the well-known tumor suppressor protein p53. Damage to a cell's DNA, by radiation or other mutagen, will induce p53, which in turn induces p21 and begins a molecular cascade that inhibits progression through the cell cycle. This allows the cell to respond to genetic damage by pausing its progression through the cell cycle while it repairs its DNA, thus helping to prevent the propagation of potentially dangerous mutations.

The *ras* gene was among the first human oncogenes to be discovered, back in 1981 by Mike Wigler's group, and last year Mike reported that the RAS protein signals to another oncogene product, the RAF protein kinase, thereby controlling cell growth and proliferation. This year, Wigler's group obtained evidence that RAS and RAF are near the beginning of a signal transduction cascade of protein kinases that passes extracellular signals to the nucleus. Staff Investigator Linda Van Aelst and Wigler also isolated several additional RAS-binding proteins. Among these was AF6, a protein that has been implicated in acute lymphoblastic leukemias. In collaboration with Senior Staff Investigator Nikolai Lisitsyn, Mike Wigler has been developing a powerful technique called representational difference analysis (RDA). This technique, which allows researchers to identify subtle differences between two DNA sequences, can detect both additions to a chromosome, such as the insertion of viral DNA, and deletions caused by mutation. Recently, the technique was used by researchers at Columbia University who found that Kaposi's sarcoma, a common complication of AIDS, may be caused by a virus that is related to the herpes simplex viruses.

In other work, Nouria Hernandez has dissected a complex of transcription factors that regulates transcription of small nuclear RNAs (snRNAs), short RNA molecules that do not code for proteins. Surprisingly, one of the components of the transcription factor for snRNAs turned out to be the same as a major mes-

senger RNA transcription factor, the TATA-box-binding protein (TBP). TBP has now been shown to have an essential role in the transcription of all genes in the nucleus of cells. Transcription is activated by other gene-specific DNA-binding proteins, and in the last year, Winship Herr's group collaborated with scientists at MIT to elucidate the crystal structure of the POU domain, an unusual bipartite DNA-binding domain common to a number of transcriptional regulatory proteins.

A major breakthrough in studies on the replication of DNA came from Carol Greider and her group's work on telomerase, a ribonucleoprotein that adds telomeric DNA sequences onto the ends of chromosomes. Telomerase has been attracting a great deal of interest because of its potential target for anti-cancer therapy. Most normal human cells have little or no telomerase because they do not need to extend the length of their chromosomes' ends, but in many kinds of cancer cells, telomerase is activated. Several years ago, Carol and her colleagues suggested that telomerase may be required for the growth of immortal cancer cells. This year, Carol's group, in collaboration with scientists at Geron Corp. in Palo Alto, California, succeeded in cloning the RNA component of the human telomerase. Using this information, they also cloned the mouse telomerase RNA and found that it is regulated during mouse development. The cloned RNAs will enable her group to pursue experiments to determine the role of telomerase in normal and cancerous cell growth. Finally, Kathy Collins and Greider, in collaboration with Senior Staff Investigator Ryuji Kobayashi, succeeded this year in cloning genes encoding two protein components of telomerase. These proteins do not have striking similarity to other polymerases, except for a distant relatedness to RNA-dependent RNA polymerases, indicating that telomerase represents an entirely new class of polymerase enzyme.

Since 1990, the Plant Group has been developing potentially powerful genetic methods for the detection and mapping of new plant genes. Called the gene trap/enhancer trap system, the technique was first developed in bacteria and has been used with impressive results in *Drosophila*. The group has succeeded in adapting this approach to the small mustard-family plant *Arabidopsis*. Their approach employs the *Ac/Ds* transposable elements discovered at Cold Spring Harbor more than 50 years ago by Barbara McClintock. These transposable elements come in two versions that have been transferred into the *Arabidopsis* genome. In one plant, the *Ds* transposable element has a genetic marker associated with it and is stable in the absence of an enzyme called transposase. In the other plant, the modified *Ac* element encodes the enzyme transposase. When these two plants are crossed, the transposase in the hybrid plants of the first generation cause the *Ds* transposon to "jump" around the genome, randomly inserting into the plant's genes. Self-fertilization produces a new generation of thousands of plants that all differ by the insertion of a single *Ds* element. If the *Ds* element is inserted into a gene, the genetic marker it carries is often expressed, telling where in the plant the gene is functional. Phenotypic alterations in the plant give clues to the function of the disrupted gene. This work is a collaboration among Venkatesan Sundaresan, Rob Martienssen, Hong Ma, and our new Cold Spring Harbor Fellow, Ueli Grossniklaus, who comes to us following graduate studies in Walter Gehring's laboratory in Switzerland. This exciting work promises to yield much new information about plant development.

Our neurobiology program has had success in teasing out the genetic basis of learning and memory. For many years, a central question in learning and memory research has been to define a difference between long-term and short-term memory. Is long-term memory simply short-term memory that lasts a long

time or is it a physiologically distinct process? An important breakthrough came when Senior Staff Investigator Tim Tully and his group were able to establish long-term memory in *Drosophila* and show that formation of memory can be divided into genetically separable components. These different forms of memory, called short-term, anesthesia-resistant, and long-term, combine to produce specific memory patterns in the brain of flies. A collaborative effort among Tim Tully, Jerry Yin, and Alcino Silva has shown that a DNA sequence-specific transcription factor in the nucleus of cells, the CREB protein, has a key role in long-term memory in both fruit flies and mice. The complementary approaches of "knockout" mice and transgenic mutant flies with inducible genes have yielded insight into what appears to be a highly conserved mechanism for producing lasting memories. In subsequent research, Yin and Tully have demonstrated that it is possible to enhance long-term memory formation in transgenic flies by inducing the expression of an activating form of CREB. Flies normally require ten (spaced) training sessions to form a permanent long-term memory. Remarkably, transgenic flies carrying the CREB activator do it after only one training session, which is functionally equivalent to a "photographic" memory.

In structural biology, Xiaodong Cheng, who has collaborated with Rich Roberts to produce a high-resolution crystal structure of the DNA methyl transferase Hha-1, has produced a structure for the important cellular enzyme casein kinase 1. Our computational biology group, headed by Senior Scientist Tom Marr, has been undertaking one of the most difficult tasks of molecular biology—identifying the genetic components of complex, multigene diseases. This effort is centered on the Laboratory's effort within the Dana Consortium for Manic Depressive Illness (MDI), a cooperative alliance with research groups at both the Johns Hopkins University and Stanford University Schools of Medicine. Tom's group has been working with Staff Investigator Harriet Feilotter to identify the genes involved in MDI by screening families with a history of MDI, documenting their genotypes, and analyzing the pedigrees statistically. An ultimate goal is to use powerful modern genetic analyses to identify the genes involved and eventually bring some relief to this all too common disease.

Finally, the Laboratory has expanded its cancer research effort into an area known as programmed cell death, or apoptosis. Two new scientists, Michael Hengartner and Yuri Lazebnik, have moved into the first-floor labs of Demerec Laboratory to pursue investigations of genetically programmed cell death, which is an essential feature of normal development but is often abnormal in human cancer. They were joined early in 1995 by Scott Lowe, our new Cold Spring Harbor Fellow, who as a graduate student at MIT studies apoptosis in cancer progression and remission.

Harold Varmus

Symposium LIX: Molecular Genetics of Cancer

More than 450 scientists gathered this year at the annual Symposium to share their insights and data on the genetic origins of cancer and how it can best be controlled. Research has progressed rapidly, and it is now possible with some cancers to define the genetic changes that occur as the disease progresses from a barely detectable growth to a vigorous metastatic tumor. This was emphasized by Harold Varmus, Director of the National Institutes of Health, in this year's annual Dorcas Cummings Lecture entitled, Why Is It Important to Understand the Genetic Basis of Cancer? Varmus, along with Michael Bishop, was awarded the 1989 Nobel prize in Physiology or Medicine for his role in demonstrating that cel-

lular genes can cause cancer. His lecture drove the point home: Cancer is fundamentally a genetic disease. "This does not mean that all cancers are inherited," he pointed out, "rather it means cancer results from defects, inherited *or* acquired, in genes." Other discussions covered basic research into how cancer cells develop, function, and proliferate. Former Cold Spring Harbor scientist Ed Harlow delivered a thoughtful summary that harbored cautious optimism that understanding the genes and their products will lead to new therapies.

An Exciting Roster of Meetings at the Banbury Center

Of all the meeting places where molecular biologists congregate, none enjoys a greater reputation than the Laboratory's Banbury Center. We strive to select topics that have reached that stage of development when critical review is necessary to prepare for further advances. There were some outstanding examples of such meetings this year. "Secretory Pathways: The Molecular Basis for Their Specificity" was a remarkable synthesis of studies across organisms and cell systems, focusing on the newly emerging molecular understanding of the transport of proteins. It was especially fitting that Nobel laureate George Palade, one of the founders of the field, attended the meeting and made the closing remarks. Another meeting that achieved the same degree of synthesis was that on "Telomeres." These structures found at the ends of chromosomes and telomerase, the enzyme complex that keeps them the correct length, are fascinating in their own right, but it now seems that they have a role in aging and cancer. Banbury Center is also noted as a neutral ground for meetings in which matters of science policy loom as large as matters of science. A meeting in the spring of 1994 on "The Genome of *Arabidopsis*" was devoted to research on the genome of this small plant whose enthusiasts see as the laboratory mouse of the plant world. The meeting reviewed progress in mapping and sequencing the *Arabidopsis* genome and, through the participation of representatives of the National Science Foundation and the United States Department of Agriculture, was able to make significant progress in setting new research priorities.

Sabin Meeting

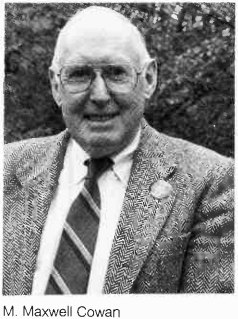
In October, the Laboratory hosted the 1994 Albert B. Sabin Vaccine Foundation Colloquium. The Sabin Foundation, named for the inventor of the oral polio vaccine, promotes the control and eradication of infectious diseases by sponsoring research and scientific meetings and by increasing public awareness of the need for vaccination. Participants at the Colloquium represented all disciplines of vaccine development, manufacture, and distribution. Among them were scientists, pharmaceutical and biotech representatives, congressional staffers, and physicians. The goal of the Colloquium was to improve the development and distribution of vaccines to American children. A highlight of the Colloquium was the presentation in Bush Auditorium of the 1994 Albert B. Sabin Gold Medal Award, to D.A. Henderson, Deputy Assistant Secretary for Health at the Health and Human Services Agency in Washington. Henderson then gave a moving lecture on arguably the world's most successful public health effort—the eradication of smallpox. The Sabin Foundation also sponsored this year's October meeting on "Molecular Approaches to the Control of Infectious Diseases."

E.O. Wilson and J.D. Watson

J.P. Morgan Executive Meeting

The Laboratory has a long and fruitful relationship with the J.P. Morgan Bank, stretching back to J.P. Morgan himself who, in 1924, was a founding patron of the Long Island Biological Association, continuing with the enormous contributions of our long-time Trustee and Morgan CEO, Walter Page, through to the present day with Sandy Warner, the current CEO of J.P. Morgan, also one of our Trustees. So it was with great pleasure, and not a little trepidation, that we learned that J.P. Morgan Bank was going to take over sponsorship of our annual Executives' Conference. The trepidation came from fear that we would not be able to reproduce the quality of the remarkable series of meetings of the past eight years. Our fears proved groundless, for the meeting's topic—The Biology of Human Behavior—could hardly have been better timed with the extraordinary current interest in human behavior and genetics. The speakers were outstanding, and it was particularly pleasing that E.O. Wilson, the celebrated Harvard zoologist, gave the keynote talk. Dean Hamer later discussed his controversial findings on a genetic basis for male homosexuality. Helen Fisher, author of *Anatomy of Love,* also gave a stimulating discussion of the evolution of human behavior and later followed with a public lecture with the provocative title of 'The Evolution of Marriage' to members of the Cold Spring Harbor Laboratory Association.

Laurie J. Landeau



M. Maxwell Cowan

A Vital Board of Trustees

At our annual Board meeting in November, we bid farewell to two Trustees, who have been members of our Board since 1988—Laurie J. Landeau, distinguished veterinarian and Assistant Director of the Program in Aquatic Veterinary Medicine at Woods Hole, Massachusetts, and W. Maxwell Cowan, Vice President and Chief Scientific Officer of the Howard Hughes Medical Institute. Mr. Cowan has been a major force in the development of the Laboratory's Neuroscience Program since the early 1970s. Happily, they are replaced by new members also of high caliber—Bruce Stillman and Henry Wendt. Bruce joins the Board as he steps into the role of Laboratory Director. Henry Wendt has just stepped down as

J.D. Watson, Henry Wendt, and David Luke III at the Wendt Cabin Dedication

chairman of SmithKline Beecham. By becoming a Trustee, he strengthens his already solid relationship with the Laboratory, which involved a major role in the Laboratory's Infrastructure Program. We also welcome to the Board our neighbor John P. Cleary, Esq., who is a distinguished local attorney associated with the firm Farrell, Fritz, Caemmerer, Cleary, Barnosky & Armentano. Mr. Cleary is a long-time member and is now Chairman of the CSHL Association.

Wendt Cabin Dedicated

On November 4, we dedicated the last of our current cabins for visiting scientists to new trustee Henry Wendt, who now divides his time between Philadelphia and his Northern California winery, Quivira Vineyards. In 1986, as the Chairman of the Board of SmithKline, Inc., Mr. Wendt participated in our first "hands-on" Banbury Conference designed to expose leading executives in the pharmaceutical and biotechnology industries to the latest ideas, results, and techniques of molecular biology. The cabin dedication was in recognition of Mr. Wendt's establishment of the Wendt Fellows program, which provides postdoctoral fellowships in neuroscience. The first two Wendt Fellows are Jim DeZazzo, in Tim Tully's group, and Zhong Wang, who works with Alcino Silva. Following the dedication, Tim Tully and Alcino Silva gave a special lecture on their exciting recent findings on the genetics of learning and memory in fruit flies and mice.

Robertson Research Fund

The Robertson Research Fund is an essential financial resource that gives the Laboratory a measure of independence essential to a small private research institution. It allows us to support scientists who are early in their careers or who are beginning untried research projects for which it is difficult to obtain federal grants—the kind of cutting-edge research that is a bit risky but has a high payoff. The Fund was established in 1973 by the generosity of Lloyd Harbor neighbor Charles Robertson and his family. After Charles Robertson died in 1981, his son Bill Robertson and daughter Anne Meier continued the family tradition of associa-

tion with the Laboratory. Through conservative management, the original endowment of $8 million has grown to nearly $48 million. This year, the Fund distributed $1.6 million in research support to Cold Spring Harbor scientists. The Robertson Research Fund supported 12 staff scientists this year, as well as our Cold Spring Harbor Laboratory Fellow, Ueli Grossniklaus. In addition, the Fund supported postdoctoral fellows, graduate students, and scientific communications via the meetings and courses programs. The Robertson family, through the Banbury Foundation, supports the Marie Robertson Memorial Fund for neuroscience research at Cold Spring Harbor, long a special interest of Charles Robertson. This year, Marie Robertson funds supported an April meeting on the Genetics of Learning and Memory at the Banbury Center as well as memory and learning research in Beckman Laboratory by Alcino Silva and Tim Tully.

Major Gifts

We gratefully acknowledge the support we received last year from individuals, foundations, and corporations. The Cancer Research Fund was brought to near completion, thanks to gifts of $300,000 from the Ira DeCamp Foundation; $75,000 from the William and Maude Pritchard Charitable Trust; $25,000 each from the Louis Berkowitz Family Foundation, the David Koch Foundation, and the Edward S. Moore Foundation; $10,000 each from Arrow Electronic, Inc., and the Daphne Seyboldt Culpepper Foundation; and $5,000 from the Dextra Baldwin McGonagle Foundation, and a bequest of $5,000 from H. Turner Slocum. Dr. David Pall donated $21,750, and the Marks Family Foundation made a gift of $10,000 in honor of Dr. Pall's 80th birthday. These contributions came to us as unrestricted funds, which allows the Laboratory to apply the funds where they are most needed. A grant of $45,000 made to the Carnegie Library by the Gladys Brooks Foundation will help address the cramped situation in our library by providing funds for microfilm.

Fellowships were funded in 1994 by the Goldring Family Foundation ($30,000) and the Seligson Foundation ($75,000). The Robert Leet and Clara Guthrie Patterson Trust awarded $50,000 to support of the research of CSHL scientist Hollis Cline. The Laboratory found a new and wonderful friend in 1-in-9: The Long Island Breast Cancer Action Coalition. 1-in-9 donated $20,000 to support cancer research in the lab of Michael Wigler and pledged their ongoing support.

In June, we launched a new planned giving program, the "Next 100 Years." Planned giving utilizes creative financial planning, enabling donors to see real lifetime benefits as a result of their charitable donations. The program is designed to help build our endowment, a step that is vital to the maintenance of the Laboratory. We currently have 30 donors in the planned giving program, with gifts in the neighborhood of $6.5 million.

In addition to their generous personal support of the capital projects of the DNA Learning Center, our long-time friends Henry and Mary Jeanne Harris have gifted $20,000 through the Edwin S. Webster Foundation for unrestricted support of Learning Center programs.

DNA Learning Center

In July, the DNALC received a five-year grant of $400,000 from the Howard Hughes Medical Institute's Pre-college Science Education Initiative for Biomedi-

cal Research Institutions. The DNALC grant was the second largest of the 42 awards given. The Hughes program will support ongoing work with local school districts to vertically integrate genetics instruction at the elementary, middle, and high school levels. Significant resources will also be used to provide intensive enrichment for minority students at several New York City schools and to link these resource-poor schools to North Shore schools that have well-developed genetics education programs. The grant provides large-scale support for the Learning Center's *BioMedia* Computer Laboratory to model uses of high-level computing in biology education, including the *Student Allele Database*, which allows students to submit electronically their own DNA fingerprints and compare them with those of other students from around the world. This grant, plus annual support from the Corporate Advisory Board, allowed the Learning Center to recruit computational scientist Robert Muratore. Robert has training in engineering and biophysics, with work experience in the aerospace industry, medical research, and academia. Robert is responsible for operating the *BioMedia* Laboratory and administering the Learning Center's local area network of 25 UNIX, Macintosh, and Pentium computers. He is also developing an Internet node, through which students and teachers will access the *Student Allele Database*. His thesis work on three-dimensional imaging of brain activity will be important in an upcoming project to develop a computer-generated tour of a human cell to play in the *Multitorium* theater.

Scientific Awards

Several of our scientists were honored with prestigious awards this year. On November 11, David Beach was elected a Fellow in the American Academy of Microbiology, the highest honor awarded in this national organization. Earlier in the year, Nouria Hernandez was appointed Associate Investigator by the Howard Hughes Medical Institute. At year-end, James Watson was honored by the Charles A. Dana Foundation with a Distinguished Achievement Award in Health. The award was in recognition of Watson's work with the Human Genome Project and his participation in the founding of the Dana Alliance for Brain Initiatives.

CSHL Press

In 1994, CSHL Press published 12 new books and 3 new videotapes, launched a new journal, doubled the frequency of both existing journals, and prepared a new publication, *The Lab Manual Source Book*. Among the notable new books was the second edition of *Manipulating The Mouse Embryo* by Brigid Hogan, Rosa Beddington, Frank Costantini, and Elizabeth Lacy. The first edition published in 1986 illustrated the techniques needed for the new molecular approaches to the study of development. The new and entirely revised edition brings those techniques completely up to date and has already joined those perennial best-sellers *Molecular Cloning* and *Antibodies* as the most sought-after lab manuals from Cold Spring Harbor.

The popularity of these manuals prompted the development of a complementary publication—an annually updated source book for the users of the techniques the manuals describe. *The Lab Manual Source Book* organizes and indexes the materials needed for work with genes, cells, and proteins and connects users with commercial suppliers of these materials. The 1995 edition has

data on 15,000 products and 1400 companies and will be distributed free to manual purchasers early in 1995.

The development of publications with a popular, educational purpose continued with the release of a videotape and teachers guide, *Stories from the Scientists*, and a beautifully illustrated book/audiotape package, *The Double Talking Helix Blues*. The scientific "talking blues," sung by twins Ira and Joel Herskowitz, was featured on both network and cable television programs. Joel is a physician and Ira is a noted molecular biologist whose association with the Laboratory goes back to the late 1960s.

In journals, both *Genes & Development* and *PCR Methods and Applications* made strong progress editorially and in subscription levels. Both fared well in the annual release of citation analysis data, a measure of the extent to which papers published in journals are subsequently referenced in the scientific literature, and confirmed their respective importance in the areas of science they serve. We increased publication of *G&D* to twice monthly and of *PCR* to bi-monthly. In both cases, the transition was managed smoothly and has resulted in more rapid publication for the papers submitted. In June, we launched a new journal entitled *Learning & Memory*. Its appearance as a forum for the new molecular and cellular analysis of higher brain functions is an indicator of the optimism within this young, growing, and immensely exciting field. The journal has started well, and its distinguished editors are dedicated to publishing only papers of a high standard. Making this publication a financial as well as an editorial success will be a challenge in this underfunded field, but everyone concerned is agreed that the task is important and also optimistic that the goals will be reached as the extraordinary science in this area continues to advance.

A Strong Year for the CSHL Association

Since the Long Island Biological Association was formed in 1924 to assume control of the Biological Laboratory at Cold Spring Harbor from the Brooklyn Institute of Arts and Sciences, loyal supporters from the local community have been an important source of strength. The Cold Spring Harbor Laboratory Association, descendant of LIBA, continues to support science with its Annual Fund, which provides unrestricted money for research. This year, the Association raised nearly $600,000 to support Cold Spring Harbor science. Special mention should be made of a $100,000 gift from Edna Davenport, widow of John Davenport, our first "angel" in the late 1960s. Mrs. Davenport continues to be an angel to the Laboratory. Under the able direction of Association President Mary Lindsay, membership in the Association reached 741. Mary's strong leadership of the Association this last year has been a boon. Although we are sad to report that she has stepped down from the presidency, we are pleased that she retains a vital presence as Vice Chairman of the Laboratory's Board of Trustees. Long-time Laboratory friend and supporter John Cleary is now the new president of the Association. We look forward to a productive term under John's able hand.

Mary Lindsay

CSHL Cruises onto the World Wide Web

This year, the Laboratory merged onto the Infobahn. A joint effort among the Press, Meetings, and Public Affairs Departments and with programming by Corp Reed from Tom Marr's group has resulted in CLIO—CSHL Information Online. CLIO is a server on the World Wide Web, the multimedia branch of the Internet.

Via CLIO, anyone with Internet access can obtain information about CSHL Press books, upcoming meetings, or general information about the Laboratory. Users can even order books or register for meetings right from their desktop. Having a "Web server" provides us with an opportunity to increase our on-line offerings and continue to serve the academic community. The URL, or Internet address, for CLIO is http://www.cshl.org/.

A Broad Spectrum of Fund-raising Events

In our perpetual search for novel ways to combine the arts or recreation with fund-raising for science, we hosted a variety of events this year. On May 1, piano virtuoso Peter Orth returned to the Laboratory for a fund-raising concert. Mr. Orth's concert raised $7400 for the Undergraduate Research Program. On June 7, we held the first CSHL Golf Tournament at the nearby Piping Rock Club. This event attracted 125 participants who helped to raise more than $48,000 for science education at the DNA Learning Center. Special thanks are due to the tournament's organizers, led by Rick Clark (who replaced Douglas Fox this year as chairman of the Corporate Advisory Board): Morgan Browne, Douglas Fox, Arthur Herman, Michael Vittorio, and William Keen. We also thank the Badge Agency and ITT Hartford for co-sponsoring a $30,000 hole-in-one opportunity on the 17th hole. In July, the CSHL Association put on "Hot Jazz at Cold Spring Harbor," a dinner and concert featuring the Big Band sounds of Jerry Jerome and his All-Stars. This concert netted over $7500 to support Laboratory scientists.

Undergraduate Research Program

Each summer the Laboratory welcomes a new group of promising college students to take part in the Laboratory's Undergraduate Research Program. In 1994, 21 students were selected from a field of 180 applicants to spend 10 weeks living and working here at the Laboratory. The program not only taught the students about molecular biology, but also acquainted them with scientific life and modern research tools and methods. As ever, the 1994 participants worked on a wide range of projects, including crystallization of biologically important proteins, the biochemistry of nerve cells, biochemical pathways involved in cancer, and plant genetics. This year, the URP projects represented a particularly good cross-section of the science at the Laboratory, with nearly every major research group represented. Funding for the Undergraduate Research Program came from Bio-Rad Laboratories, Burroughs-Wellcome Fund, C. Bliss Memorial Fund, The Garfield Internship, Hanson Industries, Libby Internship, National Science Foundation, Phillips Petroleum Foundation, Inc., Powers Foundation, William Shakespeare Internship, and Frederica von Stade Internship.

Partners for the Future

Autumn of 1994 ushered in a new group of Partners for the Future—five high school seniors who worked 10 or more hours per week in our labs after school from October through March. This was the fifth such group to have the opportunity to work in high-level molecular biology labs of noted scientists and to be paid a stipend for doing so. The students, their respective high schools, and their

Front row: Maureen Berejka, J.D. Watson, Herb Parsons
Middle row: Susan Schultz, Judy Cuddihy, Carlos Mendez
Back row: Jim Hope, Mike Ockler, Bruce Stillman, John Meyer

mentors were as follows: Michael Burstein (Syosset High School) carried out cancer research in the Lab of Dr. Michael Wigler; Jay Bikoff (Huntington High School) studied plant genetics with Dr. Venkatesan Sundaresan; Vinay Gupta (Half Hollow Hills High School) studied neuroscience with Dr. Jerry Yin; Sharon Hoffmann (Oyster Bay High School) worked with Dr. James DeZazzo on *Drosophila* learning and memory; and Brian Fox (Cold Spring Harbor High School) worked with Dr. Michael Regulski and also studied learning and memory in *Drosophila*.

Long-term Service

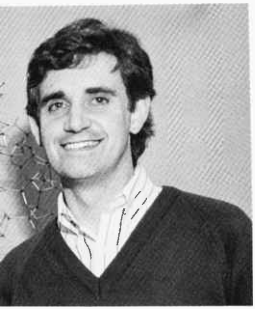
Three of our employees celebrated 20-year anniversaries with us in 1994. Annette Kirk, Print Buyer and Production Manager; Michael Mathews, Senior Staff Scientist; and Lane Smith, Lead Plumber, were each congratulated on two decades of service. Celebrating their 15-year anniversaries this year were Maureen Berejka, Administrative Assistant to the President; Judith Cuddihy, Editor of *PCR Methods and Applications*; James Hope, Director of Food Service; Carlos Mendez, Cash Management Bookkeeper; John Meyer, Painter; Michael Ockler, Art/Photo Supervisor; James Herb Parsons, Audiovisual Manager; James Sabin, Materials Supervisor; Susan Schultz, Grants Manager; and Bruce Stillman, Director.

Changes in Scientific Staff

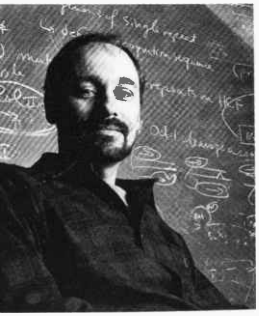
Cold Spring Harbor is traditionally a place with a high turnover of scientists. Most of our scientists are young and spend several highly productive years here before moving on to tenured positions at universities. Adding to this situation is the change in scientific funding, which in recent years has made jobs in industry increasingly appealing. This year, an unprecedented number of staff scientists took positions in industry. Michael Gilman, Senior Staff Scientist and a member of the Lab since 1986, accepted a position as Vice President and Science Director

of Ariad Pharmaceuticals in Cambridge, Massachusetts. Robert Franza, who had been with us since 1982, moved to Delmar, California, to start a new company. Dan Marshak moved to Baltimore to become Senior Vice President of Research and Development for Osiris Therapeutics, Inc. Several members of our structural biology group moved on as well. Senior Staff Investigator James Pflugrath, with us since 1986, has taken a position with Molecular Structure Corp., in The Woodlands, Texas. Senior Staff Investigator Jeff Kuret went to Molecular Geriatrics in Lake Bluff, Illinois, and Senior Staff Investigator and crystallographer John Anderson moved on to Jacksonville, Florida. We also said goodbye to the excellent crystallographer and CSHL Fellow David Barford, who is now a University Lecturer at Oxford University in the U.K. David has maintained a collaboration with Senior Staff Scientist Nick Tonks. Senior Staff Investigator Dafna Bar-Sagi has taken a tenured faculty position at SUNY Stony Brook.

Several visiting scientists have wrapped up their stays here. Saulius Klimasauskas from Rich Roberts' and Xiaodong Cheng's laboratory has gone to the Institute of Biotech Fermentas in Lithuania as a Senior Staff Scientist; Alexandre Melnikov is now a visiting scientist at the University of Georgia; Brian Cox from Bruce Futcher's lab has returned to the U.K.; and Gavin Screaton, who spent a sabbatical in Adrian Krainer's lab, returned to Oxford University. John Scott, a visiting scientist in the Stillman lab, has returned to Hawaii. Tokio Tani, visiting David Spector's lab, returned to Kyushu University in Japan. Visiting computer scientist Stephen Lincoln spent the year in Tom Marr's group and left for Molecular Tool, Inc., and Roussoundan Bourtchouladze from Alcino Silva's lab accepted a staff position at the Psychiatric Institute at Columbia University.



Bruce Stillman



Winship Herr

New Staff Members

With the arrival of Michael Hengartner and Yuri Lazebnik in the fall, the Laboratory begins a new research program in apoptosis. Hengartner comes to us straight from graduate school at MIT for a Staff Investigator position, and Lazebnik comes from postdoctoral work at Johns Hopkins as a Senior Staff Investigator. The Laboratory welcomed two new neuroscientists this year: Robert Malinow and Hollis Cline, a husband-and-wife team who come to us from the University of Iowa in Iowa City to become Senior Staff Investigators. Visiting scientists coming this year include Young-Seuk Bae from Kyungpook University in Korea, working with Dan Marshak; Boris Kuzin from the Russian Academy of Science, working with Grigori Enikolopov; and Ron Pruzan from Geron Corporation in Menlo Park, California, working in Carol Greider's lab.

Promotions

With the creation of the position of President of the Laboratory for James Watson, on January 1 Bruce Stillman assumed the directorship, while still running a large, vigorous research lab—a feat no CSHL director has managed since Milislav Demerec retired in 1960, when the Laboratory had about one-fifth the staff it does today. To aid in some of the administrative duties of running a research lab, Stillman has appointed Senior Staff Scientist Winship Herr as Assistant Director. Winship received his Ph.D. from Harvard in 1982, after which he worked with DNA sequencing pioneer Fred Sanger at the MRC in Cambridge. He came to Cold Spring Harbor as a postdoc in 1983 to work with tumor virologist Joe Sambrook. Winship was promoted to the scientific staff in 1984 and became a Senior

Staff Investigator in 1985 and Senior Staff Scientist in 1988.

Senior Staff Investigators Adrian Krainer, Carol Greider, Nick Tonks, and Tom Marr were promoted to Senior Staff Scientist. Although Cold Spring Harbor does not have a formal tenured position, Senior Staff Scientists have an appointment called "rolling five," which is renewed each day for five years. Adrian received his Ph.D. from Harvard in 1986. He came to Cold Spring Harbor that year as our first Cold Spring Harbor Laboratory Fellow and worked with Rich Roberts on RNA splicing. The Cold Spring Harbor Fellows program was modeled after Harvard's Junior Fellows program, which allows young investigators to pursue independent research. Adrian was awarded the fellowship in 1987, was promoted to the scientific staff in 1989, and then to Senior Staff Investigator in 1990. Carol Greider has followed a similar trajectory. After finishing up her Ph.D. at the University of California at Berkeley, where under Liz Blackburn she discovered the enzyme telomerase, Carol came to Cold Spring Harbor as a CSHL Fellow in 1988. She was appointed to the scientific staff the next year and to Senior Staff Investigator in 1992. Nick Tonks came to Cold Spring Harbor from the University of Washington in Seattle as a Senior Staff Investigator in 1990. Carol, Adrian, and Nick have in common the fact that they are all Pew Scholars. This prestigious program of support for young lab heads selects candidates from a small, elite group of nominating institutions. The Laboratory joined the ranks of Pew nominating institutions in 1990, and in the first three years, first Carol, then Adrian, and then Nick were elected to the Pew Scholars program. Tom Marr received his Ph.D. in 1981 from New Mexico State University. He then went on to research positions at the University of Arizona, E.G. & G., and Los Alamos National Laboratory, before coming to Cold Spring Harbor as Senior Staff Investigator in 1989. He is a leading expert on computational informatics, the science of using computers to uncover and analyze information in biological sequences. He has also become an integral part of the Dana Foundation Consortium on the genetics of manic depressive disease.

Crystallographer Xiaodong Cheng and protein chemist Ryuji Kobayashi were promoted to Senior Staff Investigator. Linda Van Aelst from Mike Wigler's lab was promoted to Staff Investigator. Rui-Ming Xu was promoted to Staff Associate II, and William Chang from Tom Marr's group was promoted to Staff Associate I.

Nick Tonks

Tom Marr

Adrian Krainer

Carol Greider

Postdoctoral Fellows

From Bruce Stillman's lab, Stephen Bell has accepted an Assistant Professorship at MIT and Thomas Melendy is now an Assistant Professor of Microbiology at SUNY Buffalo School of Medicine. Jeffrey Kazzaz finished up in David Helfman's lab and took a position at Winthrop University Hospital Cardiopulmonary Research Institute as a Research Scientist and Assistant Professor, while Mark Pittenger has gone on to work with Dan Marshak as a Senior Research Scientist at Osiris Therapeutics, Inc., in Baltimore. Timothy Connolly from David Beach's lab has also gone to Osiris as a Senior Scientist, and Tomohiro Matsumoto, also from Beach's group, has moved to Albert Einstein College of Medicine as an Assistant Professor. From Michael Mathews' group, Ben-Hao Dong returned to China as a postdoc and Claude Labrie has gone on to Lavel University Medical Center in Quebec. From Dafna Bar-Sagi's lab, Judy Cupp-Burris moved to West Palm Beach, Florida, and Michael Boyer has taken a position at Children's Hospital in Pittsburgh, Pennsylvania. Scott Henderson, from David Spector's lab, accepted a position with Mount Sinai School of Medicine as Director of Microscopy/Research

Assistant Professor. Gokul Das left Winship Herr's lab for a position at the Texas Medical Center-Baylor College of Medicine, Cancer Therapy Research Center in San Antonio as an Assistant Professor. Three of Robert Franza's staff departed: Manfred Neumann and Annette Wilisch went to Tübingen, Germany, and Judith Scheppler went to SUNY Stony Brook. From Tom Marr's lab, Wentian Li left for Columbia University Department of Psychiatry. From Mike Wigler's lab, Anthony Polverino and Stevan Marcus have moved on, Polverino as a visiting scientist to Amgen, Inc. in Thousand Oaks, California, and Marcus to the University of Texas M.D. Anderson Cancer Center as an Assistant Professor. Lin Mantell, from Carol Greider's lab, is now a research associate at Winthrop University Hospital.

Graduate Students

From Bruce Stillman's lab, Karen Fein and York Marahrens went on to postdoc positions, Karen at Columbia University and York at the Whitehead Institute at MIT. From Dafna Bar-Sagi's group, Kurt Degenhardt, Tom Joneson, and Shao-Song Yang have moved with her to Stony Brook; Nicholas Gale went to Regeneron Pharmaceuticals in Tarrytown, New York; and Linda Graziadei went to MIT in Cambridge, Massachusetts. From David Helfman's lab, George Mulligan has gone on to a postdoc position at the MIT Center for Cancer Research, while Stefan Stamm has gone on to the Max-Planck Institute for Psychiatrie in Munich, Germany. Raymond O'Keefe, from David Spector's lab, went on to postdoc work at the Medical Resource Council Laboratory of Molecular Biology in Cambridge, England. From Robert Malinow's group, Neal Hessler took a postdoc position at the University of California at San Francisco, Yuchi Li left the group to join Oncogene Science in Uniondale, New York, and Diana Pettit has gone to Duke University in North Carolina. Zhong Wang, from Alcino Silva's lab, departed for Cornell Medical College in New York City as a research assistant and Celeste Casciato went from the lab of Xiadong Cheng to a position in Philadelphia. Sridaran Natesan went with Mike Gilman to Ariad Pharmaceuticals in Cambridge, Massachusetts, and Gary Lee left Gilman's lab to become a Research Assistant at Tularik, Inc., in San Francisco. Gilles Carmel went from the lab of Jeff Kuret to Molecular Geriatrics in Lake Bluff, Illinois. Dusan Kostic left Hong Ma's lab to accept a position with the Roswell Park Cancer Institute/Grace Cancer Drug Center in Buffalo. Roberto Mariani left Jacek Skowronski's group for a position at the Aaron Diamond AIDS Research Center. From Bruce Futcher's group, Wolfgang Seufert went to University of Munich in Germany as a Heisenberg Fellow. William Thomann has left Winship Herr's group to attend NYU Law School.

Residential Transitions for the President and Director

Late this spring of 1995, both of our families completed moves to our new homes, Ballybung and Airslie. The new position of President, with its major fund-raising activities, necessitated an on-campus President's House, and construction of Ballybung commenced in June 1992. It is located on virtually the same site where the Henry de Forest house Nethermuir had stood for almost 100 years before it mysteriously burned to the ground at the end of World War II. By then, it had been vacated by the elderly Mrs. de Forest following her husband's death and the breakup of its adjoining estate lands into two-acre parcels. In its place was built an unimaginative split-level ranch-style house, inappropriate for its mag-

nificent site looking down Cold Spring Harbor and across to the Connecticut shore. No sighs were heard when it disappeared in less than two May days, since it was soon to be replaced by a much more appropriate dwelling of classical, somewhat Regency style. Preparing its plans were the talented architects, William Grover and Mahdad Saniee of Centerbrook, the widely respected Essex, Connecticut, architectural firm. During the past 20 years, Centerbrook has designed many important buildings for the Laboratory, including the Grace Auditorium and the Neuroscience Center.

The shape of the land parcel on which Ballybung was to be built dictated its almost square shape, with its key design feature being a skylight-topped two and one-half storied central hall which uses mirrors to magnify the light sent below, particularly into the eight internal windows that open into second-floor rooms and corridors. This architectural trick was employed near the end of the eighteenth century by the English architect John Soane, who was celebrated for the plans of many fine country homes as well as his London town home in Lincoln's Inn Fields and the monumental Bank of England located in the City.

Its unusual design made the construction not simple, and we were served well by the builder, William Baldwin, who previously played key roles in our Blackford Hall and McClintock Laboratory renovation-expansion projects. Equally important was the supervision by our own talented builder and Director of Buildings and Grounds, Jack Richards, who saw that the final costs closely approximated the budgeted target forecasts. Until its completion, we (Jim and Liz) remained uncertain as to what we should call it, but finally its name reverted to our first choice, Ballybung, the Gaelic name for Bungtown. From the start, we were tempted to give its exterior stucco-like "drivit" finish a strong color, finally settling on a creamy pumpkin orange hue frequently used in Italy. Helping us make the decision was our friend the English landscape architect, Elizabeth Banks, who while touring Lloyd Harbor noted the effective use of this color on a house on Middle Hollow Road. Later, Liz stood firmly behind the color choice when most of us were more faint-hearted.

The basement level of Ballybung contains windows with marvelous views of the Harbor, and adjacent to them there exists living space for visiting scientists. Currently, it is occupied by two English students, Jonathan Montagu and Greg Jefferis, who are here for a year doing research in their "gap year" between Eton College and their later university years in Oxford and Cambridge, respectively. Also within the basement is a specially designed archival space for the storage and study of the scientific and personal papers of what we anticipate will be a long line of accomplished Laboratory presidents.

Airslie was constructed in 1806 on the grounds of Nethermuir for cavalry officer and agricultural enthusiast Major William Jones, a member of the distinguished Long Island Jones family. The house and surrounding land changed hands several times over the following century—its name was found on a map dated 1855 detailing the estate of G.L. Willard, Esq. —and by the early 1900s was a part of the Henry de Forest estate. In 1942, four years after Henry de Forest's death, his widow donated the de Forest stables, built in 1914, and a parcel of land to the Laboratory, and the following year the Laboratory purchased Airslie and the surrounding seven acres.

Built in traditional Federal style, with a wide front porch and stately columns, the two and one-half story house has served as director's residence since that time. When then-director Milislav Demerec took up residence in Airslie, the century-old building underwent little change. Renovations on the house did not

take place until 1974 after former Laboratory director John Cairns departed for England. Prior to the Watsons moving into Airslie, the house was updated through structural renovations, the upgrading of mechanical services, and reconfiguration of the interior space to accommodate a young twentieth-century family. The renovations included the creation of a central staircase in the front of the house to the third floor. As they extended the twisting stairs upward, the architects opened the area in an inverted V-shape, to reveal the upper-most window of the house, a round, arched window in a sunburst design, created some 100 years before. Masterminded by architect Charles Moore and associates, this renovation was the beginning of an ongoing professional relationship between the Laboratory and Moore and his collaborators in the creative re-use of old buildings.

Despite 20 years of family living since the 1974 renovation, the stately house needed little renovation prior to the arrival of the Stillman family earlier this year. The exterior was repainted, the body of the house in its original cream color, and the trim changed from a greenish-gray to a rich, dark red. Set in the natural beauty of the harbor region, Airslie is shaded on the north side by an Amun Cork and a Kobus Magnolia, rare trees imported from the Orient, and an American Horse Chestnut, all planted for Miss Julia De Forest circa 1909 by Olmstead Brothers Landscape Artists (noted for their landscaping of Central Park and other major sites). Now mature trees, they are all among the largest of their kind on Long Island. Symposium picnics, Easter egg hunts, and other outdoor functions have been held on Airslie lawn since its acquisition in 1943, making it an integral part of our Laboratory home.

Inside Airslie, Grace has restored a cozy nineteenth century decor. With dark wood and traditional country furniture, the house is welcoming and warm. Flooded with sunlight—Airslie has large bay windows at both ends of the house and large windows all around—the house is spacious and airy. Soft mauves and greens are comfortable and soothing, making Airslie a wonderful home in which to raise a boy and a girl as well as a suitable place for the social affairs that are so vital to the Lab's function.

As we settle into our new dwellings, our residential relocations complete, we both look forward to many more productive and satisfying years at Cold Spring Harbor Laboratory.

July 19, 1995

James D. Watson, *President*
Bruce Stillman, *Director*

ADMINISTRATION

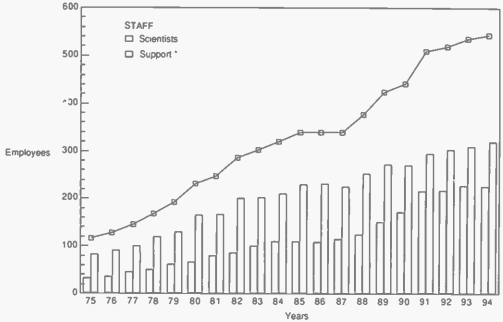
The Laboratory's science program in 1994 was once again at the leading edge of biology with remarkable new discoveries in all three of our primary fields of research—cancer, neuroscience, and plants. The latter two areas have been funded in large part by Laboratory resources, and it is particularly encouraging that these laboratory groups have become so productive. Much concern continues about the level of federal support for science as the congress in Washington focuses on deficit reduction. Clearly, there is going to be a serious need for new sources of money if our nation is to retain the leadership in basic research that has so benefited the well-being of our citizens and provided important impetus for the national economy. In any event, it will be the quality of science here at the Laboratory that will determine our ability to compete for available funding, and in this regard there is reason for confidence at Cold Spring Harbor.

Financial results for the year were good with revenues exceeding $41 million for the first time. A surplus from operations after provision for depreciation which now amounts to more than $2.6 million per year was achieved despite a 5% reduction, about $500,000 per year, in the Laboratory's recovery of indirect costs on federal grants. This stems from a 1993 agreement with the government, which approved our prior overhead procedures and established a new predetermined indirect cost rate through 1996. Results for the year were about $400,000 better than budget, once again reflecting successful competition for Federal and other grants, strong meetings attendance, better than anticipated interest and royalty receipts, and good control of costs. These results were also aided by the release of a small portion ($200,000) of the reserves set aside in prior years to provide for reduced overhead recovery and for start-up costs of the neuroscience program. 1994 was the sixth consecutive year of a balanced budget, and the cash flow from operations during this period has amounted to more than $14 million, which has been invested in facilities, equipment, and new programs.

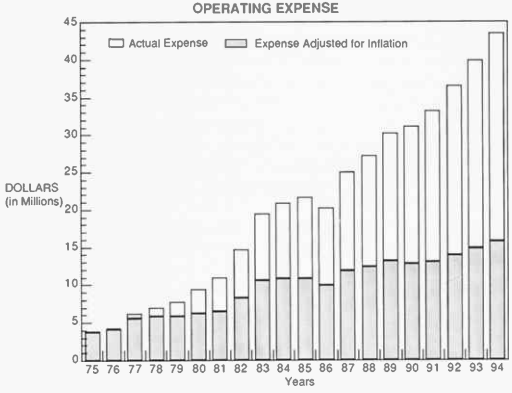
Banbury Center and the DNA Learning Center again had very good years conducting unique meetings and education programs dealing with a broad range of nationally important matters of science and ethics. Banbury meetings bring together leading authorities, often from a variety of disciplines, with the aim of focusing a range of viewpoints to shed new light on subjects of current importance. The DNA Learning Center has led the way in exciting a new generation of young people, fifth grade through freshman college, with the power of molecular biology and genetics and how the new science will change their lives. The Cold Spring Harbor Laboratory Press continued to publish first-rate science in its remarkably successful new books and journals. Much effort was directed to exploring the future potential of electronic publishing and, just after year-end, to the publication of "*The Lab Manual Source Book*," a new and comprehensive product directory for scientists in the academic, biotechnology, and pharmaceutical worlds. *The Source Book* is proving extremely popular and should make an important contribution to the operations of the Press.

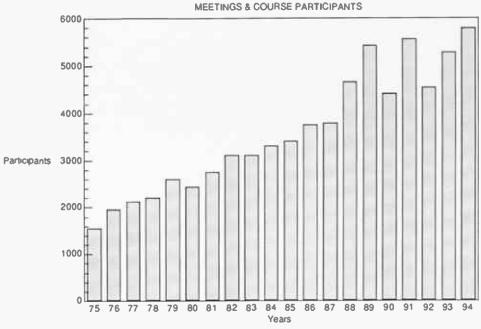
The Meetings Office has made good progress in adding new meetings in the normally less active months of fall, winter, and early spring. By making greater use of our now greatly upgraded facilities, it should be possible to generate additional revenues that can hold down the cost of our more traditional scientific symposia. Doing so is increasingly important as affordability becomes paramount for graduate students and even for many scientists with strained research budgets. David Stewart, in his first full year as Director of Meetings, has done a first-rate job of finding meetings that are appropriate and scientifically interesting while also maintaining the quality and ambience of the regular meetings schedule.

At year-end, the Laboratory's Endowment, consisting of the Robertson Funds and the Cold Spring Harbor Fund, was little changed from the prior year at approximately $91,000,000. In a year of difficult investment climate in both the equity and fixed-income markets, the Funds benefited importantly from the Laboratory's conservative policy for drawdown of income, which has averaged



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





less than 3.5% per year since inception. This has tended to stabilize the Endowment during difficult times and enabled it to grow over the years at a rate that provides both for inflation and for the support of new programs. Investments include a balanced mix of domestic and foreign equity, fixed-income securities, and short-term liquid instruments. Endowment is the primary internal source of funding for science at the Laboratory and serves as important support for $30,000,000 of outstanding tax-exempt bonds issued in recent years to help fund the construction here of new science and infrastructure facilities. During 1994, the Cold Spring Harbor Fund benefited again from a generous gift to the Charles S. Robertson Chair by the Banbury Fund and also from an addition of $500,000 from Laboratory operations.

The Cold Spring Harbor Fund now includes the year-old Science Fund, where are placed all royalties or equity shares received from a growing number of Laboratory technology transfer agreements. John Maroney, Director of Commercial Relations, manages these agreements and has had much success in working closely with Laboratory scientists to explore the commercial potential of their discoveries. The Science Fund was established by the Board of Trustees for the direct and indirect support of science here at the Laboratory, with the aim that one day royalties, and perhaps also the equity shares from our fledgling biotech participations, will make this Fund an important source of research funding.

Trends that were evident in 1994 serve to emphasize some of the financial challenges we will need to resolve during the next few years. Federal funding will continue to be extremely competitive and may actually decline on an inflation-adjusted basis. Overhead rates will trend downward. Last year, federal funding of about $11 million represented two thirds of the direct support for our science program, whereas nonfederal and Laboratory in-house resources provided the other third or about $5 million. We must further increase the percentage of nonfederal funds and we must convince the sources of such funds of the importance of carrying a larger share of indirect costs. In coming years, we should expect that our facilities-related overhead will continue to grow, as the physical plant is larger and must be properly maintained. There is an increasing need for scientific equipment, and present instruments will be replaced with more expensive new models. The rising cost of the data processing infrastructure, housing support for our scientists, and payments to local school districts and municipalities are additional concerns.

Contributing to the strong financial results of recent years has been the restraint in hiring exercised by administrative departments despite continued rapid growth of the Laboratory's programs. The result is a lean organization with individuals who should feel great satisfaction in the large amount of work they accomplish with limited resources.

As grant competition has intensified, the number of applications to multiple funding sources has soared. The Grants Department, headed by Susan Schultz, has handled the mushrooming paperwork with remarkable efficiency. In recent years, there has been a large expansion of time-consuming new federal audit requirements and reporting regulations. Much effort must also be devoted to monitoring the very complex individual laboratory budgets and to maintaining a reasonable balance of available resources with what sometimes seem the insatiable needs of world class science.

Each year, the Buildings & Grounds Department, led so capably by Jack Richards, astonishes us with their accomplishments. Although a village-wide moratorium stopped all nonresidential construction at the Laboratory for the latter half of the year, many necessary renovations were completed. In Demerec, three small laboratories for Mike Wigler were modernized and converted to a single larger one with adjoining post-doc offices. Barbara McClintock's historic space was modernized for Michael Hengartner who joined us in the fall to study apoptosis (programmed cell death), one of the newest and most intriguing areas of cancer research. A new tissue-culture facility was created in Jones by the conversion of an older cold room. The exterior of Olney was completely refurbished, and at Banbury Center, the basement of Sammis Hall was renovated to resolve the Lab-wide need for long-term record storage. Also at Banbury, the severely storm-damaged seawall and jetty were rebuilt with funding provided by the Robertson Maintenance Fund, exhibiting again

Jack Richards with montage of his 25 years at the Laboratory

the foresight of Charles Robertson in endowing the maintenance of the beautiful estate he donated to the Laboratory in 1972.

Jack Richards supervised construction of the Laboratory's wonderful new President's House ("Ballybung") where Jim and Liz Watson are now happily in residence. Together with Peter Stahl, Jack watched over the completion and start-up of our new sewage disposal system that now connects the entire Laboratory campus to the Nassau County sewage lines. Our new system has functioned virtually trouble-free for more than a year and has permanently ended the disposal of treated effluent into the inner harbor. With Cablevision Systems as prime contractor, the installation of our new high-speed ATM data transmission network was completed smoothly and on time with only a minimum disruption of the campus. This installation, consisting of a perimeter bank of 9 underground conduits and 8 manholes connecting 19 major Laboratory buildings with fiber optic cable will provide state-of-the-art data transmission, telephone, and other utility services well into the next century. The new network began operation in late fall.

As of April 1, 1995, Jack Richards completed his 25th year as Director of Buildings & Grounds and has now assumed the new position of Assistant Director for Special Projects. Hopefully, he will also be able to spend a little more time with his wife Corey and his family at their new house in Florida. Jack's contributions to the Laboratory have been too numerous to enumerate, and we feel most fortunate to have his continued availability in years to come. At a dinner on March 30th, held appropriately at the waterfront clubhouse of the Centerport Yacht Club, Jack's years at the Laboratory were celebrated by some 65 members of his department, outside contractors, family members, and friends. Jim Watson ended the memorable evening with the announcement that the new Buildings & Grounds facility will be named the Richards Building. Art Brings, who has been our very effective Director of Environmental Health & Safety, has been appointed to head the Department, succeeding Jack Richards.

The newly integrated Public Affairs and Development Department represents the Laboratory to the local community and outside world via written communications, events, and other activities. It conducts the multitude of fund-raising efforts that have become so important to the entire range of Laboratory programs from science to the DNA Learning Center. Its accomplishments would not be possible without the leadership and management skills of its Director, Susan Cooper, and her dedicated staff.

During 1994, the effort to raise $1 million for scientific equipment for the newly renovated McClintock Laboratory comfortably exceeded its goal, allowing the focus of equipment funding to now turn to the major needs of the neuroscience program. Annual Giving, conducted primarily through the CSHL Association, raised a record of almost $600,000 for science, particularly in support of our younger staff members. Nearly $100,000 was raised for the DNA Learning Center through the Corporate Advisory Board, chaired by Rick Clark, from contributions by Long Island businesses and from the hugely successful first Laboratory golf tournament held in June at

the Piping Rock Club. There was a summer jazz benefit, a return by Peter Orth for his third piano concert here, and numerous "friend-raising" events such as lectures on science and health, the latter co-sponsored by the Huntington Hospital, joint programs with area garden clubs, and tours for the community and other groups. Perhaps most importantly, a new planned giving program called the "Next 100 Years" was launched. Planned giving will be the primary focus of the Laboratory's goal to at least double the current Endowment by the year 2000. Sadly, year-end marked the departure of Joan Pesek after 8 years in the Development Department. Joan was the primary support for the CSHL Association and also made important contributions to the hugely successful Second Century capital campaign. Support for the CSHL Association will now be provided by Jean Schwind formerly in our Grants Office. Deb Rizzieri, Administrative Assistant for 7 years, also departed at year-end as did Claire Fairman. For the past 6 years, Claire was responsible for many of the friend-raising events that have so brightened the Laboratory, and she also played a key role in the capital campaign.

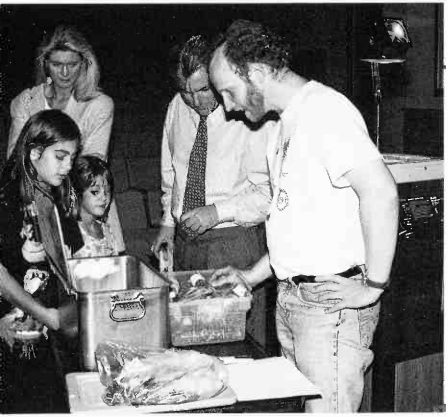
The Library continued to provide a vital resource for the scientific staff and so many others at the Laboratory who marvel at the quiet efficiency of Margaret Henderson, Head of Library Services, and her staff. In the face of the increasing costs of journal subscriptions and book purchases, she continues with limited resources to provide improved services. A particularly gratifying event was the receipt of a grant of $45,000 from the Gladys Brooks Foundation for the purchase of a microfiche, and we are most grateful for a gift of the 1994 edition of the *Encyclopedia Britannica* from Dr. and Mrs. Gervin Marahens.

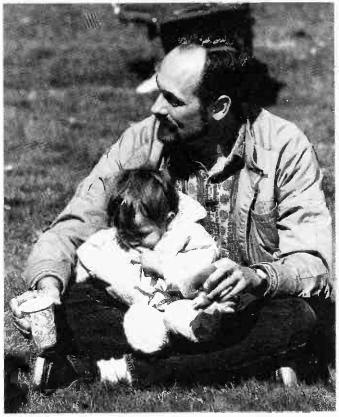
Greatly important to the functioning of the Laboratory is our Controller, Bill Keen, and his Assistant Controller, Barbara Wang, who take responsibility for much of the annual budget preparation and monitoring. Cheryl Sinclair manages the Human Resources Department with much caring and efficiency, and we welcomed in the fall Phil Lembo who joined us as Manager of Purchasing. His predecessor, Sande Chmelev, departed in November, 1994 to join her husband in Moscow. Roberta Salant provides John Maroney and myself with the assistance that is essential to our functioning and also provides for the needs of our very active Board of Trustees.

With 1995 now well under way, we are pleased with an improvement in relations between the Laboratory and the Village of Laurel Hollow. We have tried to listen carefully to concerns raised by local residents and address them fairly. Early in 1994, an agreement was reached with the Cold Spring Harbor School District regarding financial and other forms of support and interaction. New landscaping has been installed to screen a number of our buildings, improving the views from across the harbor and from Route 25A. Motion sensors have been installed to reduce nighttime lighting in many laboratories that must be visited throughout the night by scientists tending experiments or working late. A series of letters from our Chairman, David L. Luke III, and Vice Chairman, Mary D. Lindsay, were sent to local residents discussing the issues of concern, and communicating a sense of the spectacular science under way here at the Laboratory. The draft environmental impact study requested by the Village regarding the Laboratory's proposed child care center and other future buildings plans was prepared by our environmental and land use consultants, Buckhurst Fish & Jacquemart, Inc., and has been submitted for consideration and comments. We believe that village residents will find the study to be professional and very thorough. The Village moratorium on nonresidential construction was enacted in May of 1994 to remain in place pending revision of the local zoning ordinance. We understand that the Village is now progressing with the planned revision to the ordinance and we look forward to being included in discussion of the provisions directly affecting our property. Through dialogue, the Laboratory and the Village can go forward together with cooperation and mutual respect.

G. Morgan Browne
Administrative Director

June 15, 1995





RESEARCH

(*Top*) Kim Arndt; Dafna Bar-Sagi; Tao Zhong. (*Center*) Rob Martienssen (*left*) showing ears of corn to Jack Pryor and his students after Rob's talk on Maize Genetics. Winship and Isabella Herr at "Easter Hunt." (*Bottom*) John, Ian, and Mary Horton (*left*), and Rachael Frenguelli, Robert, Elinor, and Bethan Hulse (*right*) at "Easter Hunt" at Airslie.

TUMOR VIRUSES

The Tumor Virus section originated from the idea that DNA tumor viruses are excellent probes to uncover the mechanisms of cell function and to identify the changes a cell undergoes when it becomes transformed. Although much of the research has diversified as new methods to isolate cellular genes and to dissect their structure and function have developed, the guiding principles and questions remain remarkably similar. For example, Bruce Stillman and Eileen White of the DNA Synthesis section initiated studies over a decade ago that have led to the realization that the product of the adenovirus E1B 19K gene protects virally transformed cells from committing suicide by programmed cell death or apoptosis. Although we no longer study the function of the E1B 19K protein, the cellular proteins involved in the mechanics and regulation of apoptosis are now being studied by Yuri Lazebnik, a new member of this section. The research has also diversified into the study of non-DNA tumor viruses, such as herpes simplex virus (HSV) and human immunodeficiency virus (HIV). Indeed, Jacek Skowronski, a long-standing colleague of ours who this year joined this section, studies how HIV influences the regulation of cellular functions. As is evident from the following descriptions, the addition of these two new members and the continuing strength of the research by long-standing members have produced a vital research program.

DNA SYNTHESIS

B. Stillman	C. Prives	M. Hidaka	A. Verreault	J. Mitchell
	J. Scott	P. Kaufman	S. Waga	H. Rao
	M. Akiyama	R. Li	M. Weinreich	L. Borzillo
	S. Bell	C. Liang	K. Fien	V. Filadora
	G. Cullmann	T. Melendy	K. Gavin	R. Yates
	V. Ellison	C. Mirzayan	Y. Marahrens	C. Dreissens

Research over the last year continued to focus on the replication of DNA in eukaryotic cells. As in the past, we have studied in parallel two different experimental systems. The first of these is to understand the replication of DNA that has been initiated at the simian virus 40 origin of DNA replication so that we may gain insight into the mechanism of replication fork movement and the control of this process. In addition to these studies, progress on the understanding of the initiation of chromosomal DNA replication in eukaryotic cells has been facilitated by the study of this process in the yeast *Saccharomyces cerevisiae*. During the past year, we have observed links between DNA replication and other events in the cell division cycle, such as control of cell cycle progression and DNA repair. Moreover, studies on the initiation of chromosomal DNA replication are beginning to recognize links between this process and controls of cell cycle progression. Finally, we have continued to study the mechanism of assembly of chromatin during DNA replication, and work over the past year has led to considerable advances in our understanding of this replication-coupled process.

SV40 DNA Replication

L. Borzillo, G. Cullman, V. Ellison, K. Fien,
T. Melendy, C. Prives, S. Waga,
R. Yates, B. Stillman

In last year's Annual Report, we described experiments that led to the complete reconstitution with purified proteins of DNA replication from the simian virus 40 (SV40) origin. In addition to the virus-encoded SV40 T antigen, many replication proteins that have been purified from human cells are required for this process. These proteins include DNA polymerase α and δ and their accessory proteins, the

proliferating cell nuclear antigen (PCNA), replication factor C (RFC), and the single-stranded DNA-binding protein called replication protein A (RPA). Also required for complete replication are three enzymes that cooperate with the DNA polymerases to synthesize efficiently Okazaki fragments on the lagging strand. These enzymes are maturation factor 1 (MF1, henceforth called FEN1), DNA ligase 1, and ribonuclease H1. These studies have led to considerable insight into the mechanism of eukaryotic DNA replication and the realization that the essentials of this replicative process are conserved from bacteria to humans. One enigma that remains is the role of DNA polymerase ε in the replication of DNA. Experiments from other investigators in the yeast *S. cerevisiae* have demonstrated a role for this essential DNA polymerase in S-phase progression in vivo, but the polymerase appears not to be required for replication in vitro. It is possible that DNA polymerase δ can substitute for polymerase ε in the purified system or that upon fractionation of the crude cell extract, we have lost proteins that cause polymerase ε to be essential.

For many years, we have focused on the role of PCNA in the replication of SV40 DNA in vitro. In the last year, David Beach's laboratory discovered that PCNA was present in a quaternary complex with cyclin-dependent protein kinases (CDKs) and their cyclin subunits together with a novel protein called p21. It is apparent that all cyclin/CDK complexes can associate with PCNA and p21, although cyclin E/CDK2 and cyclin A/CDK2 appear to have high affinity for the p21/PCNA complex. The Beach laboratory cloned a cDNA from human cells that encodes the p21 protein and found the expressed recombinant protein to be a potent inhibitor of CDK activity. Since CDK cyclin complexes are required for progression through multiple stages of the cell cycle, expression of the p21 protein in mammalian cells inhibited cell cycle progression. cDNA encoding p21 was also isolated at about the same time in other laboratories and the protein has been alternatively called WAF1, SDI1, or CIP1. Transcription of the gene encoding the p21 protein was found to be induced following DNA damage in a process dependent on the p53 tumor suppressor protein. Moreover, p21 was found to be present at high levels in senescent and terminally differentiated cells. In collaboration with members of the Beach laboratory, we have characterized the interaction between this interesting CDK inhibitor and PCNA.

Addition of increasing amounts of p21 to DNA replication reactions containing a crude extract from human cells or purified human proteins demonstrated that the p21 CDK inhibitor was a potent repressor of SV40 origin-dependent DNA replication in vitro. The observation that p21 inhibited SV40 origin-dependent DNA replication in a highly purified reconstituted system which did not require CDK cyclin complexes suggested that p21 was having an effect on DNA replication independent of its CDK inhibitory function. Using purified human cell proteins, it was demonstrated that p21 blocked the ability of PCNA to stimulate DNA polymerase δ, thereby explaining the inhibition of SV40 DNA replication. Thus, the p21 protein appeared to be bifunctional because it could inhibit CDK activity and also inhibit PCNA's function in DNA replication. This observation created a paradox, however, because PCNA apparently is required for nucleotide excision repair in cells that express high levels of p21 protein due to transcriptional induction of the p21 gene by p53.

We therefore investigated in vitro the effect of p21 on nucleotide excision repair of UV-induced damage in DNA. Using a partially reconstituted cell-free system for repair of UV-damaged DNA that had been described previously by R. Wood and T. Lindahl at the Imperial Cancer Research Fund in England, we confirmed that these repair reactions were dependent on PCNA. Surprisingly, however, addition of increasing amounts of the p21 CDK inhibitor did not affect the repair of DNA damage in vitro. Yet in the same extract, SV40 T antigen and *ori*-dependent DNA replication was completely inhibited. Thus, the p21 protein was able to discriminate between PCNA-dependent replication and repair, allowing DNA repair while inhibiting DNA replication. Using purified proteins, we demonstrated that DNA polymerase ε, another eukaryotic cell DNA polymerase implicated in both DNA replication and DNA repair, appeared to be more refractory than polymerase δ to the inhibition of p21.

These results obtained in vitro suggest that the p21 CDK inhibitor is a key coordinator of control of cell cycle progression and DNA replication. If DNA damage occurred at any stage of the cell cycle, then a p53-dependent increase in transcription of the gene encoding p21 could lead to higher levels of the p21 protein, simultaneously blocking cell cycle progression and elongation of DNA replication, but allowing DNA repair in these cells. We have recently begun to test this model by examining the localization of p21

during the cell cycle in mammalian cells and in the nucleus following DNA damage.

A key protein that also associates with PCNA is RFC. RFC is essential for SV40 DNA replication in vitro and recognizes primer template junctions to load PCNA onto the DNA. PCNA then functions as a DNA polymerase accessory protein to stimulate the processivity of DNA polymerase δ during SV40 DNA replication. RFC is a complex protein consisting of five polypeptide subunits in both human and mammalian cells. In past years, we have reported the purification of RFC from both yeast and human cells. To examine further the biochemical functions of RFC and its role in DNA replication, we have cloned each of the cDNAs encoding the five human subunits into baculovirus vectors so that the RFC proteins may be expressed individually or in combination. Preliminary data suggest that we can reconstitute the RFC complex from these recombinant proteins. In parallel, each of the genes encoding RFC from the yeast *S. cerevisiae* has been cloned and shown to be essential for cell viability. The genes from yeast and human cells are highly related to each other, and in both species, each of the five proteins shares extensive sequence similarity. We are currently performing biochemical and genetic experiments to further examine the function of RFC and its interaction with other cellular proteins, including PCNA.

DNA Replication-dependent Chromatin Assembly

V. Filadora, P. Kaufman, A. Verreault, B. Stillman

A number of years ago, we described the establishment of a cell-free system that is capable of assembling chromatin during SV40 DNA replication in vitro. In this cell-free system, a three-subunit protein called chromatin assembly factor 1 (CAF1) is required for the assembly of nucleosomes concomitant with passage of the replication fork during DNA replication. cDNAs encoding each of the subunits from CAF1 have been isolated and expressed in insect cells by infection with a recombinant baculovirus vector. Using these recombinant proteins, we have further characterized the mechanism of DNA-replication-dependent chromatin assembly.

Both the CAF1 p150 and p60 proteins are essential for chromatin assembly. These proteins interact with each other and cooperate to assemble nucleosomes onto replicating DNA. In addition to CAF1 and the essential DNA replication proteins described above, other proteins are required for chromatin assembly in vitro. We have previously identified histones H2A and H2B as essential for nucleosome assembly, but interestingly, histones H3 and H4 purified from chromatin inhibit the DNA-replication-dependent chromatin assembly reaction. We therefore hypothesized that modified forms of histones H3 and H4 would be required for this reaction, and in the past year, we have demonstrated that CAF1 binds to acytelated forms of histone H3 and H4. These acytelated forms of histone H4 correspond to those that are newly synthesized following translation of the protein. Newly synthesized histone H4 is modified by acytelation of specific lysine residues near the amino terminus of the protein. These studies suggest that CAF1 may be a molecular link between the synthesis of newly synthesized histones H3 and H4 and chromatin assembly at the DNA replication fork. In addition to proteins associated with these modified forms of histones H3 and H4, we have identified other proteins that associate specifically with CAF1 and are currently characterizing them.

Cell Chromosome Replication

M. Akiyama, S. Bell, K. Gavin, M. Hidaka, C. Liang, Y. Marahrens, C. Mirzayan, J. Mitchell, H. Rao, J. Scott, M. Weinreich, B. Stillman

Our studies on viral DNA replication have led to an understanding of the mechanism of replication fork progression. Because the SV40 T antigen plays multiple roles in the initiation of DNA replication, an understanding of SV40 DNA replication has not led to insight into the mechanism and control of initiation of cell chromosomal DNA replication. We have therefore studied in parallel with SV40, DNA replication in the yeast *S. cerevisiae* because of the opportunity to combine biochemical and genetic approaches to understanding this complex process. In previous years, we have reported the identification of an origin recognition complex (ORC), a six-subunit protein that binds to origins of DNA replication in the yeast genome. Previous work has demonstrated that ORC binds to replicator sequences in an ATP-dependent manner and requires the degenerate 11-base-pair consensus sequence found within the A ele-

ment of all autonomously replicating sequences (ARSs) in the yeast genome.

Work over the last year has demonstrated clearly that for efficient sequence-specific DNA binding, ORC also requires the previously identified B1 DNA element that contributes to replicator function. In the absence of the B1 element, ORC binds to DNA, but binding is not sufficiently tight to allow complete protection from digestion of the DNA with a nonspecific nuclease. In contrast, in the presence of both the A and the B1 elements, ORC binds tightly to the DNA and forms a stable complex. Concomitant with this binding is the induction of several nuclease hypersensitive sites in the DNA. Work from John Diffley's laboratory at the Imperial Cancer Research Fund in the U.K. has demonstrated that these hypersensitive sites vary in intensity throughout the cell cycle, suggesting that regulation of DNA replication may be in part due to binding of proteins to ORC on the DNA only at certain stages within the cell division cycle. We conclude from these studies that ORC binds to a bipartite DNA-binding site within yeast replicators and that these sequences are conserved among the various replicators in the yeast genome. Moreover, ORC binding to these sequences probably marks the DNA as a target for establishing active origins of DNA replication.

Through collaborative studies with Jasper Rine's laboratory at the University of California, Berkeley, we have identified a number of temperature-sensitive mutants in the genes encoding *ORC2* and *ORC5*. In the last year, we have further characterized the phenotype of these temperature-sensitive mutants with respect to the role of ORC in DNA replication. In parallel, Jasper Rine's laboratory has continued to study the role of the ORC proteins in silencing of transcription at the silent mating-type loci that control yeast sexual development. Using two different physical mapping techniques that determine the location of origins of DNA replication in the yeast genome, and in particular, two-dimensional agarose gel electrophoresis techniques originally published by Bonita Brewer and Walter Fangman at the University of Washington, Seattle, we have examined the effect of mutations in the *ORC2* and *ORC5* genes on initiation of chromosomal DNA replication. At both the *ARS1* and *ARS501* loci on chromosomes IV and V, respectively, initiation of DNA replication in wild-type cells occurs with near 100% efficiency every cell cycle. In contrast, in both the *orc2-1* and *orc5-1* mutants, initiation of DNA replication is much less efficient, even at the permissive temperature for these temperature-sensitive mutants. Upon a shift to the nonpermissive temperature, initiation of replication at these two loci is reduced dramatically and the cells are no longer able to grow. We interpret these results as demonstrating that ORC determines the frequency of initiation of DNA replication in the genome by binding to specific DNA sequences and thereby locating an origin of DNA replication at that site. In the *orc* mutants where the amount of ORC is greatly reduced, the frequency of initiation in the genome is lower than in wild-type cells. Because of the redundancy of replicator sequences in the genome, at the permissive temperature, the cells can still grow and divide. In contrast, at the nonpermissive temperature where there is even less active ORC protein, the frequency of initiation in the genome is so low that cells cannot complete S phase in the time normally taken to proceed through the cell division cycle. As a consequence, these cells die.

To identify proteins that may interact with the ORC proteins, we have initiated a number of genetic screens to search for interacting proteins. One such gene identified by high-copy-number suppression of the *orc5-1* mutant was *CDC6*. Overexpression of the CDC6 protein in the *orc5-1* mutant allowed cells to grow at 37°C, the nominal nonpermissive temperature for the temperature-sensitive mutant; however, these cells grew slower than when they were complemented by the wild-type *ORC5* gene. Using two-dimensional gel electrophoresis, we have demonstrated that the frequency of initiation at replicators in the genome is increased by overexpression of CDC6 protein in the *orc5-1* cells, thereby enabling the cells to grow at 37°C. Overexpression of CDC6, however, does not restore initiation to 100%, and the cells grow more slowly than wild-type cells. These experiments led us to investigate whether a temperature-sensitive mutant in the *CDC6* gene might also be defective for initiation of DNA replication; indeed, two-dimensional gel origin-mapping techniques have demonstrated that the levels of CDC6 protein also determine the frequency of initiation of DNA replication in the genome. A link between *CDC6* and the *ORC6* gene has previously been reported by Joachim Li and Ira Herskovitz because overexpression of *ORC6* in a *cdc6* mutant strain causes inviability. Furthermore, in collaboration with Jasper Rine's laboratory, we have demonstrated that *cdc6* is synthetically lethal with both *orc2-1* and *orc5-1* mutants.

These genetic results encouraged us to search for a physical interaction between the ORC proteins and CDC6. The *CDC6* gene was cloned into a recombinant baculovirus vector, and the CDC6 protein was produced upon infection into sf9 insect cells. When an extract from these cells was made and mixed with purified ORC protein, the recombinant CDC6 protein could be immunoprecipitated with anti-ORC monoclonal antibodies. CDC6 was not immunoprecipitated with anti-ORC monoclonal antibodies or when ORC was omitted from the sf9 cell extract. These data suggest that ORC and CDC6 physically interact with each other and cooperate to determine the frequency of initiation of DNA replication in the yeast genome.

The CDC6 protein is of considerable interest because it is highly related to a protein from *Schizosaccaromyces pombe* called $cdc18^+$. $cdc18^+$ was identified previously by Tom Kelly, Stephen Martin, and Paul Nurse at the Imperial Cancer Research Fund in the U.K. as a protein that is required for entry into S phase in *S. pombe*. Interestingly, $cdc18^+$ is also required for prevention of entry into mitosis until DNA replication is completed. Other studies have suggested that CDC6 from *S. cerevisiae* may also be involved in controlling the G_2-to-M phase transition. Thus, our studies link an important cell cycle regulatory protein to the initiation of DNA replication by binding to the ORC proteins.

We have also pursued a number of other genetic studies that point to additional proteins that may interact with ORC and control DNA replication in the cell cycle. Furthermore, we have cloned cDNAs encoding the ORC proteins from a number of other species, including humans, and this will ultimately facilitate the identification of origins of DNA replication in metazoan species.

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MOLECULAR BIOLOGY OF PAPILLOMAVIRUSES

A. Stenlund M. Berg J. Sedman
T. Sedman M. DaCosta

The papillomaviruses infect and transform the basal epithelium in 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 16, 18, 31, 33, and 39) give rise to tumors that are prone to progress toward malignancy, especially frequently cervical carcinoma. In total, HPV DNA can now be found in biopsies from approximately 80% of all cervical carcinomas.

A key impediment to the study of human papillomaviruses has been the inability to define a simple in vitro cell culture system for these viruses. This is largely due to the fact that these papillomaviruses normally require specialized differentiating cells that only with great difficulty can be generated in cell culture. Therefore, a bovine papillomavirus (BPV-1) has become the prototype virus for the papillomavirus group largely because a convenient cell culture system exists for this virus where 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 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 previous years, we have reported the development of a short-term replication assay that has enabled us to define the viral components required for viral DNA replication. More recently, we have directed our attention toward detailed biochemical analysis of the replication process. We have continued to refine the expression and purification of the two viral proteins (E1 and E2) that are required for viral DNA replication as well as continued the analysis of the initiation complexes that are formed on the Ori. We have also adapted and modified a cell-free replication system that was originally developed in M. Botchan's laboratory. From these studies, we now have a detailed picture of the relative roles of the E1 and E2 proteins in replication. E1 has all the characteristics of an initiator protein, including ori recognition, DNA dependent ATPase activity, and DNA helicase activity. E1 can also function to unwind a supercoiled plasmid that contains the Ori sequence. The E2 polypeptide, whose function has remained more elusive, appears to serve simply as a specificity factor for E1. Through an interaction with both E1 and the ori, E2 provides sequence specificity in the formation of the initiation complex (Fig. 1). This year, we have also completed some work (in collaboration with T. Melendy in Bruce Stillman's laboratory) to identify the cellular factors that are required for viral DNA replication. The results from this study indicate that a number of the replication factors that have been identified as being required for replication of SV40 are also required for BPV replication; however, one or more additional factors appear to be required for BPV replication.

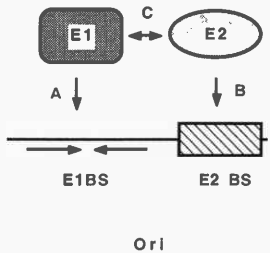

FIGURE 1 Schematic representation of the interactions between E1 and E2 proteins and the BPV origin of replication. The sum of the three interactions, E1 with DNA, E2 with DNA, and E1 with E2, determines the stability of the complex and the replication activity of the ori.

Formation of Initiation Complexes

T. Sedman, A. Stenlund

The smallest sequence element from BPV that can direct initiation of replication in vivo (ori) is approximately 60 nucleotides long. This sequence contains three different, separable, elements. Two of these elements have known functions and constitute the binding sites for the viral E1 and E2 proteins, respectively. The third element has been termed the A+T-rich region and its function is unknown. Our earlier genetic experiments have demonstrated that the presence of these elements is necessary for replication activity. We have developed a series of assays to study the binding of E1 and E2 to the ori sequence to determine the requirements for binding and for formation of productive replication complexes. By a combination of DNase protection, gel retardation, and interference assays, we have established that E1 is capable of binding to the Ori in at least two different forms, either together with E2 to form an E1/E2/ori complex or by itself to form a multimeric E1/ori complex. The ori/E1/E2 complex is formed by cooperative binding of E1 and E2 to Ori and requires low concentrations of E1. The E1/ori complex requires higher concentrations of E1 and forms on the same ori sequence, but it does not require E2- or E2-binding sites for its formation. To determine which of these complexes are important for replication in vivo, we have generated a large number of point mutations in the ori. These mutants have been tested for their ability to form the two different complexes and for replication. Mutations in the E1-binding site reduce the ability to form both complexes and reduce replication.

Mutations in the E2-binding site do not affect the formation of the E1 complex, reduce the ability to form the E1/E2/ori complex, and also reduce replication. The sum of these results indicated that the ability to form the E1/E2/ori complex is important for replication activity in vivo. The role of the E1/ori complex, however, was unclear, since all the mutations that affected the formation of the E1/ori complex also affected the E1/E2/ori complex. To circumvent this problem, we combined mutations that reduce the affinity of the E1-binding site with mutations that increase the affinity of the E2-binding site. These mutants, which have a reduced ability to form the E1/ori complex but retain the wild-type ability to form the E1/E2/ori complex, replicate at wild-type levels, indicating that the ability to form the E1/ori complex is not limiting for replication.

DNA Replication In Vitro

J. Sedman, A. Stenlund

As indicated above, we have demonstrated that replication in vivo requires the formation of an E1/E2/ori complex. The formation of this complex requires binding sites at the ori for both E1 and E2 proteins. For replication in vitro under standard conditions, E1 alone is sufficient to initiate replication in an ori-specific manner, and addition of E2 has only minor effects on replication. Furthermore, replication in vitro does not require an E2-binding site at the ori. These results present an apparent paradox; in the absence of E2 and an E2-binding site, the E1/E2/ori complex obviously cannot form. Consequently, replication can clearly be initiated in vitro without the formation of the E1/E2/ori complex. A possible resolution to this paradox could be that assembly of a replication complex is a multistep process where formation of the E1/E2/ori complex is a required early intermediate which can be bypassed under the conditions used for in vitro replication. One clear difference between the in vitro and in vivo conditions is the presence of vast quantities of competing DNA sequences under in vivo conditions. Since E1 appears to have a relatively modest sequence specificity, E2 might function to increase the selectivity of binding. By challenging binding of E1 with nonspecific competitor DNA, in the absence or presence of E2, we could demonstrate that the sequence specificity of E1 is greatly increased in the presence of E2. On the basis of these binding studies, we have modified the conditions used for replication in vitro by generating conditions where DNA binding of E1 is limiting for replication. This modification results in a complete dependence on E2 protein and the presence of an E2-binding site for replication, resulting in an in vitro system that in all important aspects reflects the requirements observed for replication in vivo. These results also establish that E2 is likely to function as a specificity factor for E1 and probably does not take part directly in the replication process.

Cellular Factors Required for Replication of BPV In Vitro

J. Sedman, A. Stenlund [in collaboration with T. Melendy and B. Stillman, Cold Spring Harbor Laboratory]

The SV40 system has for a number of years served as the paradigm for DNA replication in mammalian cells. This system relies on the viral initiator large T antigen for ori recognition, ori unwinding, and helicase activity. With the use of this system, a number of cellular factors involved in DNA synthesis have been purified and characterized. A minimal purified system, which contains all the cellular factors that are required for SV40 replication, has been developed by B. Stillman and co-workers. For a number of homologs of these factors, an involvement in chromosomal DNA replication has been demonstrated through genetic experiments in *Saccharomyces cerevisiae*.

To determine if BPV utilizes the same set of cellular replication factors that are required for SV40 replication, we have tested the requirement for a number of these factors in a BPV in vitro replication system. All the factors required for SV40 replication also appear to be involved in BPV replication; however, the purified minimal system that can replicate SV40 is not capable of replicating BPV, indicating that one or more additional factors are required for BPV replication. In addition, the degree of dependence on some of the known factors appears to be different. For example, substantial amounts of replication can be achieved in the SV40 system in the absence of RFC or PCNA: The BPV system is essentially devoid of activity in the absence of these factors. These results indicate that mechanistic differences exist between the replication systems for SV40 and BPV.

Physical Interactions between the E1 and E2 Proteins

M. Berg, A. Stenlund

In a majority of the eukaryotic replicons that have been studied so far, it has been observed that a transcriptional component is associated with the origin of replication. In most of these systems, including SV40, polyomavirus, and ARS elements from *S. cerevisiae*, the required activity can be supplied by various transcriptional *trans*-activators with little apparent specificity.

A similar requirement also exists for papillomavirus replicons; however, the requirement is much more specific and only the virus-encoded transcription factor E2 can serve as an auxiliary factor for replication. The requirement for E2 in replication of BPV extends beyond a mere requirement for E2 bound to the ori; a physical interaction with E1 is also required. This interaction can be detected as cooperative binding of the two proteins to the Ori, when the respective binding sites are located in the correct position relative to each other. The E2 proteins are well conserved between different papillomaviruses, and they appear to have a conserved overall structure. For example, the sequence specificity for DNA binding is identical for E2 proteins from all papillomaviruses tested so far. When tested for their ability to bind cooperatively with BPV E1, some E2 proteins from other papillomaviruses fail to show detectable interaction with BPV E1.

This observation presented us with an opportunity to map the regions of E2 that were required for this interaction by construction of chimeric proteins between BPV E2, on the one hand, and HPV11 E2, on the other hand. These chimeras could then be tested for their ability to interact physically with BPV E1 in a biochemical assay and for the ability to support replication in a functional assay. This approach has a distinctive advantage over conventional mutagenesis in that the overall structure of the protein can be maintained. Following this procedure we have generated and tested a large number of chimeric E2 proteins. The results from these experiments have revealed a more complex situation than we had anticipated. Multiple regions from both the amino-terminal *trans*-activation domain of E2 as well as from the carboxy-terminal "hinge" and DNA-binding/dimerization domain, cooperate to generate a strong interaction with E1. Two regions of the BPV E2 protein are specific for interaction with E1, the corresponding domains in HPV E2 do not interact. At least one other region of BPV E2 located in the amino-terminal half of the protein and coinciding with the *trans*-activation domain interacts with E1; however, this interaction is not specific to BPV E2 but is also shared with HPV E2.

TRANSCRIPTIONAL REGULATION

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We study the mechanisms of transcriptional regulation in eukaryotes. To probe these mechanisms, we use both cellular and viral regulatory factors, including two cellular POU-domain proteins, Oct-1 and Oct-2, and a herpes simplex virus (HSV) protein, VP16. Although the cellular proteins Oct-1 and Oct-2 possess the same intrinsic DNA-binding specificity, their transcriptional regulatory properties are different. For example, both proteins recognize the octamer sequence ATGCAAAT found in a variety of promoters, but the efficiencies with which they activate such promoters differ. These differences result from the activities of promoter-selective transcriptional activation domains that can discriminate among promoters. The viral protein VP16 is a component of the HSV virion that, after infection, associates with Oct-1 and a second cellular factor called HCF to form a multiprotein-DNA regulatory complex. By its association with Oct-1, VP16 alters the transcriptional regulatory properties of Oct-1 in two ways: It recruits Oct-1 to new DNA sites that are not recognized effectively by Oct-1 alone, and it provides a potent transcriptional activation domain to activate the HSV immediate-early promoters, a type of promoter that is normally resistant to activation by Oct-1.

Our studies continue to focus on four issues:

1. How does a eukaryotic transcription factor recognize a diverse and yet specific set of DNA sequences?

2. How is a multiprotein-DNA regulatory complex, such as the VP16-induced complex, assembled?

3. What are the rules for how transcriptional activation domains and the basal transcriptional apparatus communicate with one another?

4. What are the effects of variation in promoter structure in naturally occurring human immunodeficiency virus (HIV) promoters isolated from infected individuals? Below are descriptions of accomplishments we made during the past year.

POU Domain Interaction with DNA and VP16

M. Cleary, C. Huang

DNA binding by the Oct-1 protein is directed by its POU domain, a bipartite DNA-binding domain made up of two helix-turn-helix-containing DNA-binding modules that cooperate in DNA recognition: the POU-specific (POU_S) and POU-homeo (POU_H) domains. Although the highest-affinity Oct-1 DNA-binding site contains the octamer sequence ATGCAAAT, Oct-1 displays significant flexibility in the range of sequences to which it binds. For example, Oct-1 recognizes a form of the HSV VP16-responsive TAATGARAT element, called the ($OCTA^-$)TAATGARAT site, that lacks octamer site similarity.

We have been studying mechanisms by which the Oct-1 POU domain achieves sequence recognition flexibility by examining the contribution of the POU_S domain to the overall binding ability of the entire POU domain. Analysis of the effects of amino acid substitutions in the POU_S domain and base substitutions in the Oct-1 binding sites has shown that the two subdomains of the POU domain can adopt different orientations on different binding sites. We have shown that on the ($OCTA^-$)TAATGARAT site, the POU_S domain is positioned over the GARAT sequence, on the side of the POU_H domain opposite from where it is located on an octamer site.

This flexibility of the Oct-1 POU domain has an impact on its participation in the VP16-induced complex. Oct-1 POU domain residues that contact DNA have different effects on VP16-induced complex formation, depending on whether or not the VP16-responsive element involved has an overlapping octamer sequence (called [$OCTA^+$]TAATGARAT). On an ($OCTA^+$)TAATGARAT site, the POU_S domain is probably positioned over the octamer sequence, away from the GARAT sequence which is necessary for VP16-induced complex formation. Here, mutations

in the POU_S domain that affect DNA binding affect VP16-induced complex formation in a manner that parallels their effect on affinity for the site. In contrast, on the (OCTA⁻)TAATGARAT site, where the POU_S domain and VP16 may have superimposed interactions, the effects of these same mutations do not correlate directly with affinity for the site. Instead, some mutations have a much greater effect on VP16-induced complex formation than on DNA binding alone, suggesting a specific influence of these residues on VP16-induced complex formation. Thus, through its ability to bind DNA flexibly, Oct-1 can differentially influence VP16-induced complex formation on different VP16-responsive elements.

To study VP16 in more detail, we are determining its structure by X-ray crystallography in collaboration with Dr. Xiaodong Cheng and colleagues at Cold Spring Harbor Laboratory. Additionally, we continue to study VP16 structure and function by mutagenesis. A comparison of VP16 sequences from four herpesviruses has revealed five conserved regions in the core of VP16 responsible for association with Oct-1 and HCF on DNA. These regions are important for VP16-induced complex formation, because amino acid substitutions in any one of these conserved segments can disrupt formation of the VP16-induced complex.

As shown in Figure 1, we are also analyzing the activity of the HSV VP16 homolog in bovine herpesvirus (BHV). The BHV VP16 protein forms a VP16-induced complex with human Oct-1 and HCF on a binding site from the BHV ICP4 promoter but, interestingly, not on an HSV (OCTA⁺) or (OCTA⁻) TAATGARAT site. Furthermore, the HSV VP16 protein does not form a VP16-induced complex on the BHV site. This result shows that Oct-1 can function in combination with different transcriptional cofactors to generate different regulatory specificities.

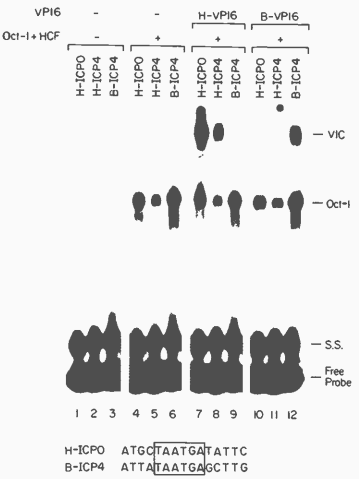

FIGURE 1 Bovine herpesvirus (BHV) VP16 displays different DNA-binding specificities for VP16-induced complex formation than herpes simplex virus (HSV) VP16 in an electrophoretic mobility retardation assay. Three different DNA probes were used. (Lanes *1, 4, 7, 10*) H-ICP0 (OCTA⁺) TAATGARAT probe from the HSV ICP0 promoter; (lanes *2, 5, 8, 11*) H-ICP4 (OCTA⁻)TAATGARAT probe from the HSV ICP4 promoter; (lanes *3, 6, 9, 12*) B-ICP4 TAATGARAT probe from the BHV ICP4 promoter. Partially purified human HeLa cell Oct-1 and HCF (lanes *4–12*) were used either alone (lanes *4–6*) or with added HSV (H-VP16; lanes *7–9*) or BHV (B-VP16; lanes *10–12*) VP16 protein. The position of the free probe, contaminating single-stranded DNA (S.S.), Oct-1, and VP16-induced complex (VIC) is shown to the right. Note that the HSV and BHV VP16-induced complexes display the opposite binding site preference (compare VIC in lanes *7–12*).

Structure and Function of HCF: A Cellular VP16 Cofactor

A. Wilson, R. Freiman, L. Peña [in collaboration with M.G. Peterson, Tularik, Inc.]

HCF is involved in initiating HSV gene expression after infection by promoting complex assembly between VP16 and Oct-1, but the normal cellular function of HCF is unknown. Its ubiquitous presence in proliferating cells and its high conservation among mammals, insects, and nematodes suggest an important role in cell function. Human HCF consists of a series of polypeptides between 110 and 300 kD in size. All of these polypeptides are encoded by a single large 2035-codon open reading frame (ORF) that initially gives rise to the largest (300 kD) polypeptide. This precursor is then processed in vivo through proteolytic cleavage to generate smaller amino- and carboxy-terminal polypeptides that remain tightly but noncovalently associated.

HCF contains two novel repeated sequence motifs: One directs HCF processing and the other is

involved in VP16-induced complex formation. The first repeat motif—the HCF repeat—is present as six nearly perfect copies located toward the center of the primary translation product. In collaboration with M.G. Peterson, we have now demonstrated that proteolytic cleavage occurs near the center of this repeat between a glutamic acid and threonine residue, in the sequence CETH, and that transfer of a single HCF repeat into an unrelated protein (Oct-1) is sufficient to signal specific cleavage of that hybrid protein. Subcellular fractionation experiments suggest that HCF processing occurs within the nucleus. The relatively slow kinetics of HCF processing combined with our inability to recapitulate processing in vitro are consistent with this being a regulated event associated with HCF function.

The second repeat motif is present as six copies clustered at the amino terminus of HCF. Unlike the HCF repeats, this second repeat motif occurs in a variety of proteins from organisms as diverse as fungi and plants. In flu virus neuraminidase and a fungal galactose oxidase, similar repeats are known to form a "super-barrel" structure of six or seven β-sheets, respectively, each made up from four β-strands. Our mapping studies show that this 380-amino-acid putative HCF super-barrel structure interacts with VP16 and is sufficient for VP16-induced complex formation. Identification of this discrete domain within HCF is an important advance in our understanding of VP16-induced complex assembly.

To explore the natural cellular function of HCF, we have taken two approaches: In one case, we are identifying and characterizing human proteins that associate with HCF, and, in the second case, we are studying the structure and function of HCF in a more genetically tractable organism, the nematode *Caenorhabditis elegans*. To identify HCF-interacting proteins, we have used the yeast two-hybrid assay. We fused the amino-terminal VP16-interaction domain of human HCF to the yeast GAL4 DNA-binding domain and used this fusion protein to screen a HeLa cDNA transcriptional activation domain fusion library for cDNAs encoding HCF-interacting proteins. In this manner, a human cDNA encoding a bZIP protein was isolated, suggesting a role for HCF in regulation of cellular transcription. To investigate the function of HCF in *C. elegans*, we have monitored its expression through *C. elegans* development by use of an electrophoretic mobility retardation assay. In extracts from developmentally staged worms, HCF activity peaked in the L1 larval stage. These results suggest that HCF is developmentally regulated.

Transcriptional Activation by Promoter-selective Activation Domains

G. Das, C. Hinkley

In eukaryotes, activation of transcription involves an interplay between activators bound to *cis*-regulatory elements and factors bound to basal elements near the Start site of transcription. The basal elements, such as the TATA box, nucleate the assembly of basal transcription complexes, components of which interact with the activation domains of activators. Although in vitro studies have addressed the general interaction of activators with the basal transcription machinery, the importance of basal elements in directing different responses to activators has not been described. We have now shown in vivo that changes in the arrangement of basal elements within a promoter can switch its response to activation domains.

We used the human U6 small nuclear RNA (snRNA) promoter as a model to study the effects of changes in basal element organization on activator function, because this promoter naturally contains both a TATA box and an snRNA-specific proximal sequence element called the PSE. Both of these elements bind multiprotein complexes containing the TATA box-binding protein TBP (see N. Hernandez, this Annual Report). By mutational analysis, we found that the presence of just the TATA box or the PSE element within the U6 snRNA promoter results in diametrically opposed responses to the acidic activation domain from VP16 and a glutamine-rich activation domain from the human transcription factor Sp1. These findings provide a mechanism for differential regulation of transcription by activators that is based on the identity of basal promoter elements rather than of upstream elements.

We have previously shown that the octamer motif-binding proteins Oct-1 and Oct-2 also display promoter-selective properties: Oct-1 activates a PSE-containing snRNA promoter better than Oct-2, and Oct-2 activates a typical TATA box-containing mRNA promoter better than Oct-1. We have now shown that the promoter-selective properties of Oct-1 and Oct-2 can be reproduced by tandem reiteration of short 19- and 18-amino-acid segments derived from

the glutamine-rich activation domains of these two proteins (called Q^{19} and Q^{18}, respectively). Because there is only a five-amino-acid difference between the Q^{19} and Q^{18} segments, we individually substituted the Oct-2 Q^{18} residues with their Oct-1 Q^{19} counterparts and examined the effect of these substitutions on activation of mRNA and snRNA promoters. Four of these substitutions had little or modest effects on selective activation of an mRNA and an snRNA promoter compared to wild-type Q^{18}. In contrast, substitution of an important phenylalanine residue in Q^{18} with the two corresponding Oct-1 residues almost completely eliminated mRNA promoter activation by Q^{18} and instead activated snRNA transcription-like Oct-1.

These results demonstrate that small differences in activation domains can have a profound influence on their ability to activate transcription in different promoter contexts.

Response of TBP to Transcriptional Activators In Vivo

W. Tansey [in collaboration with S. Ruppert and R. Tjian, University of California, Berkeley)

To elucidate mechanisms of transcriptional activation, we have studied the response of the basal transcriptional machinery to different transcriptional activator proteins in vivo. We have focused our attention on the TATA box-binding protein TBP and have analyzed the effects of mutations on the surface of TBP on its ability to respond to various types of transcriptional activators in human cells. By use of a TBP protein with an altered DNA-binding specificity, we have shown that the ability of TBP to respond to transcriptional activators in vivo is curiously resistant to clustered sets of alanine-substitution mutations in individual regions of the protein; combined sets of these mutations, however, can attenuate the activity of TBP in vivo. Remarkably, the combined sets of mutations can selectively affect the response to individual activation domains, indicating the existence of more than one way in which TBP is used by activators to stimulate transcription.

To address the mechanistic basis for this behavior, we have assayed the activity of the TBP mutants in several in vitro assays, including DNA binding, basal transcription, direct interaction with activation domains (e.g., the VP16 activation domain), and, in collaboration with S. Ruppert and R. Tjian, interaction with the largest subunit of the TBP-containing complex TFIID, the TBP-associated factor $hTAF_{II}250$. We have found that DNA binding, basal transcription, and the direct interaction between TBP and VP16 in vitro can be disrupted by single sets of mutations that have little if any effect on TBP's activity in vivo. In contrast, the interaction between TBP and $hTAF_{II}250$ in vitro is resistant to single sets of mutations in TBP but is sensitive to combined sets of mutations in TBP in a way that mirrors the in vivo activity. Taken together, these data suggest that TBP utilizes multiple interactions across its surface to respond to RNA polymerase II transcriptional activators in vivo; some of these interactions appear to involve recruitment of TBP into TFIID, whereas others are involved in the response to specific types of transcriptional activators.

Enhancement of In Vivo Promoter Occupancy of Transcriptional Activator Proteins through Their Activation Domains

M. Tanaka

Eukaryotic transcriptional activator proteins are often composed of two separate modular domains, a DNA-binding domain and an activation domain. The DNA-binding domain is generally sufficient for sequence-specific DNA binding of activator proteins in vitro. The activation domain is thought to be simply tethered to the promoter by the DNA-binding domain and to provide a surface that interacts directly or indirectly with the general transcription factors to activate transcription.

I have found, however, through an in vivo DNA-binding analysis in yeast cells, that activation domains can augment by up to two orders of magnitude the binding of activator proteins to their promoter target sites, essentially tethering the DNA-binding domain to the promoter. This function of activation domains on DNA binding contributes to the ability of potent activator proteins to activate transcription at low concentrations, concentrations at which less potent activators did not activate transcription or bind to the promoter. This observation implies that enhancement of DNA binding by activation domains may be generally important in depositing activator proteins to their target sites in vivo and can

profoundly affect how promoters respond to activator proteins.

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RNA SPLICING

A.R. Krainer A. Mayeda, J. Cáceres, A. Hanamura, D. Horowitz, M. Murray, T.-L. Tseng, S. Perera, G. Screaton, I. Watakabe, C. Sekunda, M. Wallace

MECHANISMS OF MAMMALIAN PRE-mRNA SPLICING AND SPLICE SITE SELECTION

RNA splicing is a required step in 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 that limited and dispersed sequence information present throughout introns and exons be precisely interpreted. 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, either tissue-specifically or in response to a developmental program or to extracellular stimuli, and thus alternative splicing is a common point in the control of gene expression. 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 of splicing and/or for the regulation of alternative splice site selection.

REGULATION OF ALTERNATIVE SPLICING IN VIVO BY CHANGES IN THE EXPRESSION OF SF2/ASF AND hnRNP A1

SR proteins have a characteristic carboxy-terminal Ser/Arg-rich repeat (RS domain) of variable length and constitute a family of highly conserved nuclear phosphoproteins. As was first demonstrated for human SF2/ASF, individual SR proteins have an activity required for general splicing in vitro, at the level of early spliceosome assembly. In addition, SF2/ASF and the remaining SR proteins promote utilization of proximal alternative 5′ splice sites in a concentration-dependent manner, with a variety of pre-mRNAs. The latter activity results from antagonistic interactions with the protein hnRNP A1, which stimulates use of distal 5′ splice sites in vitro. We have proposed that the intracellular ratios of

SF2/ASF (or other SR proteins) and hnRNP A1 may control the specificity of splice site selection and that in vivo regulation of one or both of these activities may have an important role in the tissue-specific or developmental regulation of alternative splicing. A. Hanamura used quantitative immunoblotting to demonstrate that the molar ratio of hnRNP A1 to SF2/ASF varies over a very wide range in different rat tissues, well above the range required to elicit splice site switching in vitro.

To investigate whether alternative splicing can be regulated by the relative amounts of these antagonistic factors in vivo, J. Cáceres transiently overexpressed human SF2/ASF or hnRNP A1 cDNAs in HeLa cells and measured the effect on the expression of several cotransfected reporter genes whose primary transcripts are processed via alternative splicing. Increased expression of SF2/ASF resulted in the selection of the most proximal of three cryptic 5′ splice sites in a thalassemia allele of human β-globin and selectively increased splicing via the proximal 13S 5′ splice site of adenovirus E1A (Fig. 1). SF2/ASF overexpression also promoted inclusion of the clathrin light-chain B neuron-specific exon and

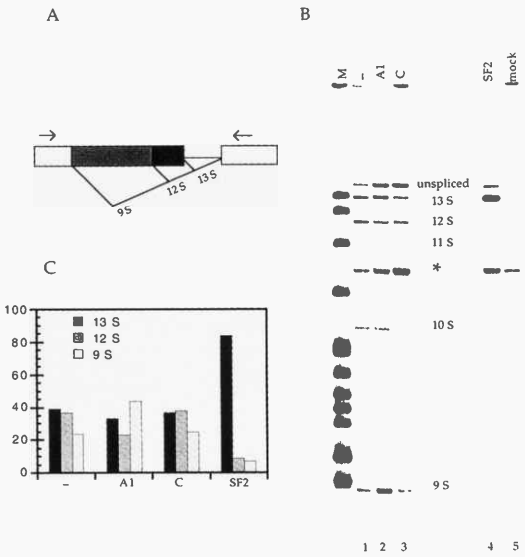

FIGURE 1 Effect of hnRNP A1 or SF2/ASF transient overexpression on alternative splicing of adenovirus E1A pre-mRNA. (*A*) Diagram of the major E1A mRNAs generated by alternative 5′ splice site selection and primers used for RT-PCR analysis. The 10S and 11S mRNAs, which arise from double-splicing events that do not involve simple competition between alternative 5′ splice sites, are not shown in the diagram. (*B*) Patterns of E1A alternative splicing upon transfection of an E1A gene (lane *1*) or cotransfection with pCG-A1 (lane *2*), pCG (lane *3*), or pCG-SF2 (lane *4*). The asterisk indicates a PCR artifact band that is also detected in mock-transfected cells (lane *5*). (*C*) The 13S, 12S, and 9S mRNA isoforms were quantitated with a phosphorimager, and the percentage of each isoform is shown.

prevented abnormal skipping of two mutually exclusive β-tropomyosin exons (in collaboration with S. Stamm and D. Helfman, CSHL). Increased expression of hnRNP A1 activated the distal 9S 5′ splice site of adenovirus E1A (Fig. 1). These results suggest that variations in the intracellular levels of antagonistic splicing factors influence different modes of alternative splicing in vivo and may be a natural mechanism for tissue-specific or developmental regulation of gene expression.

STRUCTURE, FUNCTION, AND RNA-BINDING PROPERTIES OF hnRNP A/B PROTEINS

To examine the structural requirements for hnRNP A1 function, mutagenesis of recombinant human hnRNP A1 was carried out by A. Mayeda in collaboration with Steve Munroe (Marquette University). The activity of hnRNP A1 in an alternative splicing assay was abolished by mutation of pairs of conserved phenylalanine residues in the RNP-1 submotifs of each of the two RNA recognition motifs (RRMs). The structure of the RRMs is unlikely to have been altered by these mutations, since the resulting proteins were unaffected in binding to β-globin pre-mRNA in a filter-binding assay. Analogous aromatic residues in other RRMs have been shown to stack with single-stranded bases. The above results suggest that the RRM mutations in either RRM of hnRNP A1 affect sequence-specific interactions with one or more RNA target sites and that these interactions are essential for alternative splicing function. The glycine-rich carboxyl terminus of hnRNP A1 was also found to be essential for alternative splicing activity. This portion of the protein contributes to the stability of RNA binding, since a truncated protein lacking the carboxyl terminus but containing both intact RRMs bound RNA very weakly.

To determine if other proteins, besides hnRNP A1, antagonize one or more SR proteins to select alternative 5′ splice sites, A. Mayeda measured the activities of other members of the human hnRNP A/B family, which bind most, if not all, nascent pre-mRNA transcripts. The hnRNP A2 and B1 proteins, which are alternatively spliced isoforms derived from the same gene and are related in sequence to hnRNP A1 (68% amino acid identity), were expressed in bacteria, purified, and assayed for alternative splicing in vitro. Both proteins were highly active in switching alternative 5′ splice site selection toward the distal site and were in fact more active than hnRNP A1. In contrast, hnRNP $A1^B$, an alternatively spliced isoform of hnRNP A1 that differs only in having a longer carboxy-terminal glycine-rich domain, had very limited activity. Surprisingly, there was an inverse correlation between the activities of these four proteins in alternative splicing and their binding affinity for pre-mRNA.

Although each of these abundant hnRNP A/B proteins can bind RNA, it is unclear if their effects on alternative splicing are mediated by binding to the same pre-mRNA sequences. Whether each hnRNP A/B protein regulates alternative splicing of different pre-mRNAs in vivo is also unknown at present. Recently, C. Burd and G. Dreyfuss (HHMI, University of Pennsylvania School of Medicine) demonstrated that hnRNP A1 is a sequence-specific RNA-binding protein. S. Perera, A. Hanamura, and I. Watakabe used SELEX to sample an RNA library with 4^{25}-fold degeneracy, in order to identify preferred binding sites for hnRNP A2 and B1. After six rounds of SELEX, both proteins selected RNAs that are purine-rich and, in almost all cases, contain one or more copies of the tetranucleotide UAGG. This selected sequence is part of the high-affinity binding site selected by hnRNP A1, UAGGGA/U, and also matches a portion of the vertebrate 3′ splice site consensus, UUAGG. I. Watakabe used a filter-binding assay to measure the affinity of hnRNP A/B proteins for representative RNAs. Each of the selected RNAs bound to hnRNP A2, B1, and A1 with comparable affinities, and in most cases the affinity was much greater than for control RNAs from round 0. hnRNP $A1^B$, which has an extended carboxy-terminal domain, bound all RNAs nonspecifically. We are currently testing the above-mentioned RRM1 and RRM2 mutants of hnRNP A1 to determine if the loss of alternative splicing activity correlates with the loss of sequence-specific RNA binding. In parallel SELEX experiments, A. Hanamura and I. Watakabe showed that recombinant SF2/ASF and SC35 selected different short purine-rich motifs positioned variably within a purine-rich context. This finding is consistent with the previously determined role of SF2/ASF and other SR proteins in binding to purine-rich exonic splicing enhancers and activating splicing of the preceding intron.

ISOLATION AND CHARACTERIZATION OF NOVEL SR PROTEINS

G. Screaton (a visiting scientist from J. Bell's laboratory, Oxford University) cloned a cDNA encoding a

novel human SR protein designated SRp30c, which has an unusually short RS domain. cDNA clones encoding the human homologs of *Drosophila* SRp55/B52 and rat SRp40/HRS were also isolated. Recombinant proteins expressed from these cDNA clones were shown to be active in constitutive splicing, i.e., they functionally complemented a HeLa cell S100 extract deficient in SR proteins (in collaboration with A. Mayeda). Additional cDNA clones reflect extensive alternative splicing of SRp40 and SRp55 pre-mRNAs. The predicted protein isoforms lack the carboxy-terminal RS domain and might be involved in feedback regulatory loops. The ability of human SRp30c, SRp40, and SRp55 to modulate alternative splicing was measured in a transient cotransfection assay (in collaboration with J. Cáceres). SR proteins affected the choice of alternative 5′ splice sites in the transcripts of adenovirus E1A or human β-thalassemia reporters upon overexpression in HeLa cells, and this choice was characteristic for each SR protein. Consistent with the postulated importance of SR proteins in alternative splicing in vivo, complex changes in the expression of mRNA isoforms encoding the above SR proteins were observed by RNase protection upon T-cell activation.

PURIFICATION AND CHARACTERIZATION OF FACTORS THAT CONTROL ALTERNATIVE 3′ SPLICE SITE SELECTION

In addition to their effects on spliceosome assembly and alternative 5′ splice site selection, several SR proteins have been shown to affect alternative 3′ splice site selection, favoring the use of the proximal sites. More recently, A. Mayeda showed that none of the known hnRNP A/B proteins antagonizes the alternative 3′ splice site selection activities of these SR proteins. Instead, a novel activity, designated SF7, promotes the selection of distal alternative 3′ splice sites. Following four steps of purification from HeLa cell nuclear extracts, SF7 activity has been separated into two independently active fractions, SF7A and SF7B, which are distinct from hnRNP A/B and SR proteins. Partial amino acid sequences for the four major polypeptides detected in the purest SF7A fractions were determined (in collaboration with R. Kobayashi, CSHL). The sequences of several peptides from the best candidate polypeptide match the conceptual translation of a cDNA in GenBank, which has characteristic features of an RNA-binding protein. It has a single RNA recognition motif (RRM) with well-conserved RNP-1 and RNP-2 submotifs, preceded by an extensive serine-rich domain. The candidate polypeptide appears to be highly phosphorylated, since a pronounced increase in electrophoretic mobility was seen upon phosphatase treatment. Significant SF7 activity was recovered after preparative SDS-PAGE and renaturation of the SF7A candidate polypeptide, suggesting that this polypeptide is sufficient for activity. To study the structure, function, and in vivo expression of SF7A in detail, we are currently trying to overexpress the candidate polypeptide in *Escherichia coli*. Further purification and characterization of the SF7B fraction are also in progress.

The potential combinatorial effects of variations in the concentrations of different SR proteins (eight human ones are currently known, not including alternatively spliced isoforms), each with some measure of substrate specificity, and of several antagonistic factors that act at either 5′ or 3′ splice sites (i.e., four hnRNP A/B proteins and SF7A) are very large. In addition to the 32 pairwise combinations of SR and hnRNP A/B proteins, additional specificity may arise from two or more members of each family cooperating to affect the splicing of certain substrates, or through interactions between these proteins and gene-specific positive or negative regulators. When yet more variables, such as regulation of protein accessibility or localization, and potential regulation of protein activity by changes in phosphorylation are added into the equation, the system gains a complexity perhaps sophisticated enough to control the numerous alternative splicing choices made in living cells.

YEAST AND HUMAN SPLICING FACTOR HOMOLOGS AND NEW HUMAN SPLICING FACTORS

To characterize further the relationship between pre-mRNA splicing in yeast and metazoans, we are using different approaches to identify related splicing proteins in humans and in yeast. The Prp18 protein is involved in the second step of pre-mRNA splicing in *Saccharomyces cerevisiae* and is a component of the U5 snRNP. From similarities among Prp18 and the translations of ESTs from rice and nematode, D. Horowitz designed polymerase chain reaction (PCR) primers which he used to identify a human homolog of Prp18 (termed hPrp18). A full-length cDNA encoding hPrp18 was cloned and sequenced. The

deduced amino acid sequence of hPrp18 is 30% identical to that of yeast Prp18; hPrp18 is not related to other proteins in the databases. hPrp18 has been overexpressed in *E. coli* and antibodies against it are currently being produced. These antibodies will be used to assess the role of hPrp18 in pre-mRNA splicing. We initially want to determine whether hPrp18 is required for the second step of splicing and whether it is a component of the human U5 snRNP.

T.-L. Tseng is searching for proteins with RS domains in budding and fission yeast, with the ultimate goal of studying the structure and function of RS domain-containing splicing factors by genetic methods. The rationale is based on the observation that very few known proteins in the databases contain significant repeats of alternating arginine and serine residues, and virtually all of these proteins have been directly or indirectly implicated in constitutive or regulated splicing. Using two monoclonal antibodies that recognize either phosphorylated or unphosphorylated RS domains, several yeast proteins were found to react with both antibodies and showed the appropriate sensitivity or resistance to phosphatase treatment. These observations suggest that several proteins containing phosphorylated RS domains exist in both budding and fission yeast. Several candidate clones were isolated by screening expression libraries with the antibody that recognizes unphosphorylated RS domains, and these are currently being characterized.

We are continuing to purify and characterize additional protein factors that are essential for one or both RNA cleavage-ligation reactions. M. Murray fractionated a HeLa cell nuclear extract and obtained a fraction in which a single, or a limited number of, required component(s) has been separated from known protein splicing factors and snRNPs. This fraction is required to complement cruder fractions containing the remaining essential components, thus defining a novel activity required for cleavage at the 5′ splice site and lariat formation. Further purification of this activity is in progress.

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PROTEIN SYNTHESIS

M.B. Mathews S. Brand, M. Greenberg, S. Gunnery, B. Hofmann, C. Labrie, B. Lee, H.-J. Liao, Y. Ma, L. Manche, P. Nahreini, T. Pe'ery, D. Taylor, P. Wendel, C. Yordan

Gene expression is controlled in many different ways. Our laboratory is studying several systems that illustrate the diversity of regulatory processes in human cells. All of the systems involve viral genes that interact with cellular pathways, resulting in modulation of the level of cellular or viral gene product(s) synthesized and impinging on processes such as cellular growth control and viral multiplication. The following paragraphs summarize the progress made in 1994 on each topic. During the year, Huey-Jane Liao

and Tsafrira Pe'ery joined the laboratory, and Claude Labrie left to take up a faculty position at the University of Laval in Québec.

Regulation of PCNA

B. Lee, C. Labrie, P. Wendel, M.B. Mathews

The proliferating cell nuclear antigen (PCNA) is an adenovirus E1A-inducible cellular protein that is intimately linked to the processes of DNA replication and cell cycle regulation. Initially discovered as an antigen in autoimmune disease, PCNA is also known as the DNA polymerase δ auxiliary factor, in which context it functions as a "sliding clamp" during DNA replication. PCNA synthesis is triggered by a variety of growth stimuli. Its induction by the adenovirus E1A gene was first described during the oncogenic transformation of quiescent rodent cells and has since been studied in much greater detail using transient expression experiments in HeLa cells and other cell types. Activation of the human PCNA promoter is a property of the 243-residue adenovirus E1A oncoprotein (E1A 243R).

We showed previously that *trans*-activation of the PCNA promoter by the E1A 243R protein is mediated through a novel *cis*-acting element termed the PERE (an acronym for the *P*CNA *E*1A-*r*esponsive *e*lement) which contains a sequence resembling an activating transcription factor (ATF)-binding site. This site lies about 50 nucleotides upstream of the PCNA transcriptional start site. To elucidate the mechanism whereby PCNA expression is regulated by E1A 243R, we have begun to identify cellular transcription factors that associate with the PERE. In electrophoretic mobility shift assays, an oligonucleotide probe containing the PERE site forms three major complexes (P1, P2, and P3) with proteins in nuclear extracts from HeLa or 293 cells. Mutational analysis showed that the formation of complexes P2 and P3 correlates with PCNA promoter activity in vivo and requires the ATF-binding site found within the PERE. Antibody interference experiments and mobility shift assays performed with protein synthesized in vitro indicate that the transcription factor ATF-1 is a major component of these complexes. Similar assays demonstrated that the hepatitis B virus enhancer-associated protein RFX1 constitutes a major component of the P1 complex, but at present, we have no evidence that this protein participates in the induction of the PCNA promoter.

Last year we reported that the PCNA promoter contains a p53-binding site approximately 220 nucleotides upstream of the transcription initiation site. The binding of p53 to this site is important for both the basal activity of the promoter and its response to E1A 243R. We have extended the analysis to examine the binding of proteins to the minimal E1A-responsive promoter in order to identify other factors that might be important for transcription from the PCNA promoter. Mobility shift assays revealed that a fragment encompassing this region, extending from −87 to +62 relative to the transcription initiation site, forms at least five major complexes (EH1–EH5) with HeLa cell nuclear extracts. Examination of these complexes indicated that the transcription factor YY1 associates with the initiator element of the PCNA promoter. In addition to identifying other cellular transcription factors contained within these promoter-proximal complexes, current studies are investigating the roles of cellular E1A-binding proteins in the activation of PCNA expression.

Regulation of HIV-1 Gene Expression

M. Greenberg, P. Nahreini, T. Pe'ery, C. Yordan, M.B. Mathews

The HIV-1 protein Tat stimulates transcription from the HIV promoter, located in the viral long terminal repeat (LTR), at least in part by controlling transcriptional elongation and overcoming premature transcriptional termination. These actions of Tat can be reproduced in a cell-free system using purified, bacterially expressed Tat to stimulate HIV transcription. Tat appears to achieve *trans*-activation in vitro by improving the processivity of RNA polymerase complexes, presumably by recruiting or modifying cellular elongation factors, but a detailed picture of Tat's interactions with cellular factors has yet to emerge. Recently, A. Rice and co-workers (Baylor College of Medicine) demonstrated specific binding of the activation domain of Tat to a cellular kinase, TAK, which can phosphorylate the carboxy-terminal domain (CTD) of RNA polymerase II (pol II). Since phosphorylation of the CTD is believed to trigger the transition from polymerase complex assembly to the elongation phase of transcription, this observation suggests a mechanism whereby Tat could increase elongation via TAK-catalyzed phosphorylation of the

CTD. However, it is also possible that TAK activates other transcription factors because it appears to phosphorylate other nuclear (and cytoplasmic) proteins whose nature is as yet unknown.

We have set out to explore the role of the Tat-associated kinase activity in *trans*-activation of the HIV promoter. Various GST-Tat fusion constructs, and their mutant derivatives, were used to detect TAK activity in HeLa cell nuclear extracts. We found a similar activity in the S-100 cytoplasmic fraction of human 293 and HeLa cells. TAK isolated by adsorption to GST-Tat phosphorylates a GST-yeast CTD fusion protein as well as a synthetic peptide containing three copies of the CTD repeat. We are currently working to assess the role of TAK activity in the Tat response. In related studies, conducted with D. Ostapenko (Weizmann Institute, Israel), we have demonstrated that the ability of Tat to *trans*-activate in vitro depends on preincubation with nuclear extract. This suggests that factors in the nuclear extract are required to "potentiate" Tat *trans*-activation. Potentiation could be due to the formation of a complex between Tat and nuclear protein(s) or to covalent modification of Tat. Heat inactivation of the extract or its depletion by column chromatography abrogated the potentiation effect. We are attempting to purify the factor(s) that potentiates Tat function in vitro and to determine their relationship to TAK.

In previous Annual Reports, we described experiments indicating that replication and chromosomal integration can influence transcription from the HIV-1 LTR. To explore these effects in more detail, we have exploited the ability of recombinant adeno-associated virus (AAV-1) to transfer and stably integrate a reporter gene cassette into the human genome. AAV-1 DNA inserts into the genome in a site-specific fashion on chromosome 19 as well as at apparently random sites elsewhere. We have constructed several recombinant AAVs containing the LTR-CAT reporter cassette. In some of these chimeric vectors, a selectable marker gene (the Neo^r gene, encoding neomycin resistance) was inserted to facilitate the isolation of clones containing the integrated HIV gene cassette. Such recombinant viruses were used to infect HeLa cells; in some instances, the recombinant was coinfected with wild-type AAV containing the *rep* gene, which is believed to impart site-specific integration into chromosome 19. Thus far, we have isolated 25 independent neomycin-resistant clones of HeLa cells containing the integrated recombinant virus. The basal HIV promoter activity, measured in CAT enzyme assays, varies significantly among these clones, and a few clones show no CAT activity at all, even though the linked Neo^r gene within the integrated proviral genome is biologically active. In most ways, these clones behave as expected. For instance, transfection of the Tat gene dramatically increases CAT expression in those clones that have some measurable basal CAT expression. In contrast to a report in the literature, the cell lines displayed no differences in their responses to full-length Tat (86 amino acids) and exon 1 Tat (72 amino acids). Finally, at least in some clones, coexpression of the adenovirus E1A and Tat genes synergistically stimulates CAT expression from the HIV promoter. On the basis of Southern blot analysis of genomic DNA from some clones, a single copy of the LTR-CAT cassette is integrated into the chromosomal DNA, and in at least two clones, the integration appears to be at the previously characterized chromosome 19 site. We now plan to characterize the integration sites of the proviral DNA in these clones.

Translation of an RNA Polymerase III Transcript

S. Gunnery, P. Wendel, M.B. Mathews

Last year, we showed that a transcript generated by RNA polymerase III (pol III) can be translated in vivo to produce a functional protein. The adenovirus VA RNA promoter, a pol III promoter, was placed upstream of the HIV-1 Tat-coding sequence to construct pVA-Tat. When transfected into cells, this construct produced VA-Tat RNA that was recruited by the polysomes for translation, giving rise to a Tat protein that was able to *trans*-activate the HIV-1 promoter. As would be expected of a pol III transcript, VA-Tat RNA lacks a poly(A) tract at its 3′ end and a cap structure at its 5′ end. These findings imply that functional mRNA synthesis is not restricted to pol II, that pol III transcripts can be transported within the cell and assembled with the translational components, and that neither the 5′ cap structure nor the 3′ poly(A) tail is obligatory for translation. Examination of polysome profiles suggested that the rate of initiation of translation on the pol III RNA is reduced compared to mRNA made by pol II, however. Therefore, it seems that the terminal structures characteristic of normal mRNAs enhance translational efficiency.

To study the mechanism of translation of this pol III transcript, we took advantage of a short upstream open reading frame (ORF) which is out-of-frame with that of Tat and lies in the 5′-untranslated region of VA-Tat RNA. In other RNAs, such upstream ORFs can reduce the translation of a downstream ORF, especially if the ORF begins with an AUG in a favorable context. This observation is commonly interpreted to imply that initiating ribosomal subunits bind first to the 5′ cap, then scan along the mRNA until they encounter a suitable AUG codon. To discover whether the upstream ORF has any role in the translation of VA-Tat RNA, we eliminated it by mutating the start codon from AUG to GCG (mutant I) or extended it by ten nucleotides into the Tat ORF by mutating the upstream stop codon from UAG to CAG (mutant S). The initiation codon mutant gave twofold more Tat activity than wild-type pVA-Tat and the stop codon mutant gave twofold less activity than wild-type pVA-Tat, suggesting that the upstream ORF can be translated and that its utilization decreases Tat synthesis. Consistent with this conclusion, *trans*-activation by the double mutant (I+S) was similar to that by the I mutant, indicating that the stop codon is influential only if the upstream ORF is utilized. These results indicate that scanning of the 5′ UTR may be involved in translation of the uncapped VA-Tat RNA. To test this conclusion further, we examined the effect of stem-loop structures inserted upstream of the Tat ORF. Stem-loop structures of high stability are thought to decrease the translation of a downstream ORF in capped mRNAs because scanning ribosomes are stalled by the secondary structure. Translation of the Tat ORF was inhibited by the insertion of such stem-loop structures, reaffirming the conclusion that the VA-Tat, despite being uncapped, is subject to scanning.

When the two ORFs overlap, as in mutant S, *trans*-activation of the downstream Tat ORF is reduced but not eliminated. To determine whether this is due to reinitiation after termination of translation of the upstream ORF by ribosomes that "backscan" along the RNA, we made mutations that increase the overlap of the two ORFs to 22 and 219 nucleotides. Both of these mutations further reduced the translation of the Tat ORF, consistent with backscanning. Similar results were obtained with constructs driven by a pol II promoter. Taken together, these results demonstrate that pol III transcripts can be recruited by ribosomes and translated by mechanisms similar to those used for normal (i.e., pol II-directed) mRNAs, albeit less efficiently. We are now examining the processing of the pol III transcripts to determine whether they are handled in the nucleus in the same way as conventional mRNAs.

Translational Control

S. Brand, D. Taylor, L. Manche, M.B. Mathews

The protein kinase DAI, the double-stranded (ds) RNA activated inhibitor of translation (also known as PKR, P1, p68, etc.), regulates the first step of protein synthesis by phosphorylating an initiation factor known as eIF-2 (eukaryotic initiation factor 2). Phosphorylation of eIF-2 leads to the slowing or cessation of translation, and the enzyme has been implicated in a wide range of processes from oncogenic transformation and growth control to viral infection. As its name implies, the enzyme is controlled by RNA regulators. In previous years, we described studies of the RNA-binding properties of DAI: This work continues at the biochemical level, with the aid of mutagenesis, and (together with Dr. Alan Hinnebusch's lab at the National Institutes of Health) in yeast. DAI contains two copies of a dsRNA-binding motif that exhibit different properties. It seems that RNA binding requires the cooperative action of both motifs. In collaboration with the laboratories of Dr. Tom Steitz at Yale and Dr. Peter Wright at the Scripps Institute, we are presently attempting to obtain detailed structures of the RNA-binding domain of DAI, both with and without its RNA ligands, by biophysical methods.

Recent studies lend support to the view that DAI activation requires dimerization of the enzyme, allowing intermolecular phosphorylation to occur. This autophosphorylation is correlated with the activation of DAI as an eIF-2 kinase. To understand the process and its role in activation, we are studying the autophosphorylation sites. In collaboration with Dr. Dan Marshak and Georgia Binns (Protein Chemistry Core section), the locations of several autophosphorylated sites have been identified through peptide mapping and sequencing. Some of these sites may define an autoregulatory region, separating the enzyme's RNA-binding and kinase domains. Other phosphorylated residues are located within the RNA-binding domain. To determine which, if any, of these sites are important for the activation of the enzyme, alanine substitution mutants have been made and are being tested in

vivo in the yeast *Saccharomyces cerevisiae*. Preliminary results show that some of the mutants have reduced activity, but none of the mutants tested as yet abolishes kinase activity.

Although it is usually assumed that eIF-2 is the physiologically relevant substrate for DAI, the enzyme has also been shown to phosphorylate histone H2A, a 90-kD protein found in rabbit reticulocytes, and the inhibitory subunit of the NF-κB transcription factor. In addition, it is likely that the activated kinase is responsible for the modification of as yet unidentified substrates. One of our aims is to elucidate the nature of the 90-kD phosphoprotein and to search for additional substrates of DAI. Using monoclonal antibodies to immunopurify the 90-kD protein, a partial peptide sequence has been obtained. Degenerate oligonucleotides have been synthesized based on the predicted amino acid sequence and are being used to screen a cDNA library in an attempt to clone the cDNA for this protein. Preliminary data suggest that activated DAI is also able to phosphorylate the HIV-1 *trans*-activation protein Tat. Experiments using a purified preparation of Tat-72 (exon 1 only) and a series of GST-Tat fusion proteins indicate that serine residues within the carboxy-terminal domain of the Tat protein are phosphorylated by DAI in a dsRNA-dependent manner. We will attempt to further identify the specific residues phosphorylated by DAI and to elucide the functional significance of the Tat/DAI interaction.

Virus-Host Interplay

F. Ma, H.-J. Liao, M.B. Mathews

The synthesis of DAI is induced by interferon, and this enzyme forms part of the antiviral defenses of the host organism. In response to the threat, viruses have evolved mechanisms to protect themselves against activation of the kinase. Some of these defense mechanisms involve the production of small RNA molecules that prevent DAI activation. The best known of these RNAs are the virus-associated (VA) RNAs of adenovirus. In previous years, we described how adenovirus 2 (Ad2) VA RNA_I blocks DAI activation in vivo and in vitro by binding to DAI. The function of VA RNA_I is critically dependent on its structure, which has been explored by several means including mutagenesis and probing with nucleases and chemical reagents. Most recently, we have turned to phylogenetic comparisons and site-specific mutagenesis to refine the structural model of VA RNA_I.

Sequence analysis of the VA RNA genes of all 47 human adenoviruses separates the VA RNAs into three superfamilies: Cluster 1 contains the single VA RNAs of adenoviruses in groups A and F and the VA RNA_I species of group C; cluster 2 contains the VA RNA_I species of groups B, D, and E, and six unclassified viruses, as well as the single VA RNAs of group B; and cluster 3 contains all of the VA RNA_{II} species. We have combined the sequence data with nuclease sensitivity analysis and computer-assisted RNA folding in order to derive secondary structural models for the VA RNAs. The RNAs in each cluster were compared to each other, and the impact of sequence variations on secondary structure models was evaluated. Within clusters 1 and 2, the major features of the previous VA RNA secondary structure model—the terminal stem, apical stem-loop, and central domain—are conserved despite considerable sequence variation. The central domain structure is critical for the inhibition of DAI activation. It contains a side stem-loop linked to the base of the apical stem and to a stem formed by the highly conserved tetranucleotides GGGU and ACCC reported previously. These three stems contain all the regions required for VA RNA function and interaction with DAI. Flanking this central domain are two internal loops further connecting the upper part of the apical stem-loop and the terminal stem. In contrast, the VA RNA_{II} species comprising cluster 3 apparently do not conform to the structural model developed for the other VA RNAs. Instead, they can be accommodated by a Y-shaped structure in which the conserved tetranucleotides are paired but not to each other. We suspect that these structural differences correlate with functional differences: VA RNA_{II} substitutes poorly for VA RNA_I in functional assays, and we speculate that it subserves a different role in virus infection. Current work is directed toward elucidating this role.

Although there is little sequence conservation in the VA RNAs apart from that related to the transcription signals (A box and B box), the mutually complementary tetranucleotides GGGU and ACCC are invariable except in two viruses where there is a conservative change (ACCC to ACCU) which still permits pairing. To verify the functional significance of the conserved tetranucleotides experimentally, we have made three sets of potentially compensating mutations in Ad2 VA RNA_I. Each set consists of

three mutants, one in the GGGU sequence, a corresponding mutation in the ACCC sequence, and a double mutant containing both changes. The mutations are designed such that the single mutations should disrupt pairing if it exists in the wild-type molecule, but the combination should allow pairing to reform. We compared the ability of these mutants to substitute for Ad2 VA RNA_I in two functional assays, a viral protein synthesis rescue assay and a CAT expression enhancement assay. In each case, the two single mutants displayed low activity, but the double mutants were partially active, indicating that the GGGU and ACCC sequences do indeed base-pair. The double mutants did not achieve wild-type activity levels, however, presumably because they do not restore the wild-type structure completely. Alternatively, the precise sequence of the tetranucleotide stem might be important for the interaction of VA RNA with DAI. We are presently attempting to distinguish between these two interpretations through RNA structure analysis.

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MOLECULAR MECHANISMS OF APOPTOSIS

Y. Lazebnik Yifan Xu

Our laboratory is interested in dissecting molecular mechanisms of apoptosis. Apoptosis is a type of cell death that results from the activation of a specialized intrinsic cell machinery that is aimed at the cell self-disassembling. The role of apoptosis in the development and functioning of an organism is as important as that of cell proliferation and differentiation. As a corollary, the derangement of the apoptotic machinery is a significant etiological factor and a potential pharmacological target in a variety of illnesses, in-

cluding neurodegenerative diseases, viral infections, cancer, and autoimmune disorders. However, at present, our knowledge of the biochemical mechanisms of apoptosis is too limited to make possible the design of drugs that would affect the apoptotic machinery.

The biochemical study of apoptosis has proven to be difficult. Due to the highly variable time of commitment to apoptosis (hours to days) and the relatively constant time of execution of apoptosis (1–2 hours), a cell population treated with an apoptosis-inducing factor becomes a complex mixture of viable cells, cells committed to death, cells undergoing the active phase of apoptosis, and necrotic cells. As a result, it is difficult to assign an activity that is purified from this complex mixture to a particular cell subpopulation and consequently to a process particular to apoptosis.

To overcome this problem, we have developed and characterized a cell-free system that accurately mimics the execution stage of apoptosis. This system uses an extract prepared from chicken tumor cells that have been committed to apoptosis by a transient synchronization in the S phase of the cell cycle and collected in mitosis (S/M extracts). Control extracts are prepared from cells collected in mitosis without S-phase presynchronization (RME, real mitotic extract). When isolated nuclei are added to S/M extracts, the nuclei synchronously undergo biochemical and morphological changes characteristic of apoptosis. In contrast, RME extracts are not able to induce any of these changes. Using this cell-free system as a tool, we began to dissect the biochemical mechanism of apoptotic cell execution.

Extensive genetic studies of programmed cell death in the nematode *Caenorhabditis elegans* have revealed that the mechanism of apoptosis is tightly controlled and the expression of genes responsible for commitment of cells to apoptosis is cell-type-specific. In contrast, the execution of cells committed to apoptosis is controlled by the same set of three genes in all cell types. Two of the genes, *ced-3* and *ced-9*, are conserved between worms and vertebrates. *ced-9*, an inhibitor of apoptosis, is a functional and structural homolog of the vertebrate oncogene *bcl-2*. *ced-3*, an inductor of apoptosis, has significant sequence similarity to the gene encoding the mammalian cysteine proteinase interleukin 1β-converting enzyme, or ICE. This similarity, along with the ability of some ICE inhibitors to inhibit apoptosis, suggests that the CED-3 protein may induce apoptosis by working as a proteinase and that a CED-3 homolog also induces apoptosis in vertebrates.

To date, several genes with sequences similar to those of genes encoding ICE and CED-3 have been cloned and together are referred to as the *ICE/ced-3* family. However, only ICE has been biochemically and functionally characterized. A study published recently clearly showed that ICE is not essential for apoptosis in mice. Therefore, the CED-3 equivalent in vertebrates is yet to be identified. Our laboratory has been set up to identify and purify this equivalent(s), to dissect pathways of their regulation, and to find out their substrates.

Identification of Proteinase(s) from the ICE/CED-3 Family That Are Essential for the Onset of Apoptosis

Y. Lazebnik, Y. Xu [in collaboration with Merck Research Laboratories, Rahway, NJ, and with Merck Frosst Center for Therapeutic Research, Montreal, Canada]

Using our cell-free system, we have identified a proteinase resembling ICE (prICE) that is involved in the execution of apoptosis and fulfills criteria for a vertebrate homolog of the CED-3 protein. First, biochemically, prICE belongs to the ICE family of proteinases.

Second, active prICE was detected only in extracts made from cells committed to apoptosis. Third, prICE is activated at the beginning of the execution stage of apoptosis. Finally, prICE activity appears to be pivotal in driving apoptosis in vitro. Inactivation of prICE blocks apoptotic changes in the cell-free system, including both morphological transformation and DNA cleavage.

We began a project aimed at the isolation, cloning, and molecular identification of prICE activity. We have made use of reagents that allow specific inhibition, labeling, and affinity purification of ICE-like enzymes and have found that S/M extracts actually have at least two different ICE-like proteinases, prICE-1 and prICE-2. Our preliminary data suggest that prICE-1 and prICE-2 have different substrate specificities. Further purification of the prICEs is under way.

Activation of ICE/CED-3 Proteinases at the Onset of the Execution Stage of Apoptosis

Y. Xu [in collaboration with H. Robert Horvitz, Massachusetts Institute of Technology]

ICE is expressed in cells as an inactive pro-enzyme which is then activated by a specific cleavage, presumably by another ICE-like proteinase. The mechanism of this activation is unknown. The high degree of structural similarity between members of the *ICE/ced-3* family suggests that CED-3 and the vertebrate CED-3 equivalent are also expressed as pro-enzymes and then are also activated by proteolysis. Therefore, an important step to the understanding of the apoptotic machinery is to dissect mechanisms of activation of the ICE/CED-3-like enzymes.

In an attempt to understand ICE/CED-3 activation, we are now screening for proteins that interact with subdomains of CED-3 by using the yeast two-hybrid system. This approach will be useful in identifying proteins required for ICE/CED-3 activation as well as for identifying substrates of these enzymes.

Biochemical Identification of the Substrates of the Vertebrate CED-3 Homologs

Y. Lazebnik, Y. Xu

The identification of substrates that are cleaved at the onset of apoptosis by either CED-3 or its vertebrate equivalents is important for at least two reasons. First, it will increase our knowledge about the cell execution, which in turn may result in the identification of novel markers of cell death. Second, it will provide new insight as to how the cell is organized, because it appears that the structures that are specifically affected during apoptosis are essential for cell life.

We have shown previously that nuclear enzyme poly(ADP-ribose) polymerase, or PARP, is cleaved into two fragments at the onset of apoptosis. Using the cell-free system, we found that PARP is cleaved by prICE. To date, PARP is the first and only known substrate cleaved during apoptosis by an ICE/CED-3-like enzyme. Another group of proteins that appear to be cleaved during apoptosis are nuclear lamins. We are investigating the role of prICE in this cleavage.

ACCESSORY GENES OF HUMAN IMMUNODEFICIENCY VIRUS

J. Skowronski S. Bronson A. Iafrate M. Lock R. Mariani S. Salghetti

AIDS immunodeficiency is invariably associated with depletion of the regulatory subset of T cells that express CD4 antigen on the cell surface. These $CD4^+$ T cells are preferentially infected by human immunodeficiency virus (HIV), but neither the mechanism(s) that subverts the normal function of the infected cells nor the identity of viral genes involved is well understood. Besides the *gag*, *pol*, and *env* genes found in all retroviruses, immunodeficiency viruses encode several additional "accessory" proteins. Some of these accessory genes are not required for the viral life cycle in established cell lines, and their function has been difficult to assess. Several lines of evidence indicate that these "nonessential" genes have an essential function in vivo for efficient virus replication and pathogenesis. Research in this laboratory is focused on the function and mechanism of action of these accessory proteins.

Our recent work has focused on understanding the function and mechanism of action of the HIV-1 *nef* gene. *nef* is dispensable for the viral life cycle in vitro but is strictly required for high viral load and $CD4^+$ T-cell depletion in animal models for AIDS. Our laboratory has been using approaches in vivo, using transgenic mice, and in vitro to address the consequences of Nef expression on normal cellular functions and its interaction with cellular regulatory mechanisms. We have shown previously that (1) HIV-1 Nef alleles isolated directly from peripheral blood of HIV-1-infected individuals decrease expression of CD4 antigen on the surface of human T cells and (2) HIV-1 Nef alters activation and blocks development

of CD4+ T-cell subset when expressed in T cells in transgenic mice. These effects of Nef expression in transgenic T cells correlated with abnormally low CD4 antigen expression on the cell surface.

These results have broad implications, because CD4 antigen is a cell-surface receptor for HIV that is involved in many aspects of CD4+ T-cell biology. CD4 is an integral membrane glycoprotein expressed primarily on the immature thymic T cells and the mature helper T lymphocytes. The extracellular domain of CD4 binds to a nonpolymorphic region of major histocompatibility complex (MHC) class II molecules expressed on the antigen-presenting cells. These events are associated with activation of a nonreceptor src-like protein tyrosine kinase $p56^{lck}$, associated with CD4 via its cytoplasmic tail, which couples CD4 to cellular signaling. In mature T cells, CD4 is involved in T-cell activation. In addition, under some experimental conditions, CD4 is involved in specifying programmed cell death and/or G_1 block in the cell cycle progression of CD4+ T cells.

We have shown previously that CD4 down-modulation by Nef requires a membrane proximal region in the CD4 cytoplasmic tail and involves disruption of CD4 association with $p56^{lck}$. During the last year, we continued a detailed analysis of Nef interaction with the CD4-$p56^{lck}$ complex. Moreover, we have shown that interaction of Nef with the CD4 cytoplasmic domain is required for defects in development of CD4+ T cells in CD3 Nef1 transgenic animals.

IN T CELLS, NEF-INDUCED CD4 ENDOCYTOSIS INVOLVES A CLUSTER OF HYDROPHOBIC AMINO ACID RESIDUES IN THE CD4 CYTOPLASMIC TAIL

To examine the CD4 sequences required for down-modulation by Nef, CD4 proteins bearing various deletion or point mutations in the cytoplasmic tail were expressed in the CD4(–) 171.22 T-cell hybridoma. The derived cell lines were subsequently transduced with a retroviral vector containing either an active allele of HIV-1 *nef* or an empty control vector. Expression of CD4 on the cell surface of the resultant populations was then analyzed by flow cytometry (Fig. 1). This analysis initially defined a short region between M407 and K418 as required for the effect of Nef. Interestingly, deletion of C420 and C422, which

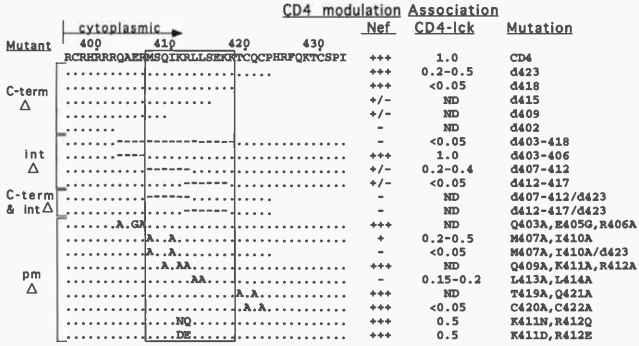

FIGURE 1 Effect of mutations in CD4 cytoplasmic domain on down-modulation by Nef and on association with $p56^{lck}$. (*A*) Amino acid sequences of the cytoplasmic tails of mutant CD4 proteins are aligned on the left with that of the wild-type human CD4. Dots indicate amino acid identities with the wild-type protein, dashes indicate the extent of internal deletions, and capital letters identify amino acid substitutions in the single-letter code. The 12-amino-acid membrane-proximal region in the CD4 cytoplasmic tail required for down-modulation by Nef is boxed. The extent of change in CD4 expression on the cell surface following transduction with HIV-1 NA13 *nef* expression vector (Nef) is indicated by (+++), (++), (+), (+/–) and (–). Quantitation of CD4-$p56^{lck}$ association was performed by Western blot analysis. The degree of $p56^{lck}$ association with mutant CD4 proteins was normalized to that observed with wild-type CD4 (1.0). ND indicates not determined.

are required for CD4 association with p56^{lck} kinase, is not essential for the effect of Nef.

To assess which amino acids in this region are required for down-modulation by Nef, mutants bearing double or triple amino acid substitutions were analyzed. As shown in Figure 1, substitutions at positions proximal to M407 and distal to K418 had no detectable effect on Nef-induced CD4 down-modulation. In contrast, replacement of leucines 413 and 414 by alanines abrogated down-modulation by Nef (L413A, L414A), whereas the double alanine substitution for methionine 407 and isoleucine 410 (M407A, I410A) still responded to Nef, but at much reduced levels. Deleting ten amino acids from the carboxy-terminal end of the latter mutant CD4 resulted in unresponsiveness to Nef (M407A, I410A/d423). Together, these data demonstrated that CD4 down-modulation by Nef requires a cluster of hydrophobic amino acids in the membrane-proximal region of CD4 cytoplasmic tail and provided evidence that the carboxy-terminal region of the tail contributes to the interaction of CD4 with Nef.

EFFICIENT CD4-p56^{LCK} ASSOCIATION REQUIRES THE NEF-RESPONSIVE ELEMENT

In T cells, the cytoplasmic tail of CD4 is associated with p56^{lck} protein tyrosine kinase, and Nef disrupts this association by an unknown mechanism. As the hydrophobic element required for Nef-induced CD4 endocytosis and the double cysteine motif (C420, C422) involved in CD4 association with p56^{lck} are located in close proximity, we tested whether the region that is involved with CD4 endocytosis also interacts with p56^{lck}.

Association of mutant CD4 proteins with endogenous p56^{lck} was analyzed by immunoblot analysis of anti-CD4 immune complexes prepared from detergent lysates of 171.22 cells expressing selected mutant CD4 proteins. Initially, analysis of mutant CD4 proteins bearing various deletions of the cytoplasmic domain revealed that mutations affecting residues M407 through K418, the region required for CD4 down-modulation, also resulted in a decrease in CD4-associated p56^{lck}, suggesting that CD4 sequences required for down-modulation by Nef also contribute to interaction with p56^{lck}. This was seen even more clearly with analysis of alanine substitution mutants. For example, substitution of alanines for M407 and I410, which dramatically reduces CD4 down-modulation by Nef, resulted in approximately a two- to fourfold reduction in CD4-associated p56^{lck}. When the same mutations were combined with deletion d423 (which lacks ten carboxy-terminal amino acids, but retains residues C420 and C422 and is completely unresponsive to Nef), no detectable p56^{lck} was coimmunoprecipitated. Moreover, when alanines were substituted for L413 and L414, which abolishes CD4 internalization induced by Nef, the amount of coimmunoprecipitated p56^{lck} was only one-eighth to one-fourth of that observed with wild-type CD4. In contrast, substitutions at K411 and R412, which had no effect on Nef-induced CD4 endocytosis, resulted in only a modest decrease in association with p56^{lck}. Therefore, the hydrophobic amino acid residues in the membrane proximal region in the CD4 cytoplasmic tail required for down-modulation by Nef are also involved with efficient recruitment of p56^{lck} into a complex with CD4 and/or with stabilization of the complex. The overlap in CD4 sequences required for interaction with Nef and p56^{lck} suggests that Nef, or cellular factors recruited by Nef, act *via* this region of CD4 to release p56^{lck} from the complex and induce CD4 endocytosis.

The CD4-p56^{lck} complex is thought to involve direct interaction between the two proteins, mediated by cysteine motifs in the amino-terminal unique domain in p56^{lck} and the CD4 cytoplasmic tail. Thus, the membrane-proximal segment in the CD4 cytoplasmic tail may form additional contacts with the p56^{lck} unique domain. Alternatively, our data are also consistent with a possibility that a third component may be required for formation or stabilization of CD4-p56^{lck} complexes. Indeed, the relatively tight correlation between the Nef-induced CD4 down-modulation and formation of CD4-p56^{lck} observed with mutant CD4 proteins suggests a model where an additional factor(s) could interact with the membrane-proximal region in CD4 cytoplasmic tail, masking the endocytotic motif and stabilizing CD4-p56^{lck} association. This possibility will be further explored in the future.

In Press

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PROTEIN CHEMISTRY

D.R. Marshak E. Araya, Y.-S. Bae, G. Binns, N. Carpino, G.-Y. Cha, N. Chester, S. Goren, J. Kahler, J.-P. Liu, C. van den Bos

The members of the Protein Chemistry laboratory conduct research on cell growth using advanced methods of analytical and preparative biochemistry. The major focus of our efforts is the biochemistry of protein kinases involved in controlling the responses of cells to external signals for growth and the interactions with the internal cell division cycle. Our interest is in the role of protein phosphorylation in normal, physiological cell division and in abnormal, pathophysiological responses, including cancer.

Some of the methods we utilize include automated protein sequence analysis, high-performance liquid chromatography, quantitative amino acid analysis, preparative electrophoresis, mass spectrometry, and peptide synthesis. Use of such procedures allows us to deduce chemical structures of proteins and to synthesize their structural and functional domains. In addition, physical analysis of proteins by mass spectrometry, in conjunction with chemical studies, permits the determination of posttranslational modifications of proteins, such as phosphorylation and acylation. This chemical approach to cell biology is highly complementary to genetic approaches in many of the other laboratories at Cold Spring Harbor.

Synthetic Peptides from Protein Kinases

D. Marshak, G. Binns, N. Carpino

Protein kinases are enzymes that catalyze the transfer of phosphates from the γ-phosphate position of ATP to a hydroxyl moiety (serine, threonine, or tyrosine) on proteins. Protein kinases are themselves often regulated by phosphorylation. We continue to use the new chemistry introduced last year that allows the synthesis of phosphorylated peptides as models of these regulatory phosphorylation sites. Such peptides, usually 10–20 residues in length, can be synthesized by solid-phase methods using automated instrumentation. They are constructed as protected molecules on polystyrene supports and then deprotected and cleaved from the support by treatment with acid. In our lab, rigorous purification and characterization are carried out to assure purity and identity of the material. We use mass spectrometric measurements to assess the molecular weight of the product to eliminate unwanted, modified side products that arise from incomplete removal of protecting groups or other modifications. The availability of this chemistry has permitted the production of several phosphopeptides that have been used to prepare antibodies specific to phosphorylation sites on proteins. We are preparing antibodies to the various phosphorylation sites on the cdc2 kinase, as well as the protein kinase CKII. In addition, for kinases that require target sites that are already phosphorylated at a site nearby the substrate hydroxyl, these synthetic phosphopeptides can be used as quantitative probes of activity. We have synthesized peptides representing the phosphorylation site for the enzyme glycogen synthase kinase 3, which requires a phosphorylated residue within the recognition site of the substrate.

Molecular Cloning and Expression of Casein Kinase II Subunits

N. Chester, D. Marshak [in collaboration with J. Horton, Cold Spring Harbor Laboratory, and J. Brooks, New England Biolabs]

The enzyme casein kinase II (CKII) is a protein serine/threonine kinase found in all eukaryotic cells. Its ubiquitous distribution among species and tissues implies a function central to all nucleated cells. The enzyme consists of two subunits, catalytic and regulatory, with molecular masses of 37–44 kD and 24–28 kD, respectively. To answer questions about the mechanism of enzyme regulation, we have cloned and expressed DNA molecules coding for the full-length forms of the subunits, using an innovative var-

iation of the polymerase chain reaction. This cloning has allowed us to insert the coding sequences for the CKII subunits into various expression vectors, including a new bicistronic vector supplied by New England Biolabs. Using these constructs, we have expressed the proteins in large amounts in bacteria. The purified, recombinant catalytic subunits are enzymatically active, and the activity is increased by the regulatory subunit. We have purified large amounts of the regulatory subunit for structural analysis. In collaboration with J. Horton, we have crystallized the regulatory subunit of CKII in forms that appear to diffract X-rays. We continue to produce larger crystals to solve the three-dimensional structure of this protein. The regulatory subunit is a unique structure that will be a novel contribution to the field.

Regulation of cdc2/CDC28 by Serine Phosphorylation

C. van den Bos, D.R. Marshak [in collaboration with A. Sutton, Cold Spring Harbor Laboratory, and G.L. Russo, Naples]

A major player in cell cycle progression and its regulation in mammalian cells is the protein kinase $p34^{cdc2}$. The enzyme has been found in all eukaryotes tested; in baker's yeast (*Saccharomyces cerevisiae*), for example, it has been termed cdc28. An important aspect of this protein kinase and its functions is its regulation by phosphorylation on multiple sites. The enzyme is highly conserved both structurally and functionally between evolutionary distant organisms such as humans and yeast, and we have exploited this by using cdc28 from *S. cerevisiae* as a model system for investigating possible functions of a phosphorylation site (Ser-39) described for the human analog earlier by our group. Previous work indicated that abolishing this particular phosphorylation by mutating Ser-46 to alanine might alter the growth behavior of *S. cerevisiae*, yet the phenotype observed was subtle as compared to, for example, *wee* mutants; in addition, Ser-46/Asp mutants seemed to behave similar to the Ser-46/Ala mutants, indicating that aspartic acid may not be able to mimic a permanent phosphorylation. We have now extended this work (1) by producing a Ser-46/Glu mutant that may have a better chance of mimicking a permanent phosphorylation, (2) by evaluating large numbers of size measurements (by linking a computer to a Coulter counter) to obtain more reliable data, (3) by challenging the yeast by means of different growth conditions (media), and (4) by testing the influence of the Ser-46 phosphorylation upon binding of ligands to cdc28 employing our mutated strains.

Regarding the size measurements, our work showed that indeed the Ser-46/Ala mutants exhibited a reduced cell size; in particular, cells from G_1 phase were affected. Exposure to different growth conditions (media) resulted in much more pronounced phenotypes: Both G_1 and G_2 cells seemed to be affected, and, furthermore, differences were observed between the Ser-46/Ala mutant and the Ser-46/Glu mutant (see Fig. 1). This might indicate a role of Ser-46 phosphorylation in *S. cerevisiae* in both G_1 and G_2 as well as a functional difference between the Ser-46/Ala mutant and the Ser-46/Glu mutant. Hence, the Ser-46/Glu mutant might indeed mimic a permanent phosphorylation.

As to possible influences of Ser-46 phosphorylation upon ligand binding to cdc28, we found that different amounts of H1 kinase activities could be precipitated from lysates of our mutant strains. This may indicate that the phosphorylation of cdc28 at Ser-46 modulates the affinity toward ligand(s) or that, depending on the phosphorylation state of cdc28 at Ser-46, different ligand(s) binds to it.

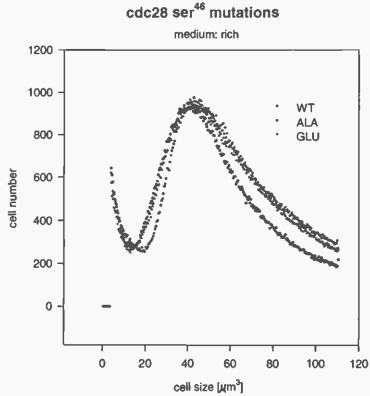

FIGURE 1 Growth curve of *S. cerevisiae* (cdc28 [Ser-46-Ala/Glu]) in rich medium; curves represent averages from 15 measurements each.

Cellular Isoforms of Protein Kinase CKII

N. Chester, D. Marshak

The CKII enzyme activity is found both in cytosol and in nuclei, and there are substrates identified in both locations. Cytosolic substrates include proteins involved in translational control (eIF-2, -3, -4B, -5), metabolic regulation (glycogen synthase), and the cytoskeleton (nonmuscle myosin heavy chain, β-tubulin). Substrates found in the nucleus include DNA topoisomerase II, RNA polymerases I and II, oncoproteins such as Myc, Myb, and SV40 large T antigen, and transcription factors such as serum response factor. The extraordinary range of substrates for this enzyme supports the contention that CKII has a significant role in cell physiology. We have developed a large set of specific antibodies to synthetic peptide antigens that react with the individual subunits of CKII.

Protein kinase CKII can be isolated as a heterotetramer, containing two catalytic (α or α′) and two regulatory (β) subunits. We have characterized the forms of CKII in HeLa cells using antibodies specific for the α or α′ subunits. Following metabolic labeling with [^{35}S]methionine, whole-cell soluble extracts were analyzed by immunoprecipitation and gel electrophoresis. Both α and α′ coprecipitate with β and with each other. However, when extracts are depleted of α, a pool of CKII containing only α′ and β is identified. Similarly, depletion of α′ revealed a pool exclusively of α and β. Therefore, we propose that there exist three distinct isoforms of CKII within HeLa cells with different catalytic subunit stoichiometries ($\alpha_2\beta_2$, $\alpha\alpha'\beta_2$ and $\alpha'_2\beta_2$). With our immunodepletion procedure, we have characterized the isoforms by activity analysis, turnover of pulse-labeled subunits, and localization in subcellular fractions obtained from labeled cells. We have also analyzed complex formation between the catalytic and regulatory subunits by examining differences in the rate of signal incorporation into subunits in immunoprecipitates obtained from continuously labeled and pulse-labeled cells. We have found that the $\alpha_2\beta_2$ and $\alpha\alpha'\beta_2$ isoforms assemble relatively slowly (12–16 hr), whereas complex formation of the $\alpha'_2\beta_2$ isoform occurs more rapidly (2–4 hr). Analysis of isoform complex formation in subcellular fractions from pulse-labeled cells revealed that the majority of nuclear CKII is assembled in the nucleus from free catalytic and regulatory subunit polypeptides, rather than being transported into the nucleus as a preassembled complex.

CKII-binding Proteins

G.-Y. Cha, Y.-S. Bae, N. Chester, D. Marshak

The regulation of protein kinase CKII may be through interactions with other proteins. N. Chester has optimized immunoprecipitation methods to allow the isolation of protein complexes involving CKII subunits. Using these methods, he has identified candidate proteins as CKII-binding proteins. In a parallel set of experiments, G.-Y. Cha and Y.-S. Bae have used the yeast two-hybrid screening system to identify CKII-binding proteins. Initially, they demonstrated that this system will reproducibly show subunit interactions within CKII, between the catalytic and regulatory subunits. They continued by screening the CKII subunits individually against a cDNA library from human cells. The goal is to isolate clones that code for proteins that interact with CKII physiologically. Bae and Cha will continue this work in collaboration from their home institution, Kyungpook University, Taegu, Korea, during 1995.

Protein Phosphorylation in Chronic Myelogenous Leukemia

N. Carpino, S. Goren, J. Kahler, D.R. Marshak
[in collaboration with B. Clarkson, Memorial Sloan-Kettering Cancer Center]

Chronic myelogenous leukemia (CML) is a disease characterized by the presence of the unregulated $p210^{bcr:abl}$ fusion protein tyrosine kinase, which is associated with an uncontrolled proliferation of myeloid cells. This project has two parts, both of which are designed to address the role of phosphorylation in CML. This project is part of a Program grant from the National Cancer Institute.

In the first project, N. Carpino and J. Kahler are purifying proteins that are potential substrates for the abnormally active bcr:abl tyrosine kinase. These proteins were first identified in leukemic cells by B. Clarkson at Memorial Sloan-Kettering Cancer Center in New York. In collaboration with that laboratory,

we are purifying two proteins, p62 and p56, that are both hyperphosphorylated in leukemic cells. Initial characterization has been done by a combination of standard chromatographic methods and affinity methods, particularly using anti-phosphotyrosine immunoblobulin.

The second study aims at mapping and characterizing $p34^{cdc2}$ phosphorylation sites on the $p210^{bcr:abl}$ protein as compared to their counterparts on the normal c-abl protein. This work is being carried out by S. Goren. Putative phosphorylation sites will be mutated, and the proteins produced will be assayed for their tyrosine kinase activity and their transformation potential using Rat-1 cells. These sites will be confirmed in in vivo phosphorylation studies of the $p210^{bcr:abl}$ protein, compared with the mutant proteins, in COS cells. In addition, transformation activity of the mutant proteins will be assayed in the human myeloid cell line MO_7 in comparison with the transformed cell line MO_7/p210, which stably expresses the $p210^{bcr:abl}$ protein. The phenotypes of the mutant proteins will be further characterized by determining their subcellular localization, identifying substrate specificity, cell division cycle dependence of c-abl, and bcr:abl phosphorylation by the $p34^{cdc2}$ kinase.

Protein Chemistry Core Facility: Peptide Synthesis

G. Binns, D. Marshak

The Protein Chemistry Core facility provides high-technology methods, equipment, and expertise for use by all of the scientists here at the Laboratory. Among the services available is the preparation of synthetic peptides for use as antigens, as enzyme substrates, as hormones and growth factors, and as entities for structural analysis. Peptides are assembled in the solid phase, usually of polystyrene supports. In this classic approach, first developed by Bruce Merrifield at The Rockefeller University, the solid support allows reagents and unwanted side products to be washed away continuously at every step without the requirement for purification of the peptide at each stage. At the end, the peptide is cleaved from the resin in a very active acid, hydrogen fluoride, under closed conditions. We then purify the peptide to homogeneity by reversed-phase HPLC. Using this approach, more than 400 peptides have been synthesized during the last few years. The structures are confirmed by amino acid analysis, mass spectrometry, and analytical HPLC. In addition, we provide assistance for investigators who are attempting to use synthetic peptides as antigens to prepare antibodies that will react with a cellular protein. There is a particular strategy that one takes to obtain good anti-peptide immunoglobulin. This strategy takes into consideration both the synthesis itself and the position in the target protein, as well as coupling methods for the antigen.

The Protein Chemistry Core facility is active in the Association of Biomolecular Resource Facilities (ABRF). This is worldwide organization of resource facilities whose purposes are (1) to promote and facilitate discussion and cooperation among facilities; (2) to provide research mechanisms for evaluation and improvement of the capabilities of facilities; and (3) to promote the education of facility staff, users, administrators, and interested members of the scientific community regarding facility functions. The ABRF exists because facilities have demanded an organization that will provide them with a mechanism for sharing information, maintaining state-of-the-art procedures, evaluating performance, and expanding to new areas. D. Marshak is a member of the Executive Board of the ABRF, and he hosted the winter board meeting. Involvement with the ABRF helps to maintain communication with other facilities around the world and improves our ability to maintain high standards of work. The ABRF provides standards that allow facilities to self-evaluate their performance and obtain expert advice on improvement, as necessary.

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MOLECULAR GENETICS OF EUKARYOTIC CELLS

The Section on *"Molecular Genetics of Eukaryotic Cells"* can boast several major accomplishments during 1994. Dr. Spector's laboratory has demonstrated the recruitment of splicing factors within the nucleus to sites of nascent RNA transcripts containing introns, but not at sites of intronless transcripts. Dr. Helfman's group has identified factors that promote muscle-specific alternative splicing and demonstrated that the dimerization pattern of tropomyosins is determined by alternative splicing. Dr. Tonk's laboratory has completed the structure of the first phosphotyrosine phosphatase complexed with a peptide substrate and utilized the MAP kinase phosphatase, MKP-1, to demonstrate the physiological roles of the MAP kinase (together with Dr. Bar-Sagi). Dr. Wigler's laboratory has demonstrated multiple pathways activated by Ras in the sexual differentiation of fission yeast and in mammalian cell transformation. Dr. Lisitsyn, in collaboration with Dr. Wigler's group, has discovered new loci in human cancers that are the frequent site of genetic deletion. Dr. Hengartner joined our section and studies the pathways of programmed cell death, or apoptosis, based on his ground-breaking observations that these pathways are conserved in metazoans.

MAMMALIAN CELL GENETICS

M. Wigler G. Asouline, P. Barker, M. Barr, J. Brodsky, E. Chang, J. Douglas, D. Esposito, K. Farina, M. Hamaguchi, V. Jung, R. Lucito, S. Marcus, C. Nicolette, K. O'Neill, T. Polverino, M. Riggs, L. Rodgers, J. Stolarov, J. Troge, H. Tu, L. Van Aelst, M. White, H.-P. Xu

During 1994, work in our group fell into two areas: signal transduction, the pathways that process the biochemical information that directs cellular activities, and the detection and characterization of genetic lesions in cancer cells. The first area stems from our long-term interest in the RAS genes, the genes that we first demonstrated were commonly activated in human cancers and that we subsequently discovered to be conserved in evolution. The second area stems from the use of representational difference analysis, or RDA, a method for comparative genomic analysis. These latter studies were conducted in collaboration with Dr. Nikolai Lisitsyn.

Signal Transduction

H.-P. Xu, L. Van Aelst, E. Chang, S. Marcus, T. Polverino, M. White, V. Jung, C. Nicolette, K. O'Neill, M. Barr, J. Stolarov, H. Tu

We have been studying RAS pathways in yeasts and vertebrates. In the budding yeast, *Saccharomyces cerevisiae*, RAS regulates adenylyl cyclase and is required for the progression of the cell through the G_1 phase of the cell cycle (Wigler et al., *Cold Spring Harbor Symp. Quant. Biol. 53:* 649 [1988]). Our studies in this organism are nearly complete. Vincent Jung (Jung et al. 1995) has cloned the gene for a new component required for normal RAS function in *S. cerevisiae*. Called *SHR5* (the fifth suppressor of hyperactive RAS), it appears to be required for the efficient localization of RAS to the membrane. Studies of *SHR5* also confirm earlier studies demonstrating multiple functions of RAS in *S. cerevisiae*. Kathy O'Neill, a graduate student from Columbia University, continues to study the cellular role of CAP (the second suppressor of hyperactive RAS), a protein that we demonstrated to be associated with adenylyl cyclase and to be required in vivo for the efficient interaction of the latter with RAS. She has identified cytoskeletal elements that interact with CAP and that may direct its localization and consequently that of adenylyl cyclase to regions of the cell where it can encounter RAS.

The majority of our recent studies of RAS have been in the fission yeast *Schizosaccharomyces pombe.* In this organism, RAS is required for sexual differentiation. It regulates gene expression, as shown by Hao-Peng Xu and others (Xu et al. 1994), by way of a protein kinase cascade (Wang et al., *Mol. Cell. Biol. 11:* 3554 [1991]), and it also regulates cellular morphology by way of a completely distinct pathway, as shown by Eric Chang (Chang et al. 1994). These two distinct pathways are the clearest demonstration of multiple RAS functions in the same organism, a conclusion that was drawn first from studies in budding yeast (Wigler et al., *Cold Spring Harbor Symp. Quant. Biol. 53:* 649 [1988]). The morphogenic pathway has a homolog in budding yeast and may involve proteins that regulate another GTP-binding protein, a member of the RHO family (Chang et al., *Cell 79:* 131 [1994]). This in turn may regulate another protein kinase, SHK1, which is likewise conserved in evolution, as shown by Stevan Marcus and others (S. Marcus et al., in press). Further studies in the fission yeast by V. Jung have led to the discovery of a new mutation in RAS that dominantly interferes with wild-type RAS function (Jung et al. 1994). These studies suggest the existence of multiple independent regulators of RAS in *S. pombe.*

The kinase cascade regulated by RAS in *S. pombe* is conserved in evolution, and it is now called a MAP kinase module. We have extended our studies to this module. Working with the budding yeast pathway required for sexual differentiation, S. Marcus discovered that STE5 is a scaffolding protein that can interact with the STE11, STE7, and FUS3 protein kinases (Marcus et al. 1994). This led us to postulate that STE5 promotes the interaction of these protein kinases and also serves as an insulator, limiting cross talk between this MAP kinase module and others with distinct functions and regulators. In *S. pombe*, byr2 is the protein kinase required for sexual differentiation that interacts directly with RAS. Maureen Barr, a graduate student from Columbia University, has identified another upstream regulator of byr2. The existence of this upstream regulator may help explain the concerted action of RAS and G proteins in the sexual differentiation of *S. pombe* (Xu et al., *Mol. Cell. Biol. 14:* 1333 [1994]). Maureen Barr and Hua Tu, a graduate student from SUNY, Stony Brook, are identifying the domains of byr2 that interact with its upstream regulators.

The observation that a MAP kinase module was conserved in evolution and that this module was regulated by RAS in both fission yeast and vertebrates led to the demonstration that RAF was an immediate downstream target of RAS in vertebrates (Van Aelst et al., *Proc. Natl. Acad. Sci. 90:* 6213 [1993]). RAF is itself encoded by a proto-oncogene and is a protein kinase capable of activating the MAP kinase module. Current work by Javor Stolarov, a graduate student from Columbia University, is directed at defining the domains of RAF that are involved in its regulation. To ask further if RAF mediates RAS effects in vertebrates, Michael White created mutant RAS proteins that fail to interact with RAF and complementary RAF mutants that restored interaction (White et al., *Cell 80:* 533 [1995]). Experiments with these mutants led to the unambiguous conclusion that RAF can mediate transformation of mammalian cells and induction of gene expression by RAS. However, these same types of experiments also led to the clear conclusion that other RAF-independent pathways can also contribute to transformation by RAS (White et al., *Cell 80:* 533 [1995]).

As in fission yeast, RAS thus has multiple targets in vertebrate cells. To begin to define these, Linda Van Aelst has used the yeast two-hybrid system of Fields and Song to identify new genes encoding proteins that bind to RAS (Van Aelst et al. 1994).

Comparative Genomic Analysis

M. Hamaguchi, P. Barker, H.-P. Xu, R. Lucito,
D. Esposito, with Dr. N. Lisitsyn

The method of representational difference analysis, which was developed here at the Laboratory by Dr. Nikolai Lisitsyn and Michael Wigler, has been described in our previous reports (Lisitsyn et al., *Science 259:* 946 [1993]). It enables the cloning of differences between two similar genomes. Recently, it has been used by other investigators to discover viruses associated with human disease, including Kaposi's sarcoma and hepatitis. We have been applying it to the analysis of the differences between the genomes of tumor cells and the normal cells from which they derived. When we seek sequences lost in tumor cells, we generally find probes for loci that have either undergone homozygous deletion or loss of heterozygosity. Two of the loci we have identified, one on chromosome 3 and one on chromosome 20, undergo frequent deletions in gastrointestional

tumors (Lisitsyn et al. 1994, 1995). We have observed lesions at these loci in breast cancer and rarely in other types of cancers. So far, RNA transcripts from these loci have not been detected.

When we seek sequences gained in tumor cells relative to normal cells, we generally have found probes that detect gene amplification. We have isolated such probes from melanomas, lung cancers, and breast cancers, mapping, respectively, to chromosomes 3, 2, and 17. The second and third may represent the N-myc and ERB-B2 locus, respectively. The first may represent a novel locus.

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CANCER GENETICS

N. Lisitsyn N. Lisitsina

We are interested in the discovery of critical genes, disrupted in cancer cells by various types of genetic alterations, and understanding the biological function of these genes. Our approach to detection of DNA changes in tumors is based on application of a new powerful technology for finding the differences between genomes, developed in Mike Wigler's laboratory (Lisitsyn et al., *Science 259:* 946 [1993]). This year, our efforts have been focused on the finding and mapping of homozygous deletions in DNA of certain types of tumors. Such genetic alterations have been regarded as evidence that the affected genomic regions are harboring tumor suppressor genes, which we have sought to identify by positional cloning.

Identification and Mapping of Homozygous Deletions in Tumors

N. Lisitsina, N. Lisitsyn [in collaboration with R. Lucito and M. Wigler, Cold Spring Harbor Laboratory]

Extensive search for DNA losses has been performed in 30 kidney cancer and colon cancer cell lines, using a new powerful technology for finding the differences between the genomes, called representational difference analysis (RDA). In 12 cases, we have searched for losses in tumor DNAs isolated from aneuploid nuclei, which have been fractionated

from tumor biopsies by the fluorescence-activated cell sorter. We have found probes, detecting homozygous losses in kidney cancer cell lines (six probes), colon cancer cell lines (three probes), and esophageal and breast cancer biopsies (one of each), and have located these probes in nine separate genomic regions (Lisitsyn et al. 1995). Two of the regions, harboring previously known tumor suppressor genes MTS 1 and DCC, have been excluded from further analysis, and the other seven regions have been cloned by screening of yeast artificial chromosome (YAC) and/or cosmid libraries.

We have searched for the most frequently deleted loci by isolating sequence-tagged sites (STSs) from YACs, using a new subtraction technology, which we have developed for this purpose, and determining the frequency of their loss in a large collection of DNAs isolated from cancer cell lines. Two STSs from two separate genomic regions, located on chromosomes 3 and 20, have been found to be deleted with remarkable frequency (~10%) in DNAs isolated from cancer cell lines established from tumors of the gastrointestinal tract. Simultaneous homozygous loss of both STSs has been detected in two tumor DNAs, indicating that potential tumor suppressor genes, encoded in these regions, are involved in different pathways.

Positional Cloning of a Potential Tumor Suppressor Gene from Region 3p21

N. Lisitsina, N. Lisitsyn [in collaboration with F. Leach and B. Vogelstein, The Johns Hopkins Oncology Center, and M. Hamaguchi, R. McCombie, and M. Wigler, Cold Spring Harbor Laboratory]

We have identified the genomic region, frequently deleted in tumors of the gastrointestinal tract, and mapped it to band p21 on human chromosome 3 by polymerase chain reaction (PCR) of radiation hybrids. On the basis of the absence of the microsatellite instability phenotype in most colon cancer cell lines with homozygous losses in this region, a potential tumor suppressor gene has proved to be different from the *mutL* gene homolog (a gene altered in patients with hereditary nonpolyposis colon cancer, which is located in the same place). Four overlapping cosmids, spanning a minimal region of homozygous loss (100 kb long), have been used for the cloning of transcribed sequences by direct cDNA selection and exon-trapping methodologies, providing five exons of candidate genes expressed in colon and stomach cells. The sequences of full-length cDNA copies of these genes will be determined and used for mutational analysis.

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TRANSMEMBRANE SIGNALING

D. Bar-Sagi M. Boyer, K. Degenhardt, J. Hong, T. Joneson, S. Kaplan, S-.S.Yang

Our interest is in the regulation of cell growth by extracellular signals. Our research has continued to focus on the mechanisms of signal transduction by Ras proteins. *ras* genes comprise a highly conserved eukaryotic gene family. They encode membrane-associated guanine nucleotide-binding proteins that function as molecular switches passing signals that control cell growth and differentiation to intracellular effector molecules. The chemical basis for this switch function involves the cycling of Ras proteins between active GTP-bound and inactive GDP-bound states. In normal quiescent cells, the Ras proteins exist primarily in the inactive GDP-bound form. Upon stimulation by a variety of mitogenic and differentiation signals, rapid and transient elevation of Ras-GTP levels are observed. Thus, the GTP/GDP cycle provides a rapid and regulatable mechanism for Ras modulation of signal transduction pathways. Mutations found in oncogenic forms of Ras proteins impair their GTPase activity, thus perturbing this cycle, and render these mutated proteins in a chronically active state. During the past year, we have continued and extended our studies on the characterization of the mechanisms by which Ras proteins mediate the transduction of signals from cell surface receptors to intracellular effector molecules. In addition, we have devoted much effort during this past year toward the identification of the cellular components that mediate the mitogenic activity of Ras proteins with the aim of furthering our understanding of the molecular events underlying the aberrant growth properties of Ras-transformed cells.

Regulation of the Mammalian Ras Exchange Factor Sos

K. Degenhardt

The Son of sevenless (Sos) proteins are guanine nucleotide exchange factors involved in the activation of Ras by cytoplasmic and receptor tyrosine kinases. In virtually all cells examined thus far, Sos is found in complex with the adaptor protein GRB2. We have previously shown that the interaction between Sos and GRB2 is mediated by the proline-rich carboxy-terminal domain of Sos and the SH3 domain of GRB2. Upon growth factor stimulation, the GRB2-Sos complex binds to the activated receptor through the SH2 domain of GRB2. However, the guanine nucleotide exchange activity of Sos is not measurably affected by growth factor stimulation. Thus, it has been postulated that receptor stimulation serves to translocate the GRB2-Sos complex to the activated receptor. This translocation could lead to an increase in the guanine nucleotide exchange on Ras by increasing the local concentration of the exchange factor in the plasma membrane where Ras is located.

Growth factor stimulation rapidly induces the phosphorylation of Sos on serine and threonine residues. Under conditions where Ras activity is inhibited, no phosphorylation of Sos occurs, suggesting that the phosphorylation of Sos may be mediated by downstream components of the Ras signaling pathway. We have characterized the phosphorylation of Sos by two-dimensional phosphopeptide mapping. Growth factor stimulation results in the phosphorylation of Sos on multiple sites. A significant proportion of these sites are also phosphorylated by MAP kinase in vitro, indicating a role for MAP kinase in mediating the growth-factor-induced Sos phosphorylation. Other cellular serine/threonine kinases of as yet unknown identity also contribute to Sos phosphorylation. Sos phosphorylation in vivo and by MAP kinase in vitro occurs at the carboxy-terminal region of the molecule. GRB2 binding to Sos does not affect the ability of MAP kinase to phosphorylate Sos. However, growth factor stimulation is accompanied by an apparent decrease in the affinity of GRB2 for Sos both in vivo and in vitro. The growth-factor-induced dissociation of the GRB2-Sos complex coincides with Sos phosphorylation. Moreover, phosphorylated Sos molecules are preferentially localized to the cytosol. These findings point to a potential negative feedback mechanism to control growth-factor-induced activation of Ras.

Differential Interaction of hSOS1 and hSOS2 with GRB2

S.-S. Yang

Mammalian cells contain two related but distinct SOS proteins designated SOS1 and SOS2. Although SOS1 and SOS2 share a high degree of overall similarity (69% identity), it is not known to what extent their biological and biochemical properties overlap. The most divergent region between these two proteins lies in their carboxyl terminus (38% identity). A number of studies have shown that the carboxy-terminal proline-rich domain of SOS1 directly interacts with the SH3 domain(s) of the adaptor protein GRB2/Sem5/Drk. The biochemical features and physiological significance of this interaction have also been well documented. In contrast, little is known about the interaction of SOS2 with GRB2. In the present study, we have compared the interaction of hSOS1 and hSOS2 with GRB2. Using the yeast two-hybrid system, we showed that the carboxy-terminal proline-rich domain of hSOS2 is necessary and sufficient for association with GRB2. As in the case of its counterpart hSOS1, the interaction of hSOS2 with GRB2 relies on the intact SH3 domains of GRB2. However, the apparent binding affinity of hSOS2 for GRB2 is significantly higher relative to that of hSOS1 both in vitro and in vivo. The region conferring this higher binding affinity has been mapped to residues 1126–1242 of the hSOS2 carboxyl terminus using chimeric proteins. This region of hSOS2 is enriched in putative high-affinity SH3-binding motifs relative to hSOS. Therefore, we propose that the higher-affinity of hSOS2 could be due to the increased abundance of high-affinity SH3-binding sites for GRB2 and/or their relative position within the carboxyl terminus of hSOS2. Our data show, for the first time, that SOS1 and SOS2 display a difference in their biochemical properties. It will be interesting to test whether this can provide a mechanism for signal diversity.

Ras Signaling and the Regulation of Cell Growth and Motility

T. Joneson [in collaboration with M. White and M. Wigler, Cold Spring Harbor Laboratory]

Genetic and biochemical studies have identified the mitogen-activated protein (MAP) kinase cascade as a critical downstream mediator of Ras signaling. Ras

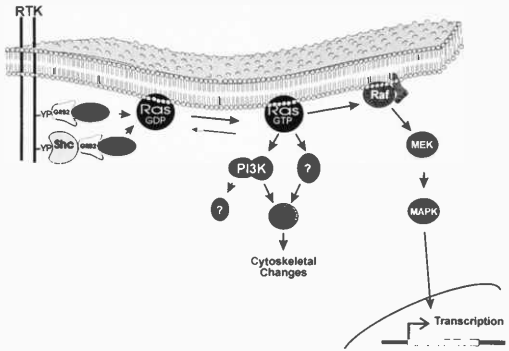

FIGURE 1 Increasing complexity of the Ras signal transduction pathway. Our studies have established that the mitogenic activity of Ras is mediated by at least two distinct signaling cascades: the MAP kinase cascade, which regulates transcriptional events in the nucleus, and the Rac cascade, which mediates changes in cytoskeletal organization. The functional relationship between these two Ras-dependent pathways will be the subject of our future studies.

proteins are also known to induce the reorganization of the actin cytoskeleton (Fig. 1). We have used a microinjection assay to analyze the functional relationship between these two Ras-dependent signals and to establish their relative contribution to the mitogenic effects of Ras. In this assay, the expression of the microinjected reporter construct containing the serum response element (SRE) is used to monitor the activation of the MAP kinase cascade, membrane ruffling is used to detect the reorganization of actin cytoskeleton, and DNA synthesis is used to score mitogenic effects. In quiescent rat embryo fibroblasts, activated Ras (RasV12) induces MAP kinase activation, membrane ruffling, and DNA synthesis. Coinjection of RasV12 and the MAP kinase phosphatase MKP-1 blocks MAP kinase activation and DNA synthesis but not membrane ruffling. Injection of activated forms of Raf (v-Raf or Raf-CAAX) induces MAP kinase activation but has no effect on membrane ruffling, suggesting that activation of the MAP kinase cascade is neither necessary nor sufficient for the induction of membrane ruffling. We have identified an effector- binding-loop mutant of Ras that is defective in Raf interaction and MAP kinase activation but retains the ability to induce membrane ruffling. An activated form of this mutant containing the V12 substitution is unable to stimulate DNA synthesis when injected into quiescent fibroblasts. Another effector-binding-loop mutant of Ras, RasV12S35, does not induce membrane ruffling but has previously been shown to interact with Raf and activate MAP kinase. This mutant is also unable to induce DNA synthesis in quiescent fibroblasts, indicating that activation of MAP kinase is not sufficient for Ras-induced mitogenesis. Coinjection of both Ras mutants results in a significant stimulation of DNA synthesis. These results indicate that stimulation of membrane ruffling and activation of the MAP kinase cascade represent two independent Ras effector systems that bifurcate at the level of the Ras protein itself. An input from both pathways is required for the mitogenic activity of Ras.

Inhibition of Ras-induced Proliferation by $p16^{INK4}$

D. Bar-Sagi [in collaboration with M. Serrano and D. Beach, Cold Spring Harbor Laboratory]

In mammalian cells, Ras proteins have a critical role in stimulating the transition from the quiescent to the DNA replication phase of the cell cycle. A key regulatory component in the progression of cells through the G_1 phase of the cell cycle is the cyclin-dependent kinase 4 (CDK4). The activity of CDK4 is controlled by the opposing effects of the D-type cyclin, an activating subunit, and $p16^{INK4}$, an inhibitory subunit. It has been postulated that $p16^{INK4}$ acts as a tumor suppressor protein because the $p16^{INK4}$ gene is frequently deleted in tumor cell lines and shows a high frequency of point mutations and small deletions in some tumor cell lines and primary tumors. We have utilized the mitogenic activity of Ras proteins to investigate whether $p16^{INK4}$ can exert growth-suppressing activity. Ectopic expression of $p16^{INK4}$ blocked entry into S phase of the cell cycle induced by oncogenic Ras. This block was relieved by coexpression of a catalytically inactive CDK4 mutant, indicating that the inhibition of Ras-induced mitogenesis by $p16^{INK4}$ is specifically mediated via its inhibitory effect on CDK4. Expression of $p16^{INK4}$ also suppressed cellular transformation of primary rat embryo fibroblasts by oncogenic Ras and Myc. Together, these observations provide evidence that the growth-promoting activity of the Ras oncogene can be effectively antagonized by $p16^{INK4}$. These findings further support the notion that $p16^{INK4}$ functions as a tumor suppressor gene.

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PROTEIN TYROSINE PHOSPHATASES AND THE CONTROL OF CELLULAR SIGNALING RESPONSES

N.K. Tonks S.M. Brady-Kalnay, M.A. Daddario, R.L. Del Vecchio, A.J. Flint, A.J. Garton, M.J. Gutch, Y.F. Hu, K.R. LaMontagne, S.N. Mamajiwalla, A.A. Samatar, H. Sun, S.H. Zhang

The phosphorylation of tyrosyl residues in proteins is a key component of the control of many fundamental physiological processes. Our lab is particularly interested in the role of tyrosine phosphorylation in transducing an extracellular signal into an intracellular response, such as proliferation or differentiation. Protein phosphorylation is a reversible, dynamic process in which the net level of phosphate observed in a target substrate reflects not only the activity of the kinases that phosphorylate it, but also the competing action of protein phosphatases that catalyze the dephosphorylation reaction. We study the expanding family of protein tyrosine phosphatases (PTPs) that, like the kinases, comprise both transmembrane, receptor-linked forms and nontransmembrane, cytosolic species. The structures of the PTPs indicate important roles in the control of key cellular functions.

During the last year, Bill Eckberg completed his sabbatical and returned to Howard University, Washington, D.C. and Peter Guida, Jr., left to begin Ph.D. studies at Albert Einstein. We were joined by Mike Gutch as a postdoctoral fellow and Martha Daddario as a technician.

Receptor PTPs: Roles in Cell-Cell Contact Phenomena

S.M. Brady-Kalnay, S.N. Mamajiwalla, A.A. Samatar, N.K. Tonks

A characteristic feature of the extracellular segment of many receptor-like PTPs is the presence of immunoglobulin (Ig)-like and fibronectin type III (FNIII) domains. These are motifs that are commonly found in cell adhesion molecules. PTPμ comprises (1) an extracellular segment containing a MAM domain, one Ig-like domain, and four FNIII domains, (2) a single transmembrane domain, and (3) an intracellular segment containing two PTP domains separated from the transmembrane domain by a juxtamembrane sequence that displays homology with the intracellular segment of the cadherin superfamily of cell adhesion molecules. We have previously shown that when expressed in Sf9 cells, PTPμ induces the formation of cell:cell aggregates through a homophilic binding mechanism, i.e., PTPμ on one cell binds to PTPμ on an adjacent cell. Such homophilic binding required only the presence of the extracellular segment and was also observed for PTPμ as normally expressed on the surface of mink lung MvLu cells. Thus, the ligand for PTPμ is another PTPμ molecule on the surface of an adjacent cell. We have now generated, expressed, and purified various fragments of the extracellular segment of PTPμ and have used fluorescent beads (covaspheres) coated with these fragments in a number of binding assays to establish that the Ig domain is both necessary and sufficient for homophilic binding. Schlessinger's group at New York University have found that when Sf9 cells expressing PTPμ and PTPκ (a close homolog of PTPμ displaying ~75% overall identity) were mixed, they sorted independently. In other words, PTPμ binds to itself but not to PTPκ, suggesting that the homophilic binding interaction is highly specific. Therefore, the reagents we have de-

veloped toward the Ig domain of PTPμ may prove very useful in defining the physiological significance of these homophilic binding interactions.

As far as we can tell, aggregation, i.e., ligand binding to the extracellular segment of PTPμ, had no detectable direct effect on the activity of the intracellular PTP domains. However, it is possible that the activity of PTPμ may be controlled indirectly by restricting its distribution in the membrane and thus restricting the spectrum of substrates with which it interacts. Our most recent data suggest that under physiological conditions of expression in mink lung cells, PTPμ is localized to adherens junctions, which are defined points of cell-cell contact at which the actin cytoskeleton is anchored. Within these junctions, we have found that PTPμ associates directly with members of the cadherin superfamily and is recovered as part of a complex with cadherin, α catenin and β catenin. Preliminary data suggest that the cadherins may be endogenous substrates for PTPμ. These data are provocative because tyrosine phosphorylation of the cadherin/catenin complex has been observed under a variety of physiological conditions and interferes with its normal adhesive function, potentially contributing to transformation and metastasis. It appears that PTPμ may provide the regulatory balance to this phorylation event, thus maintaining the complex in its normal dephosphorylated state.

We are also studying a broadly expressed receptor PTP termed DEP-1 (density-enhanced PTP). The cDNA predicts an enzyme with eight FNIII repeats in its extracellular segment and a single intracellular PTP domain. The most striking property of this enzyme is that its expression is increased dramatically in dense relative to sparse cell cultures. In a series of experiments currently under way, we have detected striking morphological changes in cells in which this enzyme is overexpressed that are not seen in cells overexpressing other members of the PTP family. We are currently trying to identify the important substrates for this effect, and preliminary data suggest a number of cytoskeletal targets.

These observations suggest that receptor PTPs may be important contributors to the mechanism of contact inhibition of cell growth. As normal cells in culture approach confluence, and adjacent cells touch each other, growth is inhibited. In cancer cells, this process is disrupted. Since tyrosine phosphorylation has been implicated in promoting cell growth and proliferation, PTPs as the natural antagonists of PTK function may exert a negative effect on such growth-promoting signals by triggering net dephosphorylation of proteins in the membrane. The advantage of the involvement of receptor PTPs in such a phenomenon is that their extracellular segments may "sense" directly cell-cell contact. Engagement of the extracellular segment of a receptor PTP in this way in confluent cells may either modulate its activity directly or target it to a particular junctional complex so as to trigger dephosphorylation of a defined subset of phosphotyrosyl proteins and initiate the growth inhibitory response.

MKP-1, a Tyr/Thr Dual Specificity Phosphatase That Dephosphorylates, and Blocks Signaling Downstream from, MAP Kinase

H. Sun and N.K. Tonks [in collaboration with D. Bar-Sagi, Cold Spring Harbor Laboratory]

Recently, progress has been made by many labs in defining signaling pathways initiated by mitogenic stimuli. Growth-factor-induced autophosphorylation of tyrosyl residues in receptor-PTKs induces the formation of multiprotein complexes in the membrane that trigger conversion of Ras from an inactive GDP-bound form to an active GTP-bound state. Activated Ras then initiates a cascade of sequential phosphorylation events in which the Ser/Thr kinase Raf phosphorylates and activates MAP kinase kinase (also known as MEK), which is a dual specificity kinase that in turn phosphorylates both Thr-183 and Tyr-185 in MAP kinase. Phosphorylation of both tyrosine and threonine regulatory sites is essential for activation. The MAP kinase family of enzymes has been implicated as common and essential components of signaling pathways induced by diverse mitogenic stimuli. Once activated, MAP kinase can phosphorylate a number of substrates, including transcription factors, that are essential for triggering the expression of genes required for the mitogenic response. Therefore, growth factor binding initiates a complex network of protein phosphorylation events that lead a quiescent cell to enter the cell cycle, undergo DNA replication, and ultimately divide. One of these immediate early genes that is activated rapidly and transiently in quiescent fibroblasts treated with serum growth factors is 3CH134, which encodes a dual specificity (Tyr/Thr) phosphatase termed MKP-

1. MKP-1 possesses intrinsic phosphatase activity that is highly specific for both the tyrosine and threonine regulatory sites in MAP kinases. It thus has the potential to feed back on the growth factor signaling pathway to prevent uncontrolled growth and proliferation.

In light of the high degree of substrate specificity displayed by MKP-1, we set out to use it as a molecular probe to investigate physiological roles for MAP kinase. Using a microinjection strategy in REF52 rat fibroblasts, we showed that expression of MKP-1 inhibits the ability of growth factors, TPA, and oncogenic Ras, which signal through the MAP kinase pathway, to *trans*-activate a promoter containing five copies of a serum response element (SRE) linked to a CAT reporter. Furthermore, MKP-1 blocked the induction of DNA synthesis in quiescent REF52 fibroblasts by the Val-12 oncogenic mutant form of Ras (Fig. 1). These results suggest an essential role for the activation of MAP kinases in the transition from quiescence to the DNA replication phase of the eukaryotic cell cycle. In similar experiments in *Xenopus* egg extracts, in collaboration with Jeremy Minshull and Andrew Murray (University of California, San Francisco), we have shown that the activation of MAP kinase is required for the establishment and maintenance of the mitotic arrest induced by depolymerization of spindle microtubles. We are currently using MKP-1 as a probe to investigate other physiological roles for MAP kinase as well as trying to characterize mechansims for posttranslational control of the activity of the phosphatase in vivo.

Cytoplasmic PTPs: Analysis of the Structure, Regulation, and Function of PTP1B

A.J. Flint, Y.F. Hu, K.R. La Montagne, N.K. Tonks

PTP1B was the first member of the family to be isolated in homogeneous form. The amino-terminal segment of the protein comprises the catalytic domain to which is fused a regulatory carboxy-terminal tail. We have previously characterized a cell-cycle-dependent phosphorylation of seryl residues in this regulatory segment. Furthermore, others have shown that the extreme carboxy-terminal 35 amino acids are highly hydrophobic and both necessary and sufficient for targeting the enzyme to the cytoplasmic face of membranes of the endoplasmic reticulum.

In collaboration with David Barford (now at the University of Oxford), we determined the crystal structure of the catalytic domain of PTP1B. It is composed of a single domain with the polypeptide chain organized into 8 α helices and 12 β strands with a 10-stranded mixed β sheet, which adopts a highly twisted conformation, spanning the entire length of the molecule. The structure illustrates that the sequence motif [I/V]HCXAGXXR[S/T]G, which contains the catalytically essential nucleophilic cysteinyl residue and uniquely defines the PTP family of enzymes, forms the phosphate recognition site and is located at the base of a cleft on the surface of the protein. We proposed that specificity for phosphotyrosine-containing substrates probably resulted from the depth of the cleft, since the structure sug-

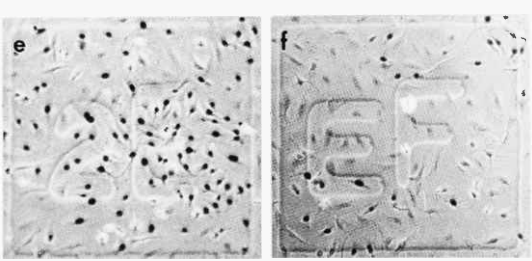

FIGURE 1 Inhibition of V^{12} Ras-induced DNA synthesis by MKP-1. Quiescent REF52 cells were microinjected with bacterially expressed V^{12} Ras protein together with control plasmid (*e*) or plasmid expressing MKP-1 (*f*). BrdU incorporation was detected by immunohistochemical staining. The fields were visualized and photographed with phase-contrast microscopy. (Reprinted, with permission, from Sun et al. *Science 265:* 285–288 [1994].)

gested that the smaller phosphoserine and phosphothreonine residues would not reach down to the phosphate-binding site.

We have now examined this issue of substrate binding by determining the structure of PTP1B complexed with a high-affinity peptide substrate representing an autophosphorylation site of the epidermal growth factor receptor. We made use of the fact that a catalytically inactive mutant of PTP1B, in which the essential cysteine from the signature motif, C215, was replaced by serine, retained the ability to bind substrate even though it could not dephosphorylate it. We observed that peptide binding to the protein was accompanied by a large conformational change in a surface loop at one end of the substrate-binding cleft, which created a recognition pocket that surrounded the phosphotyrosine in the substrate. In fact, PTP1B represents an example of Koshland's concept of "induced fit", whereby substrate binding induces a conformational change that creates the catalytically competent form of the enzyme. As we initially postulated, the depth of the pTyr binding pocket, which at ~9 Å exactly matches the length of a pTyr residue, is a major determinant of specificity for phosphotyrosine. A similar mechanism has been proposed to explain the specificity of SH2 domains for pTyr residues. Another determinant of specificity for PTP1B is the presence of nonpolar side chains lining the cleft that form hydrophobic interactions with the phenyl ring of the pTyr residue. Engagement of pTyr by the binding pocket anchors the peptide to the peptide-binding site. Hydrogen bonds between peptide main-chain atoms and the protein contribute to binding affinity, and specific interactions of acidic residues of the peptide with basic residues on the surface of the enzyme confer sequence specificity. However, the relatively open structure of the peptide-binding site is consistent with the ability of PTP1B to dephosphorylate a variety of pTyr substrates.

We are currently using this structural information as a guide for preparing various mutant forms of PTP1B in which we have altered key residues that are important for substrate recognition. Preliminary studies suggest that some of these mutants, for example, D181A, retain a high affinity for substrate but display an exceedingly poor ability to catalyze dephosphorylation. We are currently expressing such mutants to see if we can trap and thus identify physiological substrates for PTP1B. We are particularly intrigued by the possibility that PTP1B may be involved in chronic myelogenous leukemia (CML). CML is a clonal disorder of the hematopoietic stem cell characterized by the Philadelphia chromosome, in which the c-Abl proto-oncogene on chromosome 9, encoding a PTK, becomes linked to the *bcr* gene on chromosome 22. This results in the production of a fusion protein termed p210 bcr:abl, the PTK activity of which is enhanced relative to c-Abl generating abnormal patterns of tyrosine phosphorylation in CML cells. We have observed that the level of expression of PTP1B, but not TCPTP, which displays 74% identity to PTP1B, is increased severalfold in a variety of cells that express p210 bcr:abl, including lines derived from a CML patient. This effect, which is due to enhanced stability of the PTP1B protein, requires the PTK activity of p210 bcr:abl and is not seen in cells expressing v-*abl* or the v-*src* PTK or in cells transformed by *myc*, a non-PTK oncogene. Therefore, it appears that specifically following expression of bcr:abl PTK oncoproteins, there is selectively enhanced expression of PTP1B, implying an important function for this phosphatase in signaling pathways triggered by bcr:abl. We are in the process of expressing PTP1B mutants, such as D181A, in cells expressing p210 bcr:abl, to determine whether we can trap either the oncoprotein PTK or some of its signaling components as complexes with the mutant phosphatase.

Regulation of PTP Activity

R.L. Del Vecchio, A.J. Garton, M.J. Gutch, S.H. Zhang, N.K. Tonks

The activity of members of the PTP family may be regulated at several levels. In the case of the receptor-like enzymes, there is obviously the potential for modulation of activity by ligand binding to the extracellular segment. In addition, it appears that many of the members of this family will be responsive to regulation by reversible phosphorylation, intracellular targeting and association with other proteins. We are currently investigating each of these potential modes of regulation in various members of the family.

PTP-PEST is a soluble enzyme that is expressed ubiquitously in mammalian tissues. We have shown, both in vitro and in vivo, that PKA and PKC are capable of phosphorylating two sites, Ser-39 and Ser-435, in PTP-PEST. Phosphorylation of Ser-39 is inhibitory, reducing the affinity of PTP-PEST for substrate, offering a mechanism whereby signal trans-

duction pathways acting through Ser/Thr kinases (PKA or PKC) may indirectly influence the phosphorylation of tyrosyl residues in cellular proteins. We have now observed that phosphorylation of Ser-435 appears to affect the subcellular distribution of PTP-PEST, promoting association with an endosomal membrane fraction. We are currently trying to identify the components of the membranes with which PTP-PEST associates and to define which other signal transduction pathways promote the phosphorylation of Ser-435 in the phosphatase.

The importance of subcellular targeting is also seen in the case of PTPX1 and X10 from *Xenopus*. These enzymes possess amino-terminal segments that have homology with lipid-binding proteins and can associate with endosomal membranes, which correlates with an increase in activity. In addition, PTPH1 displays homology, in its amino-terminal segment, with the band-4.1 superfamily of proteins which are targeted to interfaces between the plasma membrane and the cytoskeleton. We have shown that this segment exerts a negative influence on catalytic activity and are currently trying to identify potential regulatory proteins that interact with this segment.

Finally, in collaboration with Michael Hengartner's group here at the Laboratory, we have recently initiated a study of PTPs in *Caenorhabditis elegans*. We hope to use the power of this genetic system to provide insights into the regulation and function of members of the PTP family.

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NUCLEAR SIGNAL TRANSDUCTION

M. Gilman D. Grueneberg, G. Lee, T. Nahreini, S. Natesan, A. Ryan, H. Sadowski, K. Simon, M.-L. Vignais

We continue our focus on how extracellular signals are communicated to the nucleus and how they are interpreted there into specific patterns of gene expression for the biological response requested by

each signal. Our work revolves primarily around our understanding of how the mammalian proto-oncogene c-*fos* is activated by extracellular stimuli. In past years, we have worked out aspects of the organization of the c-*fos* promoter and the identification of DNA-binding proteins and signal transduction pathways that relay signals to the gene. Most of our recent attention has focused on the serum response element (SRE), a DNA sequence required for the transcriptional response of the c-*fos* gene to multiple independent signal transduction pathways. Activity of the SRE requires binding of a protein termed serum response factor (SRF), a ubiquitously expressed MADS-box transcription factor. SRF is the nucleus for the formation of multiprotein complexes at the SRE that contain proteins whose activities are directly modulated by signals. Thus, one of the clear roles of SRF in this process is to recruit these signal-responsive proteins to the DNA.

Our work this year has focused in three general areas. First, we have studied how SRF itself is recruited to the DNA, because we believe that SRF recruitment is a critical step in determining which genes in a cell have the potential to respond to extracellular signals. We have identified two types of partners that can help SRF bind DNA—homeodomains of the Paired class and the ubiquitous zinc-finger protein YY1. Second, we have studied one of the signal transduction pathways that links growth factor receptors with the c-*fos* gene, which involves transcription factors of the STAT family. Third, we have extended our studies of nuclear signal transduction to *Drosophila*, in which we can more readily assess the role of these pathways in an intact animal. We have isolated and studied a gene encoding a fly homolog of SRF and a fly member of the JAK kinase family, thought to be involved in STAT activation by cytokines.

SRF-Homeodomain Complexes and the Specificity of Signal Transduction to the Nucleus

D. Grueneberg

A key unresolved issue in development is how similar signal transduction pathways can elicit distinct patterns of gene expression in different cells. Our working hypothesis is that such signals are interpreted at the transcriptional level by factors that regulate cell-type-specific gene expression. We believe that interactions between SRF and homeodomain proteins may serve as a model for understanding this mechanism. We have found that a novel human homeodomain protein, Phox1, and related homeodomains of the Paired class, can interact with SRF in vitro and enhance its binding to certain sequences.

We have now devised an assay to study the interaction between SRF and Phox1 in vivo. In HeLa cells, Phox1 can enable an SRE-containing reporter gene to respond to serum growth factors. By a variety of criteria, we believe that the activity of Phox1 in this assay reflects its ability to recruit functional SRF to the reporter. Although we do not know the organization of the SRF-Phox1 complex, testing of various Phox1 homeodomain mutants in this assay shows that activation of the reporter requires amino acids in all three helices of the Phox1 homeodomain. Most interestingly, the Phox1 homeodomain activates transcription from only a subset of SRE-binding sites in HeLa cells. It appears that Phox1 targets SRF to specific moderate-affinity binding sites for transcriptional activation in response to extracellular signals.

Our working model implies that ubiquitously inducible genes contain an SRE that strongly binds SRF without requirement for a homeodomain partner and that SRF in turn recruits its signal-responsive accessory proteins to confer a transcriptional response to signals. Genes induced in a cell-type-specific pattern may contain an imperfect SRE that binds SRF only in the presence of an appropriate homeodomain protein. The resulting SRF-homeodomain complexes retain the ability to recruit signal-responsive SRF accessory proteins to confer a transcriptional response to incoming signals, but only in cells expressing the correct homeodomain protein.

Structure of SRF-Homeodomain Complexes

K. Simon

We have previously cloned a novel human homeodomain protein, Phox1, that interacts with SRF to enhance its binding to DNA. Our working hypothesis is that Phox1 can recruit SRF to DNA-binding sites that SRF does not recognize alone and thereby bring the entire SRF transcriptional activation apparatus to sites that would be enhancers of cell-type-specific genes. To test this hypothesis, we have generated a

series of Phox1 mutants and tested their activities in vitro and in vivo to determine which amino acids in Phox1 are important for interaction with SRF.

The homeodomain consists of three α helices separated by short loops and an amino-terminal arm of disordered structure in solution. In cocrystals of homeodomains and DNA, both the amino-terminal arm and helix 3 make contact with the DNA molecule. Helices 1 and 2 are situated away from the DNA, toward the solvent. In scanning the Phox1 homeodomain by mutagenesis, we find that mutants fall into three classes. One class fails to bind DNA in vitro and to activate an SRE reporter gene in vivo. These mutations suggest a role for major-groove DNA contacts for activation by Phox1 in vivo. A second class of mutations, localized on the solvent-exposed surfaces of helices 1 and 2, does not affect DNA binding but still abolishes activation in vivo. These mutations suggest a requirement for contact of the Phox1 homeodomain with another protein, presumably, but not necessarily, SRF. The third class of mutations, mainly in the amino-terminal arm, abolishes DNA binding but leaves transcriptional activation intact. We believe that these mutations identify portions of the homeodomain that have a different function in vivo than is indicated in the simple binary protein-DNA structures revealed by crystallography and nuclear magnetic resonance (NMR).

We have also made mutations in the *Drosophila* homeoprotein Deformed, which is in a different homeodomain class. Deformed has no detectable activity on the SRE in vivo, but when amino acid residues on the surface of Deformed helix 2 are replaced with the corresponding residues on Phox1, the resulting chimeric proteins acquire the ability to activate the SRE in vivo. This observation suggests that the specificity of action of a homeodomain can be changed by alterations to the solvent-exposed surface of the protein. It adds to the presumed importance of protein-protein interactions in the function of the homeodomain.

Regulation of SRF-SRE Complex Formation by YY1

S. Natesan

The c-*fos* proto-oncogene is rapidly induced by a variety of growth factors, cytokines, and other extracellular stimuli. Several DNA elements in the promoter region of the gene mediate the transcriptional response to these signals. These elements are in close proximity to each other and to the transcriptional start site, and each binds one or more transcription factors responsive to multiple signals. Thus, the structural and functional organization of the c-*fos* promoter region may be crucial to its prompt and coordinated transcriptional response to extracellular signals.

We have previously shown that the zinc-finger transcription factor YY1 has a role in the structural organization of the c-*fos* promoter by bending DNA upstream of the TATA box. YY1 binds to at least three sites in the mouse c-*fos* promoter and induces DNA bends of approximately 80° in each of these sites. YY1-induced DNA bending at a site between the cAMP response element (CRE) and the TATA box in the c-*fos* promoter affects the interaction between proteins bound at these sites.

YY1 also regulates the activity of the c-*fos* SRE by promoting the assembly of a multiprotein complex at this element. YY1 binds to and induces a phased bend in the SRE. Binding of YY1 to the SRE accelerates the association of SRF with the SRE. This effect requires binding of YY1 to the SRE, and we observe a ternary complex in which YY1 and SRF co-occupy the SRE. In this complex, SRF makes predominantly minor groove contacts with the DNA, whereas YY1 contacts the major groove. We propose that YY1 binding induces a conformation in SRE DNA that is kinetically favorable for subsequent binding by SRF. Together with its role at the TATA-proximal site, this observation suggests a general role for YY1 in the building of highly organized promoter complexes. The diverse functions of YY1 in imparting structural organization to protein DNA complexes may account for its role in complex elements such as developmentally regulated silencers and highly regulated genes like c-*fos*.

Regulation of c-*fos* Transcription by Tyrosine Phosphorylation of Latent Cytoplasmic Transcription Factors

H. Sadowski

Receptor tyrosine kinases (RTKs) activate c-*fos* transcription through multiple pathways. One signal proceeds through the ras/MAP kinase pathway, resulting in the phosphorylation and activation of Elk-1 and

other ternary complex factors that bind to the SRE. But RTKs activate a second pathway that targets a distinct element in the c-*fos* promoter, termed the SIE (v/c-sis-inducible element). Treatment of cells with PDGF rapidly induces novel DNA-binding activities, termed SIF, that recognize the c-*fos* SIE. During the past 3 years, we have extensively characterized these proteins, showing that SIF DNA-binding activity is regulated by tyrosine phosphorylation and that SIF proteins are members of the STAT family of transcription factors, specifically, STAT1 and STAT3. The STAT pathway defines a novel and highly direct signal transduction pathway from the cell surface to the nucleus shared by receptors of distinct structural organization.

Our recent work has focused on the mechanism of activation of STATs by RTKs, with a particular focus on the receptor for PDGF. We have tested a set of PDGFR mutants carrying substitutions in critical tyrosine residues required for the recruitment and activation of various downstream signaling proteins. We find that SIF activation requires the protein-tyrosine kinase activity of the PDGFR. Furthermore, mutations that eliminate the recruitment, and presumably the activation, of PI3 kinase, GAP, phospholipase C-γ, and the protein tyrosine phosphatase Syp (which in turn recruits proteins required for activation of the ras pathway) have no effect on SIF activation. In contrast, a single substitution of phenylalanine for tyrosine at position 579 in the PDGFR nearly totally abolishes SIF activation by PDGF. This residue was previously identified as a site of recruitment for Src family protein tyrosine kinases, thus raising the possibility that Src kinases are intermediates in the activation of SIF by RTKs. Consistent with this hypothesis, we have observed that activation of a temperature-sensitive v-Src protein results in the rapid activation of STAT3 independently of new protein synthesis, suggesting that STAT3 activation is a direct consequence of the induction of Src activity.

Regulation of SIF/Stat Activity by Polypeptide Growth Factors

M.-L. Vignais

STAT1 and STAT3 proteins are also activated by a number of cytokines, whose receptors, unlike those of EGF and PDGF, do not harbor intrinsic protein tyrosine kinase activity. Signaling by these receptors relies on noncovalently associated protein tyrosine kinases of the JAK family. The role of specific pairs of JAK kinases in the activation of STAT1 by interferons α or γ has been demonstrated using a powerful genetic approach. We have adapted this approach to evaluate the contribution of JAK kinases to the activation of STAT proteins by the PDGFR. Using amphotrophic retroviral vectors encoding functional PDGF-β receptors, we have derived cell lines carrying comparable numbers of cell-surface receptors but lacking individual JAK kinases. We have tested these cell lines for their ability to support SIF activation by PDGF. In wild-type cells, all three ubiquitously expressed JAK kinases—JAK1, JAK2, and Tyk2—are tyrosine phosphorylated when cells are treated with PDGF. However, none of these kinases are individually required for the activation of STAT1 or STAT3 by PDGF. These results contrast with data obtained for interferon receptors, where kinases of the JAK family have been shown to be individually required for full activation of the STAT proteins.

To address the mechanism of STAT activation by the PDGFR directly, we are exploiting our previously developed cell-free assay for STAT activation. We now find that recombinant STAT1 protein produced in *Escherichia coli* can be activated in vitro by EGF in the presence of A431 membranes. Activation is not augmented by the addition of cytosol, indicating that no cellular components beyond those supplied by the membrane fraction are required. We will use conventional biochemical approaches, such as fractionation and depletion, to identify the membrane components required for STAT activation in vitro.

The Structure and Function of a *Drosophila* Homolog of SRF

A. Ryan

To study further the role of SRF in cell signaling and activation of transcription during animal development, we have isolated a full-length cDNA encoding a closely related protein from *Drosophila*. Amino acid identity in the region encompassing DNA-binding and dimerization activity is greater than 95%. The DSRF gene was mapped by in situ hybridization to the right arm of chromosome 2 at site 60C. DSRF mRNA is ubiquitously distributed in the early precellular embryo. RNA levels decline during cellulariza-

tion and increase upon gastrulation and organogenesis, during which RNA appears in localized patterns that coincide with the developing trachea. Immunocytochemistry using a monoclonal antibody against DSRF indicates that DSRF protein is found in most imaginal discs of the *Drosophila* larva.

DSRF shares many biochemical properties with the human protein, including DNA-binding specificity and the ability to form cooperative ternary complexes with the Ets-domain protein Elk-1. DSRF interacts productively with Elk-1 in transfected *Drosophila* tissue culture cells and can mediate Ras1-induced transcription of a reporter gene.

To evaluate the function of SRF during development, we are constructing transgenic flies carrying reporter genes with SRF-binding sites. We have constructed a set of reporter genes that direct *lacZ* expression under the control of an 85-base-pair element from the mouse c-*fos* promoter that is sufficient for the response to multiple signal transduction pathways emanating from receptor tyrosine kinases. This element includes binding sites for SRF, Elk1, and the STAT proteins. A set of analogous reporters carrying mutations in each of these factor binding sites has also been constructed. These plasmids are being injected into *Drosophila* embryos with the goal of establishing stable lines of flies in which *lacZ* expression is activated in response to various characterized signaling systems in the fly embryo.

Biochemical Analysis of a *Drosophila* JAK Kinase Involved in Hematopoietic Neoplasia

T. Nahreini [in collaboration with D. Harrison, R. Binari, and N. Perrimon, Harvard Medical School]

The *hopscotch* (*hop*) gene of *Drosophila melanogaster* encodes a nonreceptor tyrosine kinase of the JAK family identified in mammals. *Drosophila hop* has been implicated in the regulation of cellular proliferation. The first characterized allele of *hop*, *Tumorous-lethal* (*hopTum-l*), is a dominant temperature-sensitive mutation associated with the formation of melanotic tumors and hypertrophy of the larval lymph glands, which are the hematopoietic organs of *Drosophila*. At restrictive temperatures, *hopTum-l* is lethal, with dominant induction of melanotic tumors. At lower temperatures, *hopTum-l* mutants are viable, but they also develop tumors. *hopTum-l* is the only known dominantly acting oncogene in *Drosophila*.

Sequence analysis of a wild-type *hop* cDNA and a cDNA derived from the *hopTum-l* allele, performed by Douglas Harrison, Richard Binari, and Norbert Perrimon in the Department of Genetics at Harvard Medical School, showed that *hopTum-l* encodes a protein with a single-amino-acid substitution of glutamic acid for glycine in a region of the protein that encodes neither the kinase nor the kinase-like domain. Overexpression of either wild-type *hop* or *hopTum-l* in the larval lymph glands of flies caused melanotic tumors and lymph gland hypertrophy. This observation suggests that the *hopTum-l* lesion generates a hyperactive enzyme.

To test this hypothesis biochemically, we examined the level of phosphotyrosine in Hop and HopTum-l proteins overexpressed in *Drosophila* tissue culture cells by transient transfection. cDNAs from *hop* and *hopTum-l* were placed under the transcriptional control of the cytoplasmic actin promoter and transiently transfected into SL2 cells. Both plasmids directed the expression of similar amounts of an immunoreactive protein with a molecular weight of 130 kD, similar to that predicted for Hop. In addition, both overexpressed proteins were phosphorylated on tyrosine. The HopTum-l protein, however, exhibited a three- to fivefold elevation in tyrosine phosphorylation compared to Hop. Therefore, our results are consistent with the hypothesis that *hop* encodes a protein-tyrosine kinase and that the *hopTum-l* lesion causes hyperactivity of the enzyme. The combined genetic and biochemical data support the idea that elevated Hop activity is sufficient for formation of melanotic tumors in *Drosophila*.

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MOLECULAR CELL BIOLOGY

D.M. Helfman M. Gimona M. Selvakumar J. Wang
J. Grossman C. Temm-Grove A. Watakabe
W. Guo

The work in our group is focused on two fundamental areas in molecular and cell biology: (1) the mechanisms responsible for cell-type-specific gene expression and (2) the reason cells express specific gene products. The genes that we have been studying are among the major components of the cytoskeleton in eukaryotic cells. The cytoskeleton is involved in a multitude of cellular functions such as cell shape and motility, organelle movement, chromosome movement, and cytokinesis. In particular, our laboratory has been interested in understanding the regulation and function of tropomyosin gene expression in muscle and nonmuscle cells. Tropomyosins are a diverse group of actin filament-binding proteins with distinct isoforms present in striated muscle (skeletal and cardiac), smooth muscle, and nonmuscle cells. We now know that at least 16 different tropomyosin isoforms are expressed from four separate genes in vertebrates through a complex pattern of alternative RNA splicing. We have been studying the expression of these genes with particular attention to understanding the mechanisms of tissue-specific alternative RNA splicing. In addition, the function of these different isoforms is not known and is under study.

This year, a number of people from our lab have moved on to new positions. George Mulligan completed his Ph.D. and has taken a postdoctoral position with Tyler Jacks at the Massachusetts Institute of Technology. Three postdoctoral fellows left our group, including Stefan Stamm who moved to the Max Plank Institute for Psychiatry in Munich, Germany; Jeff Kazzaz took a position at Winthrop University Hospital on Long Island; and Mark Pittenger has moved to a biotechnology company. Below is a summary of our studies during the past year.

Exon Sequences Have a Role in Cooperation Across a 3′ and 5′ Splice Site

M. Selvakumar

Previously, it has been shown in our lab that a simple pre-mRNA substrate derived from the β-tropomyosin gene which contains exon 5–intron 5–exon 6 could not be spliced in an in vitro splicing assay. This can be overcome by three different *cis*-acting elements using HeLa cell nuclear extract: (1) a previous splicing event joining exon 6 to exon 8, (2) a poly(U) substitution in the 3′ splice site of exon 6, and (3) a 5′ consensus mutation in the 5′ splice site of exon 6 (Tsukahara et al. 1994). Examination of the sequences in exons 6 and 8 revealed the presence of purine-rich motifs that have been implicated as exonic enhancers in other systems. To determine if these motifs have a critical role in this splicing event, we made substitutions (purine to pyrimidine) in individual motifs as well as combinations of motifs. Our results show that mutating all the motifs completely abolishes splicing. Interestingly, longer pre-mRNA substrates containing multiple exons and introns (exons 5 through 8) skip the nonmuscle exon 6 in an in vitro assay using nonmuscle nuclear extract. Exon 6 was included when a poly(U) substitution was made in its 3′ splice site. However, mutating the two purine-rich motifs in exon 6, in the poly(U) context, causes exon 6 to be skipped. This is in accordance with the exon definition model which predicts that exons are recognized via their splice sites instead of across an intron. It has been shown that factors that interact with the 3′ and 5′ splice sites of an exon are involved in protein:protein interactions via bridging factors across an exon. Presumably, purine-rich motifs act by stabilizing these bridging factors. To look at effects of these mutations in an in vivo context, these mutations were made in an SV40 vector and will be transfected into HeLa cells and REF52 cells. RNA-binding assays are planned to look for proteins interacting with these purine-rich motifs.

Use of Antibodies against PTB to Detect Specific RNA-Protein Interactions

J. Grossman

We recently found that at least one protein, the polypyrimidine tract-binding protein (PTB), specifi-

cally interacts with sequences upstream of exon 7 which are involved in alternative splicing of β-TM pre-mRNA (Mulligan et al., *J. Biol. Chem. 267:* 25480 [1992]). To identify other proteins that interact with these sequences, [^{35}S]methionine-labeled nuclear extracts from HeLa cells were mixed with non-biotinylated RNAs. The RNA-protein complexes were recovered by immunoprecipitation using monoclonal antibodies to PTB, and the proteins were resolved using one- and two-dimensional gel electrophoresis. When RNAs containing intron 6 were coprecipitated with antibodies to PTB, a novel set of proteins were found to coprecipitate. In contrast, addition of RNAs containing introns 5 or 7 gave the same results as no RNA, indicating that these RNAs are unable to form a stable complex with PTB. These results are in agreement with our previous studies demonstrating that PTB interacts with sequences with intron 6 but not with sequences in introns 5 or 7. When [^{35}S]methionine-labeled nuclear extracts were mixed with biotin-RNA containing intron 6, and the RNA protein complexes recovered using avidin-agarose beads, the identical patterns of proteins were observed compared to the immunoprecipitation assay.

Finally, analysis of the proteins that assembled on introns 5, 6, or 7 using biotin-RNA revealed a unique set of proteins that interact with each of these sequences, indicating that different hnRNP proteins are bound to different regions of the pre-mRNA. Experiments are in progress to study the interaction of proteins obtained from myogenic cells in order to identify cell-type-specific factors that interact with distinct regions in the β-TM pre-mRNA.

Analysis of Factors from Myogenic Cells That Promote the Recognition of a Muscle-specific Exon in β-Tropomyosin Pre-mRNA

Y.-C. Wang

The β-tropomyosin (β-TM) gene in rat and mouse contains two pairs of alternative exons that are spliced in a tissue-specific manner. The regulation of the internal exon pair (exons 6 and 7) has been extensively studied. Exon 6 is incorporated in non-muscle and smooth muscle cells, whereas exon 7 is exclusively used in skeletal muscle cells. To date, in vitro studies have been limited to extracts derived from HeLa cells. Previous studies suggest that the splicing of exons 5 to 7 (skeletal muscle-type splice) is suppressed in HeLa cells by a negative factor (Guo et al., *Genes Dev. 5:* 2096 [1991]). Muscle cells may simply lack this negative factor or express a muscle-specific factor that promotes the inclusion of exon 7. To understand the regulation of the splicing of β-TM pre-mRNA in skeletal muscle cells, we have been developing an in vitro splicing system from the mouse BC3H1 myogenic cell line.

We are also utilizing a complementation assay of the BC3H1 nuclear extract or its fractions added to the human 293 nonmuscle cell nuclear extract to identify factors involved in the regulation. The addition of BC3H1 nuclear extracts to 293 nuclear extracts stimulated the splicing of exons 5 to 7 in a β-TM minigene transcript. This stimulatory activity was also detected in nuclear extracts from the mouse C2C12 skeletal muscle cell line but not in 3T3 fibroblasts or HeLa cells. This activity was resistant to treatment with micrococcal nuclease and was precipitated in 20 mM of $MgCl_2$, which is a characteristic feature of the SR protein family. When the SR proteins purified from HeLa cells were added to the 293 nuclear extract, the splicing of exon 5 to 7 was not stimulated. But when the SR proteins were added to the BC3H1 nuclear extract, stimulation was detected. Thus, a stimulatory factor(s) present in skeletal muscle cells may cooperate with the SR proteins in promoting the splicing of exons 5 to 7 in β-TM pre-mRNA. Work is in progress to further characterize this activity.

Study of PTB-binding Proteins using the Yeast Two-hybrid System

W. Guo

The functional role of PTB in the regulation of alternative splicing of exons 6 and 7 remains to be elucidated. We are using the yeast two-hybrid system to identify proteins interacting with PTB. Fusion proteins in which PTB1 and PTB3 fused to GAL4-binding domain (pGBT9PTB) have been constructed and used as baits to screen a HeLa cDNA library that has been fused to GAL4 transcriptional activation domain (GAD). We have detected protein S20, a

mammalian ribosome small subunit protein, interacting with PTB1 and PTB3 bait proteins and turning blue in the filter color assay. The interaction between PTB and S20 detected by the two-hybrid system seems to be specific, as we did not detect any interactions between PTB and Ras, PTB and SNF4, S20 with Raf, and S20 with SNF1 in similar pairwise two-hybrid assays.

We are currently studying the binding of PTB with S20 in an in vitro system to see if the interaction detected in vivo occurs in vitro. In picornavirus, PTB has been shown to bind to IRES (internal ribosomal entry site) and is essential for IRES function in the translation of viral RNA. It has been proposed that PTB will specifically attach to the IRES and bring the 40S ribosomal subunit into the vicinity of a potential initiation codon. The interaction between PTB and S20 detected by the two-hybrid system suggests that S20 could be a potential candidate that brings the 40S ribosomal subunit to IRES via PTB and initiates the viral RNA translation.

Isolation and Characterization of cDNA Clones Encoding a Low-molecular-weight Fibroblast Tropomyosin TM-5

W. Guo, C. Temm-Grove

Last year we reported the isolation and characterization of a cDNA clone encoding a seventh rat fibroblast TM, herein referred to as TM-5, that is the product of a fourth gene which is homologous to the human hTMnm gene. Expression of TM-5 in a variety of rat tissues and transformed cell lines was examined using Northern blot and RNase protection assays. The gene encoding TM-5 is expressed in all tissues examined including skeletal muscle, stomach, heart, liver, kidney, uterus, spleen, brain, and diaphragm. However, RNase protection analysis revealed the presence of different mRNAs in fibroblasts, striated muscle (skeletal and diaphragm), and brain, which are expressed via alternative RNA splic-

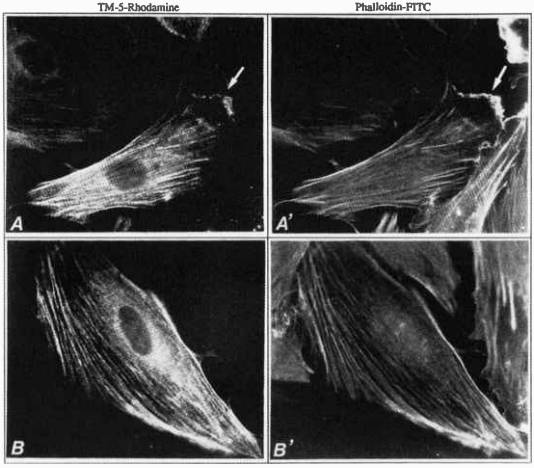

FIGURE 1 Labeling of microfilaments in rat fibroblasts by microinjection of fluorescently tagged TM-5. Bacterially expressed TM-5 was conjugated with rhodamine and microinjected into REF52 cells to study in vivo actin filament binding and localization. Rhodamine-labeled TM-5 can be seen along the stress fibers and at the leading lamella (arrow) of the cells (*A*, *B*). The cells were double-stained with phalloidin-FITC to demonstrate the localization of F-actin (*1A'*, *B'*).

ing and the use of alternative promoters. The TM-5 protein was expressed in a bacterial system and tested for its ability to bind actin in vitro and in vivo. It was found that TM-5 bound to actin with a similarly high affinity as TM-5b and it localized to the stress fibers and ruffles of the leading lamella (Fig. 1).

Characterization of Brain-type Isoforms of Tropomyosin

A. Watakabe

Among numerous isoforms of tropomyosin, TmBr1 and TmBr3 are distinguished in that they are highly tissue-specific. These isoforms are expressed only in brain or cells derived from the nervous system. Both of these isoforms contain unique carboxy-terminal coding sequences due to the use of exon 9c. To begin to study the role of these isoforms, we examined if these tropomyosins bind to F-actin. In vitro experiments using purified muscle F-actin and recombinant TmBr1 and TmBr3 showed that TmBr1 and 3 bind to F-actin at reduced efficiency compared with other tropomyosin isoforms. To determine if this weaker binding would also occur in vivo, we transfected fibroblast with either TmBr1 or 3 and examined if these isoforms are incorporated into actin-based structures such as stress fibers. Consistent with the in vitro results, both of these isoforms were poorly incorporated into stress fibers, whereas other tropomyosin isoforms such as Tm2 and Tm5a were efficiently incorporated. Collectively, these studies show that TmBr1 and 3 have intrinsically weak affinity for F-actin. We are also examining if there is any protein that binds to the brain-type tropomyosin isoforms using antibody-based blot overlay (AnBO) assay. In this AnBO assay, brain proteins are run on SDS gels and transferred to a nitrocellulose membrane, which is probed with epitope-tagged recombinant tropomyosin. Subsequently, the binding of tropomyosin to the blotted protein was detected by Western blotting with anti-epitope antibody. Using this assay, we found that TmBr3 as well as Tm5a bind to tropomodulin, a protein originally identified as the tropomyosin-binding protein in platelets. In addition, we detected another protein that binds preferentially to TmBr3 and does not bind to TmBr1 or Tm2. AnBO assay showed that this protein is specific to brain. We have partially purified this protein for microsequencing. Future characterization of this protein will provide greater insights into understanding the function of brain-type tropomyosins. These studies will increase our understanding of the role of the cytoskeleton in nervous system functions.

Tropomyosin Isoform Diversity in Epithelial Cells

C.J. Temm-Grove [in collaboration with B.M. Jockusch, Braunschweig Technical University, Germany]

Tropomyosin isoform expression is highly cell- and tissue-specific. One interest of our work has been to characterize and understand the significance of the tropomyosin (TM) diversity in renal brush border epithelial cells (BB). Previously, it was reported that the porcine kidney epithelial cell line, LLC-PK1, has retained much of the character of renal brush border cells. We have characterized the TM isoform expression of these cells and found that primary BB cells and LLC-PK1 cells express the same isoforms of TM (TM-1, TM-2, TM-3, TM-5, TM-5a, TM-5b, TM-6), whereas the other cell lines exhibited some differences in the patterns of TMs expressed. Furthermore, it was found that the high-molecular-weight (HMW) isoforms TM-1 and TM-3 are present in larger amounts relative to the other isoforms in BB-type cells, in contrast to those of rat fibroblasts, where TM-1 and TM-2 are predominant. In addition, whereas fibroblasts express primarily TM-5a, the BB cells express only TM-5b among the low-molecular-weight (LMW) products of the α gene. Immunofluorescence analysis using antibodies directed against HMW TMs (TM-1, TM-2, TM-3, and TM-6) and LMW TMs (TM-5, TM-5a, TM-5b) revealed that HMW isoforms were localized to stress fibers but not in adhesion belts, whereas the LMW isoforms were found in the adhesion belts. These results were confirmed using the epitope-tagged TM constructs. The dynamic distribution of these isoforms was verified by the microinjection of representative fluorescently labeled recombinant TM isoforms expressed in the pET bacterial expression system.

Tropomyosin Characterization and Expression during Differentiation of LLC-Pk1 Cells

C.J. Temm-Grove

The relationship of TM expression and localization to cell growth and differentiation during morphological-

ly differentiating stages has been tested in the renal epithelial cell line, LLC-PK1, using available isoform-specific antibodies. It was found that at least the low-molecular-weight isoform, TM-5b, switches on at a certain stage of development. These biochemical assays were correlated with fluorescence microscopy and F-actin structure (as seen with phalloidin staining). It was found that the expression of TM-5b begins with the stable development of adhesion belts. This suggests that these isoforms may fulfill a specific function at that site, which is not required at stress fibers. Signal transduction pathways that are stimulated by cell-cell contact or default pathways induced by removal of serum probably cause the development of the adhesion belt. These may also be responsible for TM-5b expression simultaneous with adhesion belt/terminal web formation.

The Specificity of Dimer Formation of Tropomyosin

M. Gimona, A. Watakabe

Tropomyosins consist of nearly 100% α-helix and assemble into parallel and in-register coiled-coil dimers. In vitro, it has been established that nonmuscle as well as native muscle TMs can form homodimers but that the mixture of both α and β muscle TMs rapidly adopts the more stable α/β heterodimer conformation. Although the assembly preference of the muscle TM heterodimer can be understood thermodynamically, the significance of its predominance in several muscles is not known. Studies of TMs from fibroblasts demonstrate that these isoforms exist as homodimers. TM functions as a dimer and isoform diversity allows a variety of different dimers to be formed. Because specificity is determined additionally by the relative thermodynamic stability of all possible protein-protein interactions, understanding this process requires identifying forces that stabilize preferentially the favored complex as well as forces that destabilize the incorrect one. Several groups have previously addressed the question of homo- versus heterodimer formation using purified muscle or nonmuscle TMs. However, all of these approaches had to either denature or chemically stabilize the TM chains. We have investigated the dimerization of tropomyosins in vivo at low temperature where chain exchange is minimized using epitope tagging with a 16-amino-acid sequence of influenza hemagglutinin (HA-tag). We used immunoprecipitation from extracts of transfected cells to analyze the composition of the dimers, taking advantage of the slight changes in electrophoretic mobility and differential antibody specificity conferred to the TMs due to the epitope tag. Single transfections demonstrate the preference of the 284-amino-acid HMW nonmuscle isoforms TM-1, TM-2, and TM-3 to form homodimers. Cotransfections of tagged TM constructs in parallel with untagged TMs did not alter the homodimer preference of both HMW and LMW 248-amino-acid nonmuscle isoforms. When tagged smooth muscle α-TM or skeletal muscle α-TM isoforms were cotransfected with untagged HMW nonmuscle TM isoforms, the formation of heterodimer was predominant. Similarly, the β-skeletal muscle TM was found to form heterodimers with TM-1, TM-2, and TM-3. Cotransfection of tagged muscle (α-smooth and β-skeletal) or tagged nonmuscle TM-2 together with the LMW nonmuscle variants TM-4, TM-5a, or TM-5b failed to form stable heterodimers in the cells.

Our results indicate that the selectivity for homo- versus heterodimerization is a property intrinsic to the TM molecule itself and that this information is conveyed by combinatorial alternative splicing of exons. These results have important implications for models of the regulation of cytoskeletal dynamics.

Expressing Functional Domains of Mouse Calponin

M. Gimona

Calponin (CaP) is a 34-kD actin, calmodulin (CaM)-, and tropomyosin (TM)-binding protein that inhibits the actomyosin ATPase activity, as well as the movement of actin filaments over coated myosin in in vitro enzyme and motility assays, respectively. Previous work (Mezgueldi et al., *J. Biol. Chem. 267:* 15943 [1992]) reported indirect evidence for the binding of F-actin to the 38-residue stretch of gizzard CaP encompassing the sequence A145-Y182 and postulated the hexapeptide motif VKYAEK, representing residues 142–147, as the putative actin-binding site. In collaboration with Drs. Mezgueldi and Fattoum at the University of Montpellier (France), we have investigated size and location of the actomyosin ATPase inhibitory domain of smooth muscle h1CaP by expressing fragments of mouse h1CaP with or without substitution or deletion at the latter motif using cosedimentation with F-actin, actomyosin S1-

ATPase assays, cross-linking reactions, and affinity chromatography over immobilized CaM or TM.

Collectively, the data show that the polypeptide stretch Ala-131–Ile-228 contains the entire actin-binding site and inhibitory sequence. This segment also interacts with TM and Ca^{++}-CaM, the binding of the latter protein resulting in the reversal of ATPase inhibition. Furthermore, structural alterations of the VKYAEK motif by site-directed mutagenesis strongly affect the inhibitory activity of CaP with only a slight decrease of actin binding.

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CELL BIOLOGY OF THE NUCLEUS

D.L. Spector S. Huang, R.T. O'Keefe, P. Mintz, A. Chi, R. Derby, S. Kaurin

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 Robert Derby.

The Spatial Association of Nascent Pre-mRNA with Splicing Factors Is Intron-dependent

S. Huang, D.L. Spector

We have been interested in the organization of RNA polymerase II transcripts within the mammalian cell nucleus. Several models have been proposed for the functional organization of RNA within the eukaryotic nucleus and for the relationship of this organization to the distribution of pre-mRNA splicing factors. One model suggests that RNAs which must be spliced are capable of recruiting splicing factors to the sites of transcription from storage and/or reassembly sites (Jiménez-García and Spector, *Cell 73:* 47 [1993]). To further evaluate this model, we have transiently transfected HeLa cells with constructs that express RNA transcripts containing introns, lacking introns, or containing an intron with a deletion at the 3′ splice site. The expression of RNAs was detected by in situ hybridization, and their association with splicing factors was evaluated by immunostaining using specific antibodies (Y12, SC35) in the same cells. We have found that the majority of the RNA transcripts produced from constructs that express intron-containing genes such as β-globin, tropomyosin, and

HIV tat are associated with splicing factors. In contrast, RNAs lacking introns, such as β-galactosidase and adenovirus VA_I, are not associated with splicing factors in the nucleus. Furthermore, the majority of HIV *tat* RNA, which contains a deletion in its intron at the 3′ splice site, showed little association with splicing factors. These observations suggest that the spatial association of RNA transcripts with splicing factors is intron-dependent. Such an association is functionally significant as transcripts containing mutated introns, which are incapable of being spliced, are not associated with splicing factors. Therefore, we propose that the organization of RNA and splicing factors in the cell nucleus reflects the transcriptional activity of the cell and the localization patterns of splicing factors will change to reflect changes in transcriptional activity.

Organization of Polyadenylation in the Mammalian Cell Nucleus

P. Mintz, D.L. Spector

Most RNAs transcribed by RNA polymerase II are spliced and polyadenylated prior to their export from the nucleus. During the past few years, we have been concentrating our studies on the organization of pre-mRNA splicing in the cell nucleus. We have found

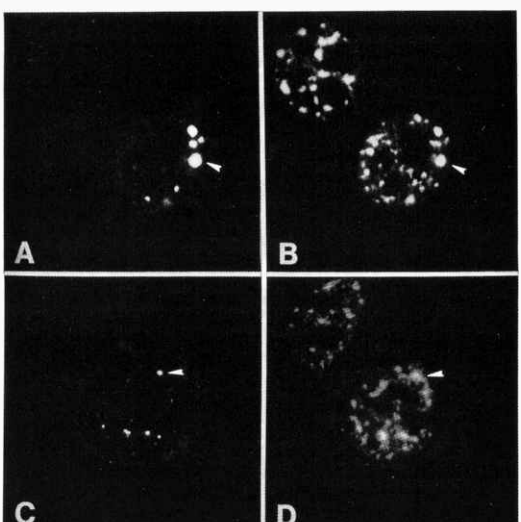

FIGURE 1 A close association is observed between the localization of transiently expressed RNA transcripts with introns and the localization of splicing factors, suggesting that the sites of association represent the sites of splicing. HeLa cells were transfected with expression vectors that encode RNA transcripts with introns such as CG-Tat (*A* and *B*) or β-globin (*C* and *D*). The localization of these RNAs was examined 7–8 hr posttransfection by in situ hybridization with biotinylated probes (*A* and *C*). The localization of splicing factors in the same cells was detected by immunostaining with monoclonal antibody specifically recognizing SC35 (*B* and *D*). RNAs and splicing factors are colocalized to the same nuclear regions (arrowheads). Bar, 10 μm.

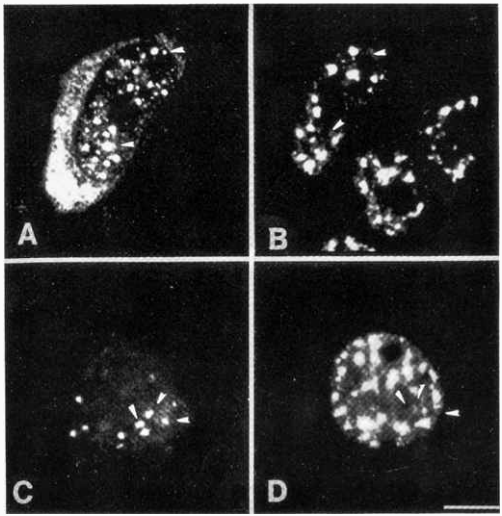

FIGURE 2 The majority of transiently expressed intron-less RNAs or RNAs with a truncated intron do not associate with splicing factors in the cell nucleus. HeLa cells were transfected with expression vectors that encode RNA transcripts without introns, β-galactosidase (*A* and *B*) or RNA transcripts containing an intron with a deleted 3′ splice site, CMV Tat (*C* and *D*). The localization of these RNAs in the cell nucleus was observed 7–8 hr posttransfection by in situ hybridization with biotinylated probes (*A* and *C*). The localization of splicing factors in the same cells was detected by the monoclonal antibody specifically recognizing SC35 (*B* and *D*). Little to no colocalization was observed (arrowheads). Bar, 10 μm.

that factors involved in pre-mRNA splicing are concentrated in a speckled pattern in interphase nuclei. When this pattern was examined by immunoelectron microscopy, it was found to correspond to interchromatin granule clusters and perichromatin fibrils. Interchromatin granule clusters are thought to represent sites of splicing factor storage and/or reassembly, whereas perichromatin fibrils are thought to represent transcription sites.

During the past year, we have been interested in determining where in the nucleus polyadenylation occurs. Work from our laboratory has previously shown that poly(A)$^+$ RNA is localized to the entire speckled pattern. However, we know that a portion of this poly(A)$^+$ RNA is stable nuclear RNA and therefore it is not localized at the site of transcription. We were interested in determining where in the nucleus polyadenylation occurred and the relationship of the sites of polyadenylation to those of transcription, pre-mRNA splicing, and total poly(A)$^+$ RNA localization. Using an antibody obtained from Jim Manley (Columbia University), we have found poly(A) polymerase to be localized in a discrete pattern of dots which extend throughout the nucleoplasm but which are excluded from the nucleoli. Studies are currently under way to determine the relationship of these sites of poly(A) polymerase to sites of RNA polymerase II transcription.

The Association of RNAs with SC35

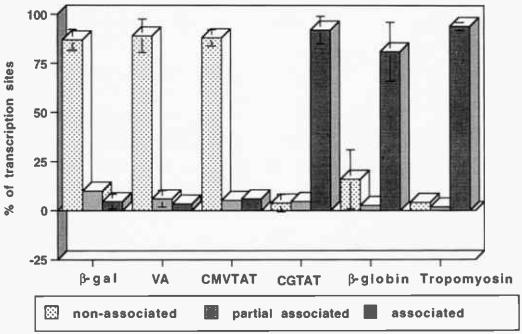

FIGURE 3 Histogram showing a statistical evaluation of the association of RNA transcripts with the splicing factor SC35 in HeLa cell nuclei.

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PROGRAMMED CELL DEATH IN *CAENORHABDITIS ELEGANS*

M. Hengartner T. Gumienny J. Keller
D. Hoeppner Q. Liu
G. Jefferis M. Spector

We are interested in understanding the molecular basis of programmed cell death (apoptosis). This process, which allows multicellular organisms to eliminate cells that are not needed or are potentially dangerous, is evolutionarily highly conserved. Proper control of programmed cell death is crucial, and breakdown in the regulation of this process has been associated with several types of cancer, autoimmunity, and possibly neurodegenerative diseases.

We have taken a genetic approach to identify genes that function in programmed cell death in the small nematode *Caenorhabditis elegans*. The worm provides an attractive system for the study of this problem, as its development has been extensively characterized, and the animal is readily amenable to genetic and molecular manipulations. So far, we and other investigators have identified 14 genes that affect programmed cell death during *C. elegans* devel-

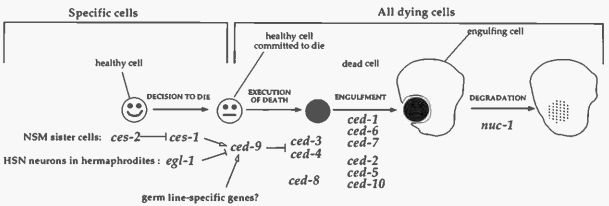

FIGURE 1 The genetic pathway for programmed cell death in *C. elegans*. Mutations in 14 genes affect programmed cell deaths. These mutations divide the process of programmed cell death into four steps; genes that act in the last three steps are common to all programmed cell deaths, whereas genes that act in the first step affect only a few cells. Regulatory interactions deduced from genetic studies are shown. (→) Positive regulation; (—I) negative regulation.

opment (Fig. 1), and we have begun the molecular characterization of these genes. For example, we found that the gene *ced-9*, which is required to prevent cells that normally survive from undergoing programmed cell death, is a homolog of the mammalian proto-oncogene *bcl-2*. Interestingly, *bcl-2* is thought to have a function in mammals similar to that of *ced-9* in *C. elegans*. Similarly, the cell death gene *ced-3* is homologous to the mammalian cysteine protease interleukin-1β-converting enzyme (ICE), and overexpression of either CED-3 or ICE induces apoptotic death in rat fibroblasts. The conservation in sequence and function of these two *C. elegans* cell death genes strongly suggests that nematodes and mammals share a common molecular pathway for programmed cell death.

We are now searching for and characterizing additional genes that function in this pathway and are developing tools to continue these studies at the biochemical level. The conservation of the molecular pathway between nematodes and mammals suggests that the genes we will identify in our screens for *C. elegans* cell death mutants will have homologs that have similar roles in mammalian apoptosis. Thus, the knowledge gained about programmed cell death in *C. elegans* promises to be relevant to our understanding of apoptosis in human development and disease.

Identification of Proteins That Interact with CED-9

G. Jefferis, D. Hoeppner, J. Keller

The CED-9/Bcl-2 family members possess no known enzymatic activity, suggesting that these proteins might regulate programmed cell death by binding to other proteins and altering their activities. To identify proteins that interact with CED-9, we are using the yeast two-hybrid system to screen a *C. elegans* cDNA library using CED-9 fused to the GAL4 DNA-binding domain as a bait. To complement and assist the two-hybrid studies, we are developing antibodies against CED-9 that will be used to identify proteins that coimmunoprecipitate with CED-9.

Molecular Genetic Analysis of the Gain-of-Function Mutation *ced-9(n1950)*

M. Hengartner [in collaboration with Bob Horvitz, Massachusetts Institute of Technology]

Dominant gain-of-function mutations have been described in both *ced-9* and *bcl-2*. In *bcl-2*, such mutations—which are commonly found in follicular lymphoma—are invariably translocations that result in overexpression of normal Bcl-2 protein. In contrast, we found that the *ced-9(n1950)* gain-of-function mutation affects the open reading frame of *ced-9* and results in a glycine-to-glutamate substitution in a region highly conserved among all *ced-9/bcl-2* family members. This region has been shown to be required for heterodimerization of Bcl-2 with other members of the CED-9/Bcl-2 family, suggesting that the *n1950* mutation might affect the ability of CED-9 to interact with other proteins. We are planning to test this hypothesis by identifying proteins that show preferential binding to either the wild-type or mutated form of CED-9. Such interactions will be

tested by coimmunoprecipitation with CED-9 and by use of the yeast two-hybrid system.

Isolation and Characterization of *ced-9* Suppressors

M. Spector, M. Hengartner, T. Gumienny, G. Jefferis

We have previously shown that *ced-9* is required to protect *C. elegans* cells from programmed cell death: In the absence of *ced-9*, many cells that normally live undergo programmed cell death, eventually resulting in the death of the animal. Mutations in *ced-3* or *ced-4* block the ectopic deaths and the lethality associated with *ced-9* loss-of-function (*lf*) mutants. To identify additional genes that function in programmed cell death, we have screened for suppressors of the *ced-9(lf)* maternal-effect lethality phenotype. So far, we have isolated over three dozen suppressors. As expected, some of these are mutations in *ced-3* and *ced-4*. We have also identified one mutation, *n2431*, that identifies a new cell death gene, which we are currently characterizing (see below). To increase the proportion of suppressors that are in novel cell death genes, we are now setting up screens for dominant suppressors of *ced-9(lf)*, as well as screens in strains carrying additional copies of *ced-3*.

Characterization of *ced(n2431)*, a New *ced-9* Suppressor

D. Hoeppner, M. Hengartner

The mutation *n2341* suppresses the maternal-effect lethality of the weak loss-of-function allele *ced-9 (n1950 n2161)*. We have mapped *n2431* to the gene cluster on chromosome *III*. No known cell death gene resides in this region, indicating that *n2431* identifies a new *C. elegans ced* gene. Although unable to suppress *ced-9* null alleles, *n2431* can also suppress *n1653*, another weak *ced-9* allele. Furthermore, *n2431* has no obvious phenotype on its own. These observations suggest that *ced(n2431)* is only a weak suppressor of cell death and that the wild-type gene might have a partially redundant role in *C. elegans* cell death. We are currently determining the precise position of *ced(n2431)* on the genetic map as a prelude to its cloning.

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

T. Gumienny, D. Hoeppner, M. Hengartner [in collaboration with Erika Hartwieg, Massachusetts Institute of Technology]

We have recently undertaken an in-depth study of programmed cell death in the germ line of *C. elegans*. The germ line tissue offers a number of advantages over the *C. elegans* soma, including a more vertebrate-like regulation of proliferation and the possibility of isolating significant amounts of dying cells for biochemical analysis. Even though little attention had been given to germ cell death in the past, we found that programmed cell death has a major role in the germ line. In fact, our results suggest that programmed cell death is the most common fate in the germ line. Germ cells are by far the most common cell type to undergo programmed cell death: During development, 131 somatic cells die. In contrast, more than 300 germ cells die during adulthood. Thus, during an animal's lifetime, more programmed cell deaths occur in the germ line than in all the somatic tissues combined.

We have found that most, but not all, mutations that affect programmed cell death in the soma also affect germ cell death. *ced-3* and *ced-4* are both required for germ cells to undergo programmed cell death, whereas *ced-9* is required to protect oocytes from death: In the absence of *ced-9*, too many germ cells die, resulting in sterility. In contrast, genes that affect programmed cell death only in specific cells (such as the *ces* genes and *egl-1*) do not affect germ cell death.

To understand how germ cells make the decision between life and death, we are screening for mutations that affect germ cell death. Characterization of the genes identified by these mutations promises to further our understanding of how cells control the activation of the death machinery.

Reverse Genetic Studies of Programmed Cell Death

Q. Liu, M. Spector, T. Gumienny

A number of cell death genes have been identified in other organisms, such as mammals and *Drosophila*. The observation that the pathway for programmed cell death is conserved between nematodes and mam-

mals suggests that these cell death genes might also function in *C. elegans*. We have been using a variety of techniques and approaches to test this hypothesis. The simplest involves the generation of transgenic worms that express the "foreign" cell death gene. We have shown that this approach is feasible by demonstrating that overexpression of human Bcl-2 from a *C. elegans* heat-shock promoter is able to prevent programmed cell death in *C. elegans*. We are now pursuing a number of other genes that have been implicated in programmed cell death, including mammalian Bcl-x, Bax, and sphingomyelinase, as well as the *Drosophila* gene *reaper*. Studies of this type not only promise to confirm that the gene in question interacts with, or is part of, a conserved part of the cell death machinery, but they can also be used to order the point of action of these genes in the pathway by testing the effect of the transgene in the various cell death mutant backgrounds available in *C. elegans*.

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QUEST PROTEIN DATABASE CENTER

J.I. Garrels B. Futcher N. Sareen
G.I. Latter R. Kobayashi B. Schwender
P.J. Monardo T. Volpe
N. Bizios L. Jokhan

Our research focuses on the construction and analysis of two-dimensional gel (2D gel) protein databases. The 2D gel (see Fig. 1) serves as an assay system which allows us to visualize and measure the in vivo abundance of a large proportion of the proteins in an organism at one time. Utilizing the Quest II computer software that we have designed and constructed, we are able to measure the volume of each protein as well. A 2D gel database is a collection of 2D gels along with the measured volumes of the proteins under various cellular conditions. We are able to use these databases to study the functional changes of gene expression across changed states of the cell.

As the genomes of organisms are sequenced, the value of these protein databases begins to increase. We have been focusing our efforts on the study of yeast (*Saccharomyces cerevisiae*) for more than 1 year now. More than one half of the genomic sequence of *S. cerevisiae* is now available, and the entire genome of this organism is expected to be sequenced by 1996. The availability of this sequence data has had major consequences in our work. The techniques that we employ for identification, such as amino acid analysis and microsequencing, and the use of overexpression plasmids all benefit from the availability of sequence data. We have in a short time been able to identify more than 100 spots on the yeast 2D gels. These identifications in turn aid our study of function.

We have developed databases and tools to help us

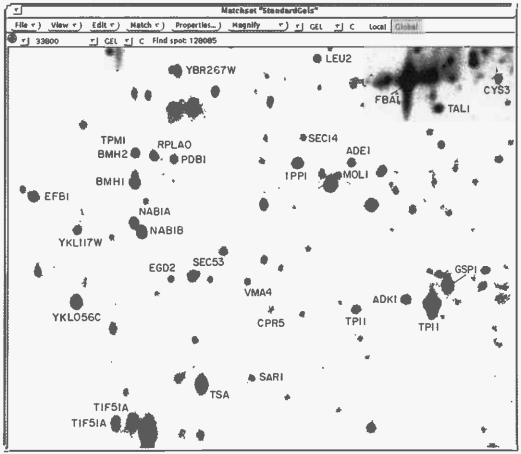

FIGURE 1 A region of a 2D gel of the yeast *Saccharomyces cerevisiae* viewed from the Quest II software showing the identified spots. Of particular interest are BMH1, BMH2, and GSP1.

in our identification of proteins on 2D gels and for the distribution of these data to scientists over the Internet. Additionally, we have developed a YPD database which is a nonredundant database of *S. cerevisiae* proteins that lists information about their properties, which will also assist us in identifications. Our identification data as well as YPD are being made available to the scientific community via the Internet in a number of ways including the World Wide Web.

Protein Identifications

J.I. Garrels, B. Futcher, R. Kobayashi, G.I. Latter, T. Volpe, B. Schwender

We have now identified more than 120 of the most highly expressed yeast proteins on our 2D gel maps. Using combined approaches of protein sequencing, gene overexpression from high-copy-number plasmids, and amino acid composition analysis, we have been able to identify many of the prominent proteins. These efforts are also guided by the YPD database, which can be used to predict which known proteins should appear in each region of the gel based on predicted molecular weight, isoelectric point, and codon bias.

Identification by amino acid analysis has used a combination of single-label and double-label analyses. Both methods (see 1993 Annual Report) use radioactive labeling with ^{14}C- and ^{35}S-labeled amino acids and image quantitation to determine partial amino acid compositions. A scoring system was developed to determine which proteins of known sequence best match to the amino acid composition data for each protein. When the scoring system was first tested on proteins already known by other means, 25 of 43 known proteins were correctly and uniquely predicted. In five cases, two or more proteins, including the correct one, were predicted. In only one case did the scoring system make a unique but incorrect prediction. Analysis of 163 unknown proteins by the same scoring system has resulted in 34 new unique predictions. Subsequent studies using new sequences that have appeared from genomic sequencing have led to even more proteins identified through amino acid composition.

Many of the identified proteins fall into categories that are expected to contain abundant proteins. These include glycolytic enzymes, ribosomal proteins, protein synthesis factors, enzymes of amino acid metabolism, and heat-shock proteins. Other identified proteins of interest are BMH1 and BMH2 (homologs of mammalian 14-3-3 protein), GSP1 (the Ras-like protein involved in regulating nuclear import), SRP1 (also called "importin" for its role in nuclear import), CYPD (a cyclophilin of the endoplasmic reticulum), TCP20 (a cytoplasmic chaperonin), and CIM5 (a 26S protease subunit).

Not all of the highly expressed proteins are known in yeast. One of the major proteins (YEPD 6260) is an unknown acidic ribosomal protein. It is present in purified ribosomes and, like most ribosomal proteins, its gene has an intron. Partial sequence data has been obtained but does not match to any known protein. Another major protein (YEPD 7260) is a close match to two sequences (YIL053W and YER062C) that have recently appeared from genomic sequencing, but this protein probably represents a third member of this newly discovered protein family of unknown function.

The various observations for the major proteins of yeast are being assembled into the Yeast Electrophoretic Protein Database (YEPD). So far, YEPD contains information on amino-terminal modification, splicing, heat shock, glucose stimulation, phosphorylation, and subcellular fractionation (ribosomes, mitochondria, soluble vs. pellet fractions).

Quest II Software

P. Monardo, G.I. Latter

Quest II software is a software system that we have designed and built for creating and analyzing 2D gel protein databases. Figure 1 shows a view of Quest II which depicts a region of the gel showing some of the identified proteins discussed above. Using the Quest II system and the growing number of identifications on the gel, we have been able not only to begin to perform detailed quantitative studies on the variation of protein expression across an experiment, but also to begin to attach identity to the proteins that show interesting patterns of variation. Using the cluster analysis feature that has now been built into our system, we can automatically find these coregulated patterns and create sets of spots for use in further studies. The Quest II software is now in use at a growing number of institutions in the United States and Europe.

Network Protein Information Resource

P. Monardo, T. Boutell, G.I. Latter

We continue to make our 2D gel identifications available via the Internet using the World Wide Web system. We are now also making the YPD database available (discussed below). Documentation on our Quest II software and miscellaneous software tools developed for the QUEST project are also being provided by this server. The total accesses to our World Wide Web server have grown to more than 30,000 per week.

The YPD Database for Yeast Proteins

J.I. Garrels, G.I. Latter

The yeast protein database (YPD) is a compilation of protein information for all yeast proteins of known sequence, including many sequences known only through genomic sequencing projects. The original aim was to collect information from the protein sequence databases and from the literature that would be useful in 2D gel interpretation. Such information includes calculated molecular weight, calculated isoelectric point, calculated codon bias, amino acid composition, known subcellular localization, known modifications, and key regulatory information. The scope of YPD has since been expanded to be a general purpose database for yeast protein information.

Some new features in YPD are the addition of genetic information (chromosome number, presence of introns, effect of knockout mutation); accession numbers to the 2D gel database YEPD; more localization and functional categories; precursor peptide lengths; motif information (CDC28 sites, *N*-glycosylation sites, and transmembrane domains), longer protein descriptions, and a reference list. YPD continues to be distributed as a spreadsheet, in which each protein is presented as a single nonredundant entry. In the spreadsheet format, users can easily search or sort the proteins by localization, modification, size, amino-

terminal sequence, etc. The files are available by ftp from isis.cshl.org.

YPD is available on-line through the World Wide Web (WWW) server of the QUEST Protein Database Center (http://siva.cshl.org). The on-line version allows proteins to be selected by various functional, localization, or modification categories. In addition, proteins can be selected by the region of the gel within which they are predicted to run. YPD data have also been incorporated into the Stanford Saccharomyces Genome Database (http://genome-www.stan ford.edu).

The latest release (3.0) of YPD contains entries for 3512 proteins. Of these, 1871 are proteins that have been characterized genetically or biochemically, 524 are proteins known only by homology with known proteins, and 1117 are proteins of unknown function (known only from DNA sequence). Table 1 shows the breakdown of the proteins by localization and by functional category.

Table 1 Yeast Proteins by Subcellular Localization

A. Of the 1871 proteins known from genetic or biochemical studies[a]

401	(21.4%)	Nuclear
377	(20.1%)	Cytoplasmic
240	(12.8%)	Mitochondrial
82	(4.4%)	Plasma membrane
56	(3.0%)	Endoplasmic reticulum
51	(2.7%)	Unspecified membrane
45	(2.4%)	Cytoskeletal
34	(1.8%)	Extracellular or cell wall
28	(1.5%)	Vacuolar
23	(1.2%)	Vesicles of secretory pathway
22	(1.2%)	Golgi
15	(0.8%)	Peroxisomal
497	(26.6%)	Unknown

B. Of the 2395 proteins known by genetics, biochemistry, or homology

By molecular environment		
318	(13.3%)	Integral membrane
299	(12.5%)	DNA-associated (not necessarily direct DNA-binding)
130	(5.4%)	Ribosomal
83	(3.5%)	Peripheral membrane
80	(3.3%)	RNA-associated
39	(1.6%)	Protein synthesis factors
16	(0.7%)	Actin cytoskeleton-associated
13	(0.5%)	Tubulin cytoskeleton-associated
By functional category		
106	(4.4%)	Transcription factors
81	(3.4%)	Protein kinases
61	(2.5%)	Enzymes of amino acid metabolism
43	(1.8%)	GTPases
31	(1.3%)	Heat shock
30	(1.3%)	tRNA synthetases
27	(1.1%)	Protein phosphatases
24	(1.0%)	Proteases other than proteasome subunits
20	(0.8%)	Conserved ATPase domain family (Sec18p/Pas1p/Sug1p)
16	(0.7%)	Enzymes of glucose metabolism
16	(0.7%)	Serine-alanine-rich proteins (Srp1/Tip1p family)
15	(0.6%)	Cyclins
14	(0.6%)	Proteasome components
10	(0.4%)	Ubiquitin-conjugating enzymes
9	(0.4%)	GTPase-activating proteins
8	(0.3%)	Guanine nucleotide exchange factors

[a]The unknown category contains many metabolic and housekeeping proteins are likely to be cytoplasmic, but definitive studies on their localization are difficult to find.

Spot Identification by Gene Overexpression

T. Volpe, B. Futcher

We have been identifying spots on the 2D gel map of yeast proteins by overexpressing particular genes on high-copy-number plasmids and looking to see which spots get darker. Preliminary experiments have been done to test the abilities of this method. In a screen of about 30 genes cloned on 2-mm circle-based, high-copy-number LEU2 plasmids, we found that success is correlated with the codon bias of the cloned gene. For genes with a codon bias of 0.3 or greater, the method was almost always successful; for genes with a codon bias between 0.1 and 0.3, the method was sometimes successful, and for genes with a codon bias less than 0.1, the method has not worked. In a recent test of 18 genes with a codon bias greater than 0.3, we unambiguously identified 15 of the gene products (often as a set of multiple spots). Gene products identified by this method in the last year include EFT1, PRT1, TEF1, TIF1, TPM1, HOM2, PGI1, PFK1, TPI1, ADE1, LEU2, SAM1, SAM2, PYK1, and HIS3.

Global Experiments: Phosphorylation and Subcellular Fractionation

B. Futcher, T . Volpe

We have labeled yeast with ^{32}P and run the extracts on 2D gels. Mixing experiments with ^{35}S-labeled ex-

tracts allowed us to align the ^{32}P map with the ^{35}S map. A large number of phosphorylated spots were noted, and some of these are known proteins.

We have also started doing subcellular fractionation experiments to see which spots go into which fractions. Simple centrifugation experiments have been particularly successful. The pellet and supernatant fractions of a 100,000*g* centrifugation have been compared. The experiment is easy to do, and results have been highly reproducible. A large number of spots fractionate into the supernatant only. Some are split between supernatant and pellet, and other spots are found primarily in the pellet (although very few are solely in the pellet). In many cases, the identity of the protein is known. The behavior of certain translation factors has been particularly interesting. We have found that many of these factors have a soluble pool and an insoluble pool, suggesting that they may be cycling on and off the endoplasmic reticulum. Our methods will allow quantification of these two pools under different conditions.

Microsequencing

R. Kobayashi, B. Schwender

We continue to apply in-gel digestion techniques to obtain internal amino acid sequence analysis of protein after SDS-PAGE for use in the construction of the yeast database. We have up to now been utilizing 1–2 μg of protein to obtain sequence. This requires from one to six gels. We are working on techniques that will allow us to microsequence with a smaller amount of protein (see Kobayashi, Structure and Computation Section).

We have now identified 38 proteins from *S. cerevisiae* 2D gels using microsequencing and have obtained sequence data on five proteins that are not yet in the sequence databases and thus are unknown proteins. Three of these unknown proteins for which we have obtained clear sequences are major yeast proteins as seen on the 2D gel pattern.

Quantitative Analysis of Apoptosis in Jurkat T Lymphoblasts

G.I. Latter, S.D. Patterson

In a previous annual report, we described the induction of apoptosis in Jurkat T lymphoblasts using two reagents, lovastatin and EGTA. Following separation of the apoptotic cells using Percoll density gradient centrifugation, the control and apoptotic cells were each labeled with [^{35}S]methionine, lysed, and separated by 2D PAGE. Surprisingly, the 2D PAGE patterns generated from the apoptotic cells did not differ dramatically from those of control cells. Thus, apoptotic Jurkat cells are able to synthesize new proteins and do not exhibit extensive proteolysis. Qualitative analysis revealed that one protein clearly displayed decreased labeling in the apoptotic cells following both treatments, and this was subsequently identified as the abundant nucleolar phosphoprotein numatrin/B23/nucleophosmin (NPM) by amino acid sequence analysis, immunoblotting using monospecific antiserum, and laser desorption/ionization time-of-flight mass spectrometry.

We are interested in proteins that may be components of a putative final common pathway in apoptosis. Therefore, we undertook a quantitative computer analysis of the 2D PAGE patterns using the Quest II software to look for proteins that exhibit repeatable coordinate regulation (either increase or decrease) in both apoptotic inductions. Stringent criteria for both fold-change and level were used for the analysis. This resulted in only five proteins displaying decreases in turnover that were common to the two treatments (and replicates). One of these is NPM. No increases in protein turnover were able to be confirmed across the replicate experiments, but this is most likely due to the stringent analysis criteria imposed. NPM is implicated in a range of diverse cellular functions, but its role in apoptosis is unclear. The other proteins await identification.

2D Gel Core Facility

J.I. Garrels, N. Bizios, N. Sareen, G.I. Latter

The 2D gel laboratory facility produced 1625 gels in 1994. These gels were produced for 17 groups within Cold Spring Harbor Laboratory and 8 external groups.

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GENETICS

The three immutables of present human existence have been posed as Birth, Death, and Taxes. It seems to be true, and given the debate about the allocation of resources toward military activities versus others, it is not clear whether we have learned much about raising or spending taxes since the Romans. I can even hear our forebears debating the value of investing in new technologies, unless perhaps for military uses.

We have, however, learned a great deal in the last 2000 years about birth and death. We know more than ever what it means to be human, how we are physically and biologically constructed, and how we pass our genetic nature to our children. We have discovered an extraordinary biological relatedness not only to our closest animal relatives, which Darwin's contemporaries so enjoyed lampooning, but also to all other biological beings. Perhaps this in some way contributes to our deepening sense that we should let species other than ourselves also survive.

All of this is due to biological science, the slow, painstaking process of understanding how cells and organisms are made, how they function, and how they propagate. The work done by scientists rarely leads them to be as rich or famous as film stars or baseball players, but an accurate contribution to our understanding of biological mechanisms is an achievement that lasts forever, and upon which the edifice of science will continue to build. The recent contribution of the local geneticists to this process is described below.

EUKARYOTIC CELL CYCLE CONTROL

D. Beach S. Allan, M. Caligiuri, D. Casso, T. Connolly, K. Dai, S. Davey, D. Demetrick, K. Galaktionov, C. Gawel, G. Hannon, G. Hannon, J. Hofmann, J. Hudson, T.K. Kim, D. Lombardi, B. Nefsky, T. Matsumoto, S. Matsumoto, K. Okamoto, S. Salghetti, M. Serrano, H. Zhang

During the course of the year, Greg Hannon was promoted to Staff Scientist II at Cold Spring Harbor, Tim Connolly left to take a Senior Staff Scientist position at Osiris Therapeutics, Inc., and Tomo Matsumoto became a Staff Scientist at Albert Einstein College of Medicine. We were joined by two new postdocs, Tae Kook Kim and Kang Dai, a new lab technician, Simone Salghetti, and a computer programmer, Diane Lombardi.

Cell Cycle Kinases in Nontransformed and Transformed Cells

H. Zhang, D. Beach

In normal proliferating cells, the various functionally distinct cell cycle kinases exist primarily in the form of a quaternary complex of cyclin/CDK/p21/PCNA. Our study indicated that p21 is a universal inhibitor for all these cell cycle kinases. p21 is transcriptionally regulated by the p53 tumor suppressor protein, which leads to the proposal that p21 may serve as the effector of cell cycle arrest during the activation of p53-mediated checkpoint control during DNA-damage response. However, our in vivo analysis of the quaternary complex suggested that in normal cells, the majority of the cell cycle kinases are in complex with p21 and PCNA. This raises a paradox as to how these cells divide at a normal rate if the majority of their cell cycle kinases are in complex with an inhibitor.

Our subsequent study suggested that p21-containing kinases can exist in both active and inactive states. The binding of single p21 molecules to the cyclin/CDK kinase does not inhibit the kinase ac-

tivity. The binding of more than one p21 molecule to the kinase leads to kinase inhibition. We therefore established that in normal cells, the quaternary complex is the primary active kinase form that regulates cell cycle progression. Furthermore, our in vitro study also indicated that p21 can promote active cyclin/CDK kinase formation, raising the possibility that in vivo, p21 may have both positive and negative regulatory effects on the cell cycle kinases.

In certain transformed cells, especially the viral oncogene-transformed cells, p21 and PCNA disappear from the cell cycle kinase complex. In these cells, the cell cycle kinases associate with new proteins. For example, in transformed cells, cyclin A/CDK2 kinase primarily associates with a new set of proteins of 9 kD (p9), 19 kD (p19), and 45 kD (p45). The association of these proteins to the cyclin A/CDK2 kinase is S-phase-dependent. We have purified this protein complex and obtained peptide sequences. p9 is a human homolog of yeast suc1 protein that binds to cdc2 kinase; both p19 and p45 are novel proteins. Based on the peptide sequences, we have isolated the cDNA clones that encode the full-length p19 and p45 proteins. We have successfully reconstituted the five protein complexes in vitro. Both p9 and p45 can bind to the cyclin A/CDK2 kinase independently. However, the binding of p19 to the kinase depends on the prior binding of p45 to the kinase. In HeLa cells, the expression of p9, p19, and p45, similar to cyclin A, are S-phase-specific. The expressions of p9 and p45 are highly enhanced in transformed cells. Further study suggested that p9 is an inhibitor of the cyclin A/CDK2 kinase that behaves differently from p21. There are no detectable effects of p45 and p19 on the kinase activity in vitro. However, microinjection of anti-p45 antibody into G_1 cells blocks S-phase entry, whereas no DNA synthesis inhibition was observed if the antibody was injected during S phase, suggesting that p45 is essential for the establishment of S phase. Detailed analysis of this five-protein complex is still under way.

Using a genetic approach that aims at isolating new cyclins, we have successfully isolated one more human cyclin. This cyclin, which shares 40% identity with human cyclin A, can cooperate with human CDK5 to complement the cell cycle defect of a *cdc28* temperature-sensitive mutant at a nonpermissive temperature, although neither the cyclin itself nor CDK5 alone can rescue the mutant. The characterization of this new human cyclin is also under way.

The Tumor Suppressor and Cell Cycle Inhibitor $p16^{INK4}$

M. Serrano, D. Beach

The cyclin-D-dependent kinase 4, CDK4, is required for progression through the cell cycle. The activity of CDK4 is positively regulated by association with a D-type cyclin and is negatively regulated by binding to the $p16^{INK4}$ tumor suppressor protein. We have studied the effect of $p16^{INK4}$ overexpression on the proliferation of normal rat fibroblasts. Microinjection of $p16^{INK4}$-encoding DNA inhibited entry into S phase of the cell cycle induced by oncogenic Ha-Ras, and this inhibition was relieved by coexpression of a catalytically inactive CDK4 mutant (studies done in collaboration with Dr. Dafna Bar-Sagi, CSHL). This observation provides direct evidence that $p16^{INK4}$ can inhibit cell growth.

We are also interested in the characterization of the CDK4-$p16^{INK4}$ interaction. We have studied the phenotype of a CDK4 mutation found in a sporadic melanoma. This mutation does not affect the catalytic activity of CDK4 or its interaction with D-type cyclins or with the cell cycle inhibitors p21WAF1 and p27KIP1. However, this tumor-specific mutation renders CDK4 unable to associate with $p16^{INK4}$ and, consequently, insensitive to its inhibitory effect. This is the first tumor-specific dominant mutation found in a CDC2-like kinase. Guided by the position of the above-mentioned mutation, we have identified by site-directed mutagenesis other residues in CDK4 that are involved in the interaction with $p16^{INK4}$. A three-dimensional model of the CDK4 structure based on the known structure of CDK2 has allowed us to locate the $p16^{INK4}$-binding surface in CDK4 (studies done in collaboration with Dr. Rui-Ming Xu, CSHL). The $p16^{INK4}$-binding site is located at the junction between the small and large lobes of CDK4, near the ATP-binding site but distant from the substrate-binding site or the cyclin-binding site. We propose that $p16^{INK4}$ induces a conformational change in the ATP-binding site of CDK4 that is responsible for inhibition of CDK4 kinase activity.

Cell Cycle Genes and Cancer

D.J. Demetrick, D. Beach

The gene for cyclin D1 (*CCND1*) is the first cell cycle regulatory gene identified to have a role in can-

cer. Translocations affecting the regulation of CCND1 are found in lymphomas (centrocytic) and solid tumors (parathyroid adenomas). The *CCND1* gene is also amplified in numerous types of solid tumors including breast, esophageal, and squamous carcinomas of the head and neck, where it presumably gives the malignant cells a survival advantage. During the last year, a diagnostic test for *CCND1* amplification, utilizing fluorescence in situ hybridization (FISH), was developed. This method was applied to frozen tissue specimens from breast carcinomas and clearly demonstrated cyclin D1 amplification in individual tumor cells. This test may be applied to fine-needle aspirates and, if shown to be prognostically useful, may provide surgeons and oncologists with prognostic information about a breast carcinoma before it is even excised.

In addition to *CCND1*, several other cell cycle regulatory genes are considered likely to be involved in cancer. The approach utilized to study these genes involved first obtaining genomic clones of the candidate cDNA and mapping the gene by FISH to determine chromosomal location (and potential involvement at sites of known karyotypic abnormalities). Genes that are mapped to known sites of deletion or loss of heterozygosity are partially sequenced to determine intron-exon boundaries. Cell lines or tumor samples, potentially having alterations of the candidate tumor suppressor gene, are studied by single-stranded conformational polymorphism (SSCP) analysis and polymerase chain reaction (PCR)-based sequencing to detect mutations or homozygous deletions. Using this methodology, the $p16^{INK4}$ gene was mapped to 9p21–22 by FISH and PCR from a somatic cell hybrid panel. Analysis of many cell lines found homozygous deletions in approximately 35%. Studies done in collaboration with the C. Harris laboratory (National Institutes of Health) demonstrated that although many cell lines were found to have deletions or other alterations of the $p16^{INK4}$ gene, alterations were not found in numerous tumor specimens from esophagus, liver, and colon, whereas rare deletions were present in non-small cell lung carcinomas.

Interestingly, the deletions of $p16^{INK4}$ in cell lines were inversely correlated to regulatory abnormalities of the *Rb* gene, implying that disruption of the Rb regulatory pathway at more than one point was potentially oncogenic. Recently, $p15^{INK4b}$, a homolog of $p16^{INK4}$, was isolated and mapped to the same chromosomal area by our laboratory. This gene may also be involved in the cancer-associated abnormalities at 9p21.

Several other cell cycle regulatory genes have been mapped to areas of cancer-associated karyotypic abnormality, including *CDK2* and *CDK4* (12q13), *RBr-2* (16q12-13), *CCNE* (19q12-13), *CCNC* (6q21), p21 (6p21.2), and *HCKS2* (9q22). Studies of the potential role for some of these genes are in progress.

$p15^{INK4B}$ Is an Effector of TGF-β-induced Cell Cycle Arrest

G.J. Hannon, D. Beach

In an animal, cell proliferation is held in check by a number of mechanisms. These include cell-cell contact, irreversible cell cycle withdrawal that accompanies terminal differentiation, the presence of growth inhibitory cytokines and tissue homeostasis factors, and, failing all else, a genetically programmed limit on division potential (senescence). One fundamental characteristic of tumor cells is that they are not subject to many of these growth controls. With the ultimate goal of understanding how tumor cells escape the inhibitory signals that constrain the proliferation of normal cells, we have explored the mechanisms by which one growth inhibitory cytokine, transforming growth factor-β (TGF-β), prevents cell proliferation.

TGF-β is a multifunctional polypeptide that elicits different responses in different cell types. For most cells, TGF-β is a negative growth factor that induces cell cycle arrest in the G_1 phase. Previous studies in other laboratories had indicated that one of the ultimate effects of TGF-β treatment was the accumulation of the product of the retinoblastoma susceptibility gene (*Rb*) in its hypophosphorylated form. In this state, *Rb* prevents passage of cells from G_1 into S phase.

The phosphorylation state of Rb during G_1 (and consequently the G_1 to S transition) is controlled by a pair of related cyclin-dependent kinases, CDK4 and CDK6, in concert with their associated D-type cyclins. Since the sole critical responsibility of these kinases appears to be Rb phosphorylation, we asked whether treatment of cells with TGF-β had any effect on the activity of the CDK4 and CDK6 enzymes. We found that addition of TGF-β to HaCaT cells (an immortal but nontumorigenic human keratinocyte cell line) suppressed the activity of CDK6 kinase (CDK4 cannot yet be reliably assayed in vivo). Coincident

with this loss of activity, CDK6 became associated with a protein with a molecular mass of 15 kD (p15).

The p15 protein was weakly cross-reactive with an antiserum raised against a known CDK4/CDK6 inhibitor, $p16^{INK4}$, suggesting that these two proteins shared some sequence homology. The cDNA encoding p15 was cloned based on this homology, and sequence analysis confirmed that p15 and p16 were closely related proteins. Functional studies demonstrated that the two were essentially indistinguishable in their ability to inhibit CDK4 and CDK6 kinases in vitro, and p15 was therefore designated $p15^{INK4B}$ (*in*hibitor of CD*K4-B*).

Treatment of cells with TGF-β is known to alter the expression of a number of genes, including PAI, whose activity is related to the role of TGF-β in promoting wound healing. Similarly, TGF-β treatment induced the mRNA encoding p15 to greater than 30 times its level in untreated cells. Considered together, our results suggest that p15 may function as the effector of TGF-β-induced cell cycle arrest.

The gene encoding p15 was mapped to human chromosome 9 at position p21, adjacent to the gene encoding p16. Cytogenetic abnormalities at 9p21 are common in many types of human tumors, suggesting the presence of a tumor suppressor gene at this locus. $p16^{INK4}$ was initially proposed as a candidate for this gene, and p16 has been conclusively demonstrated to be the familial melanoma susceptibility gene. The presence of a second member of the p16 gene family at 9p21 raises the possibility that, in some cases, loss of tumor suppression may involve inactivation of either or both genes. In this regard, homozygous deletion of both the p15 and p16 genes is common in a number of cancers (e.g., in gliomas and leukemias). The response of p16 to viral oncoproteins indicates that it may function in intracellular growth regulatory pathways, and the response of p15 to TGF-β indicates that p15 may act as an effector of extracellular growth inhibitory signals. Thus, deletions that remove both genes could simultaneously negate two major proliferation control pathways.

Regulation of Cell Cycle Commitment in Fission Yeast

T. Connolly, M. Caligiuri, D. Beach

In the fission yeast *Schizosaccharomyces pombe*, passage through Start and subsequent commitment to the mitotic cell cycle requires the function of the Cdc2 protein kinase ($p34^{cdc2}$) and the Cdc10/Sct1 transcription complex ($p85^{cdc10}/p72^{sct1}$). We have identified the presumed G_1 form of the kinase as a complex containing $p34^{cdc2}$ and the $p40^{puc1}$ cyclin. $p40^{puc1}$ activates $p34^{cdc2}$ as a histone H1 kinase, an activity that is thermolabile in strains carrying one specific temperature-sensitive allele of *cdc2*. We have found that when a strain carrying this mutation is grown under restrictive conditions, the cells arrest predominately in G_1 due to the dissociation of the $p85^{cdc10}/p72^{sct1}$ complex. The binding of this transcription complex to the *cdc22* promoter oscillates during the cell cycle and is absent when cells carrying a temperature-sensitive mutation in *cdc2* are raised to the nonpermissive temperature. Therefore, we considered the possibility that a component of this transcription complex may be a substrate of the $p40^{puc1}/p34^{cdc2}$ kinase, and we found that $p40^{puc1}$ is in fact associated with $p85^{cdc10}$ in vivo. We have explored the biochemical consequences of amino acid substitutions at a canonical $p34^{cdc2}$ recognition site in $p85^{cdc10}$. Our results indicate that phosphorylation at this site is required for the stabilization of the $p85^{cdc10}/p72^{sct1}$ complex, a necessary prerequisite for the formation of a transcriptionally active DNA-binding complex.

In addition to *cdc2*, *sct1*, and *cdc10*, progression through the G_1 phase of the cell cycle requires the *ran1* gene product. *ran1* encodes a protein kinase that functions as a negative regulator of the meiotic pathway under conditions that promote vegetative growth. Inactivating mutations in *ran1* cause cells to undergo meiosis from the haploid state. A genetic interaction between *puc1* and *ran1* prompted biochemical studies that led to our finding that $p40^{puc1}$ is also associated $p52^{ran1}$ in vivo and that $p40^{puc1}$ may in fact be a substrate of the Ran1 kinase. We have investigated the consequences of the interaction between $p52^{ran1}$ and $p40^{puc1}$ and have found that $p52^{ran1}$ functions at least in part to control the association between $p40^{puc1}$ and $p85^{cdc10}$. On the basis of these data, we suggest a molecular model for Start in which the Puc1 cyclin is phosphorylated by Ran1 which enables it to associate with and phosphorylate Cdc10. This either promotes or stabilizes the association between Cdc10 and Sct1, allowing the transcriptional activation of genes required for DNA replication and commitment to a subsequent round of cell division.

The *sct1* gene protein has a pivotal role in the cell

cycle both as an activator of the mitotic cell cycle and as a repressor of the cell's alternative developmental fate leading to sexual differentiation. This gene therefore provides a means to dissect the elements of Start genetically. We have initiated a suppression analysis of the *sct1* null allele and have isolated two *b*ypass suppressors of *sct1*, *bst1-10* and *bst1-12*. The characterization of these mutants indicates that at least one of them (*bst1-12*) is dominant to its wild-type counterpart, providing a genetic screen for the molecular cloning of this gene that is currently under way. In addition, strains carrying *sct1* and *bst1-12* are cold-sensitive for growth, whereas those carrying *bst1-12* in a wild-type genetic background are not. This strain therefore provides another means to dissect the regulation of Start at the genetic level and has been used for the isolation of multicopy suppressors of the cold-sensitive phenotype, which may lead to the identification of additional elements of the pathway leading to cell cycle commitment.

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CELL CYCLE CONTROL IN *SACCHAROMYCES CEREVISIAE*

B. Futcher B. Elliott, D. Germain, K. Hidaka, B. Schneider, B. Steiner, G. Tokiwa, T. Volpe, H. Wijnen, Q.-H. Yang

Our main interest continues to be 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 Cdc28 and a cyclin. More than a dozen known yeast cyclins now exist, and there are probably more to come. These fall into two broad groups: the G_1 cyclins, including Cln1, Cln2, and Cln3, that regulate Start and the mitotic, B-type cyclins Clb1, Clb2, Clb3, and Clb4. Two other cyclins, Clb5 and Clb6, are very important for DNA replication, but they also have roles at Start and perhaps also in early mitosis.

Interaction of Cyclin-Cdc28 Complexes with G_1 and Mitotic Inhibitors

D. Germain [in collaboration with M. Mendenhall, University of Kentucky]

It has recently been shown that the protein $p40^{Sic1}$ is an inhibitor of at least some cyclin-Cdc28 complexes. Passage into S phase requires destruction of Sic1 so that S-, G_2-, and M-phase cyclin-Cdc28 complexes can be activated. It is thought that destruction of Sic1 is triggered by phosphorylation, allowing recognition by Cdc34, a ubiquitin-conjugating enzyme.

We have studied the interactions of Sic1 with various cyclin-Cdc28 complexes and have found that it is phosphorylated by all tested complexes, including the G_1 cyclin complexes (Cln1, Cln2, and Cln3), the S-phase complexes (Clb5 and Clb6), and the mitotic complexes (Clb, 2, 3, and 4). Although Sic1 has only five consensus Cdc28 phosphorylation sites, two-dimensional gels showed that as many as 12 phosphates can be added by Cdc28. Sic1 inhibited only the Clb-Cdc28 complexes and did not inhibit Cln-Cdc28 complexes. This supports a model where Cln-Cdc28 complexes are responsible for phosphorylating Sic1, leading to degradation and allowing activation of S-, G_2-, and M-phase kinase activities.

We also examined the effects of p21 (a gift from D. Beach and co-workers, CSHL), a mammalian inhibitor of G_1 cyclin–cdk complexes. This protein strongly inhibited the three Cln-Cdc28 complexes and also inhibited Clb5 and Clb6 complexes, but failed to inhibit Clb1, 2, 3, or 4 complexes. Thus, perhaps surprisingly, p21 recognizes some feature of G_1- and S-phase cyclins, but not other cyclins, conserved from yeast to humans. p21 and p40 seem to be largely cyclin-specific rather than cdk-specific.

Critical Thresholds of G_1 Cyclins for Start

B. Schneider

We are interested in knowing how much G_1 cyclin is required for Start. Is a critical level required? Or will a small amount suffice if given long enough to work? As a first step, we measured the amount of G_1 cyclin at Start in different cells. Surprisingly, the amount varied tremendously with growth rate: Rapidly growing cells had at least 50-fold more Cln1 or Cln2 than slowly growing cells. This difference may be due to the very short half-lives of the Cln proteins and their mRNAs: Short half-lives make steady-state levels very sensitive to rates of synthesis.

In normal cells, CLN expression is size-dependent—larger cells make more CLN than smaller cells. We constructed a strain with genotype *cln1 cln2 cln3 GAL-CLN1 gal1*. In this strain, very small amounts of galactose can be used to turn on the *GAL-CLN1* construct, and the amount of expression is proportional to the amount of galactose used. In this strain, CLN expression is independent of size. We are now using this strain to measure the level of CLN required for Start under different conditions (growth rate, etc.).

The RAS/cAMP Pathway Connects G_1 Cyclins to Growth

G. Tokiwa, T. Volpe [in collaboration with M. Tyers, University of Toronto]

As described in the section above, slowly growing cells have relatively low levels of Cln protein. This may be a simple consequence of the short half-lives of Cln mRNA and protein. The difference in Cln levels between slowly growing and rapidly growing cells might be even greater than the difference observed, except that rapidly growing cells seem to have mechanisms for partially repressing *CLN* expression. This mechanism works through the cAMP pathway: The faster the growth rate, the higher the intracellular levels of cAMP, and the greater the *CLN* repression. This repression is only relative—rapidly growing, "repressed" cells actually have more Cln protein than slowly growing, nonrepressed cells. The fact that there is repression at all is demonstrated by the fact that (1) *CLN* mRNA levels drop briefly but sharply when glucose is first added to a culture and (2) cells lacking a RAS/cAMP signaling pathway have more *CLN* at high growth rates than do wild-type cells.

It has been known for many years that rapidly growing cells are larger than slowly growing cells. This now appears to be an effect of the cAMP-mediated repression of *CLNs*. In addition to *CLN1* and *CLN2*, all of the other known genes dependent on the SBF or MBF transcription factors are also at least partly repressed by cAMP (Fig. 1). The significance of this is not yet clear.

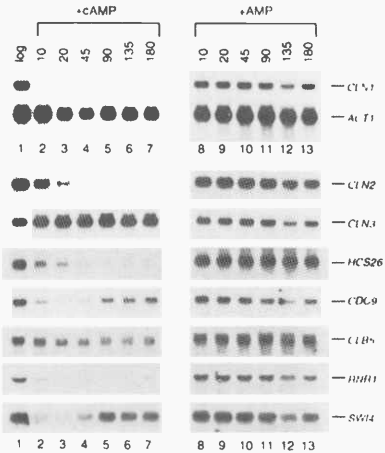

FIGURE 1 High levels of cAMP repress many SBF- and MBF-regulated genes. Either cAMP or 3′ AMP was added to W303 *pde2:TRP1* cells growing in raffinose, and samples were analyzed for mRNA abundance by Northern blotting.

Mechanisms of Transcriptional Activation by Cyclin-Cdc28 Complexes

H. Wijnen

One effect of the Cln3-Cdc28 complex—perhaps the only effect—is to induce transcription of a family of genes that includes *CLN1*, *CLN2*, *CLB5*, and *CLB6*. We are trying to discover the mechanism of induction. The promoters of the *CLN3*-inducible genes all include binding sites for the Swi4 transcription factor or for its close relative Mbp1. Thus, we imagine that Cln3 somehow allows activation from Swi4/Mbp1-regulated promoters. To examine this, we constructed a reporter consisting of an *HIS3* open reading frame downstream from a set of binding sites for Swi4 or Mbp1. Surprisingly, it appears that this reporter is not significantly induced by *CLN3*. It may be that Swi4/Mbp1 sites are necessary but not sufficient for *CLN3* inducibility, consistent with previous results of C. DiComo and K. Arndt (CSHL). Addition of other elements found in the *CLN1* and *CLN2* promoters to the reporter may allow induction by *CLN3* and in this way point to the mechanism of activation.

G_1 Cyclins Are the Only Limiting Factors for Start

Q.-H. Yang

When the G_1 cyclin *CLN3* is overexpressed in rapidly growing cells, it accelerates all or nearly all of the cells through Start, so that there are almost no cells in G_1 phase. However, in slowly growing cells, about half the population is in G_1 even when *CLN3* is overexpressed. This could mean that some proteins other than *CLN*s are limiting for Start in slowly growing cells. Alternatively, since the main function of *CLN3* is to promote transcription of *CLN1* and *CLN2*, it is possible that under poor conditions, insufficient *CLN1* and *CLN2* are synthesized to make Start constitutive. Therefore, we overexpressed *CLN1* and *CLN2* in slowly growing cells. In this case, we found that all of the cells were in S, G_2, or M.

In a more extreme experiment, cells were starved for carbon source so that they were unable to grow at all. Galactose was then used to induce a *GAL-CLN1* construct in a strain carrying a *gal1* mutation so that galactose could not be used as a carbon source. The cells then proceeded past Start into S, even though they were synthesizing very little protein and under normal circumstances would have arrested quantitatively in G_1 phase.

It appears that the only limiting factors for Start, for budding, for DNA synthesis, and for spindle pole body duplication are the *CLN*s. All other needed components must be present even in very small, very slowly growing G_1 cells. This is somewhat surprising, given the large number of genes known to be cell-cycle-regulated at the G_1/S transition.

Cell Cycle Exit and Stress Resistance

B. Elliott

Previously, we identified *TPS2*, which encodes trehalose-6-phosphate phosphatase, as a gene needed for stress resistance in yeast. Yeast are known to accumulate trehalose in response to stress, but it has been controversial whether or not the trehalose is a cause or an effect of the stress-resistant state. Our *tps2* mutant is stress-sensitive and accumulates much less trehalose than a wild-type cell, but it also accumulates trehalose-6-phosphate (tre-6-P). Sugar phosphates are often toxic, and tre-6-P is known to be

an inhibitor of glycolysis. We therefore considered the possibility that the stress sensitivity of the *tps2* mutant was due to accumulation of tre-6-P. We compared *tps1* mutants (which are defective in the first step of trehalose synthesis and do not make tre-6-P) with *tps2* mutants and *tps1 tps2* double mutants. All three were stress-sensitive, but the *tps2* single mutant was significantly more sensitive than the other two. Furthermore, a novel gene isolated as a partial suppressor of *tps2* encodes a phosphomutase and partially converts tre-6-P into trehalose. These results suggest that although part of the stress sensitivity of *tps2* is due to the accumulation of tre-6-P, another part is genuinely due to the lack of trehalose. Thus, we believe that trehalose is a stress protectant.

Est1 Is a Protein Component of Yeast Telomerase

B. Steiner, K. Hidaka

The *EST1* gene (*E*ver *S*horter *T*elomeres) was identified many years ago in a screen for telomere maintenance mutants. Cells lacking *EST1* suffer a gradual shortening of telomeres, then senesce and die. This has led to speculation that the Est1 protein might be a component of yeast telomerase.

We tested this idea by tagging Est1 with an epitope and then immunoprecipitating. Northern blots show that the immunoprecipitates contain the yeast telomerase RNA. When immunoprecipitates are resuspended in a reaction mix containing telomere-like oligonucleotides, dTTP, and ^{32}P-labeled dGTP, the oligonucleotides are elongated to give a telomerase-like ladder of reaction products. The activity is sensitive to RNase digestion, it is specific for immunoprecipitates from tagged Est1 strains, and it fails to elongate most nontelomeric oligonucleotides.

At present, we are working to confirm that the activity is telomerase by asking whether we can change the nucleotides that are incorporated by changing the sequence of the telomerase RNA.

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REGULATION OF GROWTH AND CELL CYCLE COMMITMENT IN BUDDING YEAST

K.T. Arndt A. Sutton, C.J. Di Como, C. Devlin, A. Doseff, F. Lin, M. Luke, F. Della Seta, T. Zhong

Our research focuses on the regulation of the G_1 phase of the cell cycle. For most eukaryotic organisms, it is during G_1 that decisions are made as to whether or not to divide. For these studies, we use the model organism *Saccharomyces cerevisiae*, which is commonly called budding yeast. Budding yeast cells that have executed the late G_1 event(s) termed Start are committed to initiate DNA synthesis, form a bud, and divide. Start is the point where growth signals and mating pheromones control entry

into the cell division cycle. The execution of Start seems to require some threshold level of G_1 cyclin/CDC28 kinase activity, which is determined in large part by the rate and levels at which the G_1 cyclin RNAs accumulate during late G_1. However, very little is known about the mechanisms that determine *if* G_1 cyclin RNAs accumulate and the *rate* at which G_1 cyclin RNAs accumulate.

Many cell cycle events are controlled by the regulation of the phosphorylation of certain key cell cycle substrates. We have found that the SIT4 protein phosphatase, which is a type-2A-related phosphatase that removes phosphate groups from phospho-serine and phospho-threonine residues, is required during G_1 for the execution of Start, for bud formation, for the initiation of DNA synthesis, and for spindle pole body duplication. Our major goals are to determine how growth signals control the ability of SIT4 to promote Start and bud initiation and to determine the downstream targets of SIT4 that function for Start and bud initiation.

SIT4 Is Required for G_1 Cyclin Accumulation

K. Arndt, C.J. Di Como

SIT4 is required for the execution of Start because it is required for the expression of the *CLN1* and *CLN2* G_1 cyclin genes. *CLN1* and *CLN2* proteins bind to CDC28, thereby activating the kinase activity of CDC28. When some threshold level of CLN/CDC28 kinase activity is achieved, Start is executed and the cells are committed for DNA synthesis and the completion of the cell cycle. During early G_1, the levels of *CLN1* and *CLN2* RNAs are very low. During late G_1, the levels of *CLN1* and *CLN2* RNAs increase at a very rapid rate. The rate at which *CLN1* and *CLN2* RNAs increase is determined in part by the activity of the *CLN3* gene, which encodes a cyclin-like protein. Hyperactive alleles of *CLN3* cause a more rapid rate of increase of *CLN1* and *CLN2* RNA levels, whereas loss-of-function alleles of *CLN3* cause a slower rate of increase.

Much evidence shows that *CLN3* and *SIT4* provide additive pathways for the activation of *CLN1* and *CLN2* expression. In fact, mutation of both *SIT4* and *CLN3* causes an essentially lethal effect. Moreover, this lethal effect is due only to a defect in G_1 cyclin expression because it is completely cured by expression of *CLN2* from a *SIT4*-independent promoter. Like a *sit4* mutation, a mutation in any gene required in the *SIT4* pathway for G_1 cyclin expression should also be lethal in combination with a *cln3* mutation. We used the colony sectoring assay to isolate 400 mutants that could not grow in the absence of *CLN3*. These mutants, termed *ctr* for *C*LN *T*hree *R*equiring, fell into 12 complementation groups. So far, we have analyzed *CTR7* in the most depth. The *CTR7* gene is the same gene as *BCK2*, which was originally isolated as a gene that in high copy number can suppress the temperature-sensitive cell lysis defect of mutations in the protein kinase C pathway.

BCK2 and CLN3 have similar effects on Start regulation. Cells containing a single deletion of either *BCK2* or *CLN3* have a modest defect in *CLN1* and *CLN2* expression that causes the cells to be more sensitive of α-factor and to execute Start at a larger than normal cell volume. Therefore, either BCK2 alone (without CLN3) or CLN3 alone (without BCK2) can activate *CLN1* and *CLN2* expression. However, in the absence of both BCK2 and CLN3, *CLN1* and *CLN2* expression is so low that the cells have a severe growth rate defect. These findings suggest that BCK2 and CLN3 activate G_1 cyclin expression by parallel pathways. Moreover, additional genetic tests show that unlike CLN3, BCK2 functions within the SIT4 pathway for *CLN1* and *CLN2* expression. Overexpression of either BCK2 or CLN3 hyperactivates *CLN1* and *CLN2* expression. This effect gave us a convenient way to determine the *CLN2* promoter elements through which BCK2 functions. The *CLN2* promoter contains DNA sequence elements to which SWI4/SWI6 and possibly MBP1/SWI6 complexes bind to activate *CLN2* expression. We have found that overexpression of BCK2 activates these elements about twofold. However, overexpression of BCK2 activates the intact *CLN2* promoter about tenfold. We have determined that a region that lies between the SWI4/SWI6- and MBP1/SWI6-binding sites and the TATA element has no substantial UAS activity on its own, but modulates the ability of the SWI4 and MBP1 elements to activate *CLN2* expression. How BCK2 modulates the SWI4/SWI6 and MBP1/SWI6 elements is not currently known. Our future goals will be to determine if BCK2 function is regulated by SIT4 and to obtain additional components of the SIT4-BCK2 pathway for *CLN1* and *CLN2* expression.

Function and Regulation of the SIT4 Phosphatase during the Cell Cycle

M. Luke, F. Della Seta

SIT4 is a protein phosphatase catalytic subunit whose levels are constant throughout the cell cycle. Therefore, the activity of SIT4 might be regulated in the cell cycle by association with regulatory subunits. In G_1 daughter cells, SIT4 exists mostly as monomeric uncomplexed SIT4. During late G_1, at a time close to Start, SIT4 is found in separate complexes with two high-molecular-mass phosphoproteins, termed SAP-155 and SAP190. After mitosis, SIT4 is again found primarily as monomeric uncomplexed SIT4.

SAP155 and SAP190 were purified by large-scale affinity purification of epitope-tagged SIT4 followed by SDS-gel separation. R. Kobayashi (CSHL) obtained partial peptide sequences for both proteins and this information was used to clone the *SAP155* and *SAP190* genes. The predicted SAP155 and SAP190 proteins show no significant homology with other proteins in the current databases. However, SAP155 and SAP190 are homologous to each other, with about 30% identical amino acids over the entire lengths of the proteins. This finding raises the possibility that SAP155 and SAP190 share common functions. Indeed, deletion of *SAP190* causes no growth-rate defects, deletion of *SAP155* causes a slight growth-rate defect, but deletion of both *SAP155* and *SAP190* causes a strong growth-rate defect.

We have prepared antibodies to both SAP155 and SAP190. With the tools (antibodies and mutations in the genes) to analyze SAP155 and SAP190 in hand, we can now address the following important questions.

Do SAP155 and SAP190 regulate, positively or negatively, the activity of SIT4 toward its in vivo substrates? Are SAP155 and SAP190 substrates of SIT4? How is the association of SIT4 with SAP155 and SAP190 regulated: via regulation of the association itself (as by phosphorylation of SAP155 and SAP190) or via regulation of the abundance of SAP155 and SAP190? How do growth signals and cell cycle signals regulate the association of SAP155 and SAP190 with SIT4? Our future experiments will be directed toward answering these questions.

Identification of Genes Functioning in the SIT4 Pathway for Bud Formation

A. Doseff

sit4 mutants arrest as large unbudded G_1 cells blocked at Start. In contrast, if *CLN2* is expressed from a SIT4-independent promoter, *sit4* mutants are able to execute Start and replicate their DNA. However, they are still blocked for bud initiation. We used a synthetic lethal screen to identify genes that function in the SIT4 budding pathway. This approach was based on the fact that whereas in certain strain backgrounds, *SIT4* is essential, in some backgrounds, *SIT4* is not essential, but the cells grow very slowly with a greatly expanded G_1 phase. The viability or inviability of Δ*sit4* strains results from a polymorphism at a single genetic locus that we termed *SSD1*. The alleles of *SSD1* that allow viability in the absence of *SIT4* are called *SSD1-v*. We isolated mutations that, like *sit4* mutations, are lethal in the absence of *SSD1-v*. From this screen, we isolated 75 mutants termed *LAS* (for *L*ethal in the *A*bsence of S*SD1*) that fell into six complementation groups. Importantly, one of the complementation groups contained mutations in the *SIT4* gene, confirming the rationale of the screen. The complementation group with the most members had mutations in the *LAS1* gene. *LAS1* encodes an essential protein with a predicted molecular mass of 59 kD. Loss of *Las1* function causes the cells to arrest as 80% unbudded cells and 20% large budded cells. The large budded cells have many secretory vesicles at the mother-daughter neck. Presumably, these vesicles accumulate because they are blocked for fusion with the cell membrane. Overexpression of Las1 results in the formation of extra cell surface projections in the mother cell, alterations in the localization of components (such as actin and Spa2) that are known to be involved in bud formation, and the accumulation of electron-dense structures along the periphery of the cell. Further evidence of a role of Las1 in bud formation is that overexpression of Las1 promotes bud formation in *sit4* mutants. Because LAS1 localizes to the nucleus, our current model is that LAS1 functions in bud formation and morphogenesis via the regulation of the expression of components that function directly in these processes. Very little is known about how components involved in bud initiation are regulated during the cell cycle. LAS1 is a phosphoprotein

and our future experiments will determine if LAS1 is a substrate of SIT4. In addition, we will try to identify genes involved in bud initiation that are regulated by LAS1.

Identification of Genes That Interact with *SIT4*

C.J. Di Como

To identify cellular substrates and/or regulatory subunits of SIT4, we isolated genes that, when present on a high-copy-number plasmid, suppress the temperature-sensitive phenotype of a *sit4* mutant. From this scheme, we isolated three genes. One gene is *SAP155*, which encodes the 155-kD subunit of SIT4. High-copy-number *SAP190* also suppresses the *sit4* mutant, but we did not isolate *SAP190* from this screen because *SAP190* is not present in the high-copy-number library. The second gene is *HCS26*. *HCS26* encodes a G_1 cyclin that binds not to CDC28, but to the CDC28-related kinase PHO85. Other cyclins (*CLN1*, *CLN2*, *ORFD*, and *CLB5*) in high copy number are not able to rescue the temperature-sensitive phenotype of the *sit4* mutant. It is not yet clear by what mechanism HCS26 suppresses the *sit4* mutant, but suppression does require *PHO85*. The third gene is a previously unidentified gene which we term *SAP42* (*S*IT4-*a*ssociated *p*rotein) because it encodes a 42-kD protein that associates with SIT4. The *SAP42* gene is essential, and germinating Δ*sap42* cells arrest as four to eight unbudded or large budded cells. We are currently testing if SAP42 is a substrate or target of SIT4 or if SAP42 regulates SIT4. In addition, we are determining the essential cellular function provided by SAP42.

Overexpression of SIS2, Which Contains an Extremely Acidic Region, Increases the Expression of *SWI4*, *CLN1*, and *CLN2* in *sit4* mutants

C.J. Di Como

The *SIS2* gene was identified by its ability, when present on a high-copy-number plasmid, to greatly increase the growth rate of *sit4* mutants. Overexpression of SIS2, which contains an extremely acidic carboxy-terminal region, stimulated the rate of *CLN1*, *CLN2*, *SWI4*, and *CLB5* expression in *sit4* mutants. In addition, overexpression of SIS2 in a *CLN1 cln2 cln3* strain stimulated the growth rate and the rate of *CLN1* and *CLB5* RNA accumulation during late G_1. Therefore, SIS2 has a role in the expression of the G_1 cyclins. The SIS2 protein fractionated with nuclei and was released from the nuclear fraction by treatment with either DNase I or micrococcal nuclease, but not with RNase A. This result, combined with the finding that overexpression of SIS2 is extremely toxic to a strain containing lower than normal levels of histones H2A and H2B, suggests that SIS2 might function to stimulate transcription via an interaction with chromatin.

PDL3 Is Required for G_2 Cyclin Expression

A. Sutton

In an effort to determine additional functions of SIT4 in the cell cycle, we carried out a genetic approach. This approach was based on the fact that whereas in certain strain backgrounds *SIT4* is essential, in some backgrounds, *SIT4* is not essential, but the cells grow very slowly with a greatly expanded G_1 phase. The viability or inviability of Δ*sit4* strains results from a polymorphism at a single genetic locus which we termed *SSD1*. The alleles of *SSD1* that allow viability in the absence of *SIT4* are called *SSD1-v*. We isolated mutations that cause a requirement for *SIT4* for viability in an *SSD1-v* background. This synthetic lethal screen should identify genes (such as *SSD1-v*) whose products carry out overlapping functions with *SIT4*.

One of the genes we obtained in this screen is called *PDL3* (*p*hosphatase-*d*eficient-*l*ethal). *PDL3* encodes a novel, essential protein kinase with several unique structural features. The predicted PDL3 protein lacks a highly conserved GXGXXG sequence found in protein kinases that is required for nucleotide binding. PDL3 also lacks another invariant G residue within the kinase catalytic domain. Despite these features, mutational analysis and in vitro kinase assays show that PDL3 functions as a protein kinase in vivo and in vitro. Several lines of evidence suggest that the essential function of the PDL3 kinase is for cell cycle progression from G_2 into M phase. First, the phenotypes caused by the *pdl3-1* mutation are

identical to those of a strain deleted for *CLB2*. *CLB2* encodes a B-type cyclin whose interaction with the CDC28 kinase late in G_2 is crucial for progression from G_2 into M. Both *pdl3* mutants and Δ*clb2* mutants have a cell cycle delay in late G_2 and an elongated cell shape. Second, *pdl3* mutants are defective in the accumulation of *CLB2* RNA, which normally occurs late in G_2. In addition, temperature-sensitive *pdl3* strains arrest with low CLB2/CDC28 kinase activity. Third, the temperature-sensitive phenotype of a *pdl3* mutant is suppressed by expression of *CLB2* from a heterologous promoter. These data strongly implicate the PDL3 kinase in control of cell cycle progression at G_2/M and suggest that its role in the cell cycle is via the activation of the CLB/CDC28 protein kinase. We are currently investigating the mechanism by which PDL3 regulates CLB/CDC28 activity and why mutations in *PDL3* cause a requirement for *SIT4* for viability.

Transcriptional Regulation of *SIS1*

T. Zhong

Heat shock normally results in the rapid transcriptional activation of heat shock genes and the preferential translation of heat shock messages. The essential *SIS1* gene encodes a DnaJ homolog whose transcription is activated by heat shock. Our interest in *SIS1* lies in the ability of overexpressed SIS1 to increase the growth rate of *sit4* mutants. Interestingly, *sis1* mutants have greatly increased transcription of the *SIS1* promoter. In addition, overexpression of SIS1 represses transcription of *SIS1*. Therefore, *SIS1* autoregulates its own transcription. We have determined that the *cis*-acting *SIS1* promoter sequences responsible for *SIS1* autoregulation are localized to a 39-base region. This 39-base promoter region contains the *SIS1* heat shock consensus element. However, the heat shock consensus element is not by itself sufficient for SIS1 regulation. An additional eight bases are required on the upstream side of the heat shock element to obtain regulation by SIS1.

In addition, *sis1* mutants have greatly increased transcription of the *SSA4* gene, which encodes a member of the A class of HSP70 heat shock proteins in budding yeast. In contrast, transcription of *SSB1* or *SSB2* (encode B class of HSP70) or the two genes encoding HSP90 homologs (*HSP82* and *HSC82*) is not altered in *sis1* mutants. The reverse is also true: Transcription of *SIS1* is activated in *ssa* mutants, but not in strains defective for SSB or HSP90 function. These findings raise the possibility that SIS1 (DnaJ) and SSA (A class of HSP70) may function together for a common process.

Role of Type-2A Phosphatase in the Actin Cytoskeleton and in Entry into Mitosis

F. Lin

PPH22, a gene encoding a catalytic subunit of PP2A (type-2A protein phosphatase) in budding yeast, had been previously isolated in our lab. Later, we identified a second gene, *PPH21*, that encodes a PP2A catalytic subunit. Deletion of either *PPH21* or *PPH22* alone caused no detectable effect, but deletion of both genes resulted in a slow growth defect and a leaky temperature-sensitive phenotype at 37°C. Only deletion of *PPH21*, *PPH22*, and a PP2A-related gene, *PPH3*, was lethal. To investigate whether PP2A is involved in cell cycle events, we prepared a temperature-sensitive PP2A mutant, *pph21-102*. At the restrictive temperature, the *pph21-102* cells arrested predominately with small or aberrant buds, and their actin cytoskeleton and chitin deposition were abnormal. The defects in actin organization might be responsible for both the aberrant bud morphology and the abnormal chitin deposition in the arrested *pph21-102* cells. Three possible functional pathways are proposed. First, PP2A may function directly on the actin cytoskeleton during the cell cycle and subsequently might direct the mode of cell surface growth to determine bud shape. Second, PP2A might regulate the bud site complex, which is responsible for bud emergence and growth, possibly through a regulation of the actin distribution. Third, the effect of PP2A on the actin distribution may be via the effect of PP2A on Clb/Cdc28 kinase activity. An active Clb/Cdc28 kinase was reported to be necessary for switching to spherical growth of the bud by a depolarization of the cortical actin patches.

Moreover, after a shift to the nonpermissive temperature, the *pph21-102* cells were blocked in G_2, with inactive Clb2/Cdc28 complexes. Even though CLB2 and CDC28 were present at normal levels and interacted normally with each other, they had a dramatically reduced kinase activity. Therefore,

PP2A is required, directly or indirectly, for activating the kinase activity of CLB2/CDC28 complexes, possibly via a posttranslational modification. In addition, the Tyr-19 to phenylalanine alteration in CDC28 does not cure the defect in CLB2/CDC28 kinase activity seen in the absence of PP2A function. Therefore, PP2A is required for the activation of the kinase activity of CLB2/CDC28 complexes via a mechanism that does not involve dephosphorylation of Tyr-19.

Mutations in the Yeast GMP Synthase Reveal Altered *HIS4* Transcription Initiation

C. Devlin

In budding yeast, the absence of the DNA-binding transcription factors GCN4, BAS1, and BAS2/PHO2 results in a His^- phenotype that is due to very low *HIS4* RNA levels. Under these conditions, no detectable *HIS4* RNA initiates at the wild-type initiation site. Mutations in the *GUA1* gene, which encodes GMP synthetase, increased GCN4, BAS1, and BAS2-independent initiation of *HIS4* transcription at the wild-type and additional initiation sites and caused a His^+ phenotype. This effect on *HIS4* transcription initiation was reversible by the addition of guanosine to the growth medium. Adenine limitation did not cause a His^+ phenotype in the absence of GCN4, BAS1, and BAS2. GMP synthetase RNA levels remained constant throughout the cell cycle, with different levels of guanosine or adenine in the culture medium, and in *bas1-2* and *bas2-2* mutants. Limitation for guanine nucleotides also increased the amount of *HIS3* mRNA that initiated at a +4 site. The mechanism by which guanine nucleotide starvation alters transcription initiation is under investigation.

In Press

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

N. Hernandez T.L. Calhoun, E. Ford, R.W. Henry, B. Ma, R. Mital, V. Mital, D.J. Morrison, P.S. Pendergrast, F.C. Pessler, C.L. Sadowski, S. Sepheri

SMALL NUCLEAR RNA GENES

In eukaryotes, transcription is carried out by three RNA polymerases, RNA polymerases I, II, and III, each of which recognizes a specific subset of cellular genes. Since RNA polymerase I, II, and III promoters are usually very different in structure, the simplest model to account for RNA polymerase specificity is one in which promoters from each different class are recognized by different transcription factors, which in turn recruit the correct RNA polymerase. It is now clear, however, that promoters from different classes all recruit a common transcription factor, the TATA box-binding protein (TBP). TBP was first identified as the DNA-binding subunit of TFIID, a multiprotein complex that recognizes the TATA box present in a number of RNA polymerase II mRNA promoters. We now know that TBP is also recruited to RNA polymerase I promoters as part of the SL1 complex and to TATA-less RNA polymerase III promoters as part of the TFIIIB complex. Thus, in general, RNA polymerase specificity appears to be determined by the type of TBP-containing complex that is recruited to a given promoter.

The human snRNA genes U1, U2, and U6 are unusual in that they have very similar promoters, yet the U1 and U2 genes are transcribed by RNA polymerase II, whereas the U6 gene is transcribed by

RNA polymerase III. To unravel how transcription of these genes is regulated and how RNA polymerase specificity is determined, we are identifying and characterizing the transcription factors that recognize the U1, U2, and U6 promoters and comparing them to those that recognize other RNA polymerase II and III promoters.

Characterization of the SNAP Complex

R.W. Henry, C.L. Sadowski, R. Kobayashi, N. Hernandez

The RNA polymerase II small nuclear RNA (snRNA) promoters consist of a proximal sequence element (PSE) and a distal sequence element (DSE), located 50 and 200 nucleotides upstream of the transcription start site, respectively. The PSE is sufficient to direct basal levels of transcription and to localize the start site, whereas the DSE is an enhancer of transcription. Thus, in these genes, basal transcription depends on a single element, the PSE. In the RNA polymerase III snRNA promoters, there is one additional promoter element, a TATA box located 20 nucleotides upstream of the start site. This element is part of the basal promoter and determines RNA polymerase III specificity.

We have shown previously that the PSE recruits a novel TBP-containing complex we refer to as the SNAP complex or SNAPc. This complex is unique in that unlike SL1, TFIID, and TFIIIB, which are dedicated to transcription by a single RNA polymerase, it is involved in transcription by two classes of RNA polymerases, RNA polymerases II and III. Purification of this complex to near homogeneity reveals that it consists of TBP and at least three polypeptides with apparent molecular masses of 43, 45, and 50 kD. We have isolated a cDNA corresponding to the 43-kD polypeptide. Peptide antibodies directed against p43 supershift the SNAP complex bound to a PSE probe, confirming that this polypeptide is indeed part of SNAPc. We therefore refer to it as SNAP43. The SNAP43 open reading frame predicts a new protein with a highly charged carboxy-terminal domain. The protein contains weak homology with regions in RAP30 (a subunit of the RNA polymerase II transcription factor IIF) and σ factor implicated in binding to RNA polymerase II and to *Escherichia coli* core RNA polymerase, respectively. Antibody depletion experiments show that, as expected, SNAP43 and/or associated proteins are required for RNA polymerase II and III snRNA gene transcription. The depletions have no effect on transcription from the VAI gene, an adenovirus-2 RNA polymerase III gene with gene internal promoter elements, nor on transcription from the adenovirus-2 major late promoter, a typical RNA polymerase II promoter, suggesting that SNAP43 is not involved in transcription from promoters that do not contain a PSE. We are now in the process of obtaining cDNAs corresponding to the other SNAPc subunits.

Characterization of TFIIIB

R. Mital, R. Kobayashi, N. Hernandez

Most RNA polymerase III promoters including the 5S, tRNA, and VAI contain gene internal elements. On these genes, the assembly of an RNA polymerase III transcription complex is initiated by the binding of TFIIIA and/or TFIIIC to the gene internal promoter elements. These transcription factors then recruit the TFIIIB factor, which in turn recruits RNA polymerase III. TFIIIB is, then, a key factor in RNA polymerase III transcription because it probably contacts RNA polymerase III directly. Despite its importance, the mammalian TFIIIB factor is poorly characterized. We have shown previously that TFIIIB can be separated chromatographically into two fractions, which we refer to as 0.38 M-TFIIIB and 0.48 M-TFIIIB according to their salt elution profile on the column. 0.38 M-TFIIIB is a TBP-containing complex; 0.48 M-TFIIIB is uncharacterized. We have used immunoprecipitations with monoclonal antibodies directed against TBP to purify an 86-kD factor tightly associated with TBP in the 0.38 M-TFIIIB fraction. We obtained peptide sequences and used the information to design degenerate oligonucleotides and obtain a polymerase chain reaction (PCR) probe. The probe was then used to screen a library. The open reading frame reveals a protein that has homology with BRF, a yeast protein associated with yeast TBP and involved in RNA polymerase III transcription. We are currently obtaining antibodies directed against this protein. Such reagents will allow us to determine whether this protein is indeed required for RNA polymerase III transcription of genes with internal promoter elements and may help us identify other components of the complex.

The POU Domain Potentiates the Binding of the SNAP Complex

V. Mittal, M. Cleary, W. Herr, N. Hernandez

Both the RNA polymerase II and III snRNA promoters contain a distance sequence element (DSE) that serves as an enhancer. The DSE is characterized by the presence of an octamer motif, the binding site for the *trans*-activator Oct-1, a POU domain protein. R. Roeder and colleagues (Rockefeller University), have shown that on a probe containing adjacent octamer and PSE sites, the POU domain of Oct-1 binds cooperatively with PTF, an uncharacterized factor that recognizes the PSE and may correspond to the SNAP complex. Indeed, we have shown that the same is true for SNAPc. We are screening for mutations in the Oct-1 POU domain that disrupt cooperative binding and determining their effect on *trans*-activation in vivo.

Characterization of a Novel PSE-binding Activity

E. Ford, N. Hernandez

In addition to SNAPc, we can detect another complex that binds specifically to the PSE. We are characterizing its composition and properties.

Characterization of Human RNA Polymerase III

S. Sepheri, N. Hernandez

Although more and more of the mammalian RNA polymerase III transcription factors are purified and cloned, little is known about RNA polymerase III itself. In particular, there is no modern method to purify the enzyme, and no good antibodies are available. We have therefore started to purify human RNA polymerase III. We are also using PCR methods to try to obtain cDNA clones corresponding to the largest subunit. We could then overexpress the protein and generate antibodies. These reagents will be invaluable for reconstitution of RNA polymerase III transcription in vitro and identification of all the *trans*-acting factors required.

Factors Required for snRNA Transcription by RNA Polymerase II

T.L. Calhoun, N. Hernandez

In the RNA polymerase II snRNA promoters, a single element, the PSE, is capable of directing basal levels of transcription. We know that the SNAP complex binds to the PSE and is required for RNA polymerase II transcription. Which other transcription factors are involved? To answer this question, we are testing general RNA polymerase II transcription factors involved in mRNA transcription for their ability to reconstitute RNA polymerase II snRNA gene transcription in vitro and to assemble on the U1 snRNA promoter.

HUMAN IMMUNODEFICIENCY VIRUS

Transcription from the human immunodeficiency virus (HIV) is regulated to a large extent by a viral protein named Tat. Tat is unusual in that instead of binding to a DNA element, like other *trans*-activators of transcription, it binds to an RNA target, the TAR element, encoded within the first 60 nucleotides downstream from the transcription start site. In the absence of Tat, the HIV-1 promoter generates a large number of short transcripts prematurely terminated around position +60. The role of these molecules is not understood, but they contain the TAR element and may therefore be involved in the regulation of Tat *trans*-activation.

We have identified an element, the inducer of short transcripts (IST), which is required for formation of the short transcripts. When present, this element activates transcription from the HIV-1 promoter, but the RNA molecules resulting from this enhancement in transcription are all short. We are interested in elucidating the biochemical mechanisms that govern formation of the short transcripts and in determining if similar elements exist in cellular promoters.

Identification of *Trans*-acting Factors Potentially Involved in the Formation of Short Transcripts

F.C. Pessler, N. Hernandez

We have identified a putative IST factor whose binding to wild-type and mutated versions of the IST cor-

relates well with short transcript formation. This factor has been purified to near homogeneity and consists of a single polypeptide whose size is identical to that of a polypeptide that can be specifically cross-linked to the IST in partially purified fractions. We are testing the role of this protein in in vitro assays.

TBP Domains Required for Tat *Trans*-activation and IST Function

P.S. Pendergrast, D.J. Morrison, W.P. Tansey, M. Sheldon, N. Hernandez

Tat is an unusual transcriptional activator in that (1) it binds to RNA rather than DNA and (2) it seems to improve the elongation properties of RNA polymerase II. We are therefore interested in comparing Tat to other *trans*-activators with respect to both its effect on short and long transcripts and its sensitivity to various mutations in TBP.

In the absence of Tat, the HIV-1 promoter generates mainly short transcripts. Upon Tat *trans*-activation, the amounts of short transcripts are decreased, whereas the amounts of full-length transcripts are greatly increased. We find that when Tat is targeted to the DNA as a GAL4 DNA-binding domain–Tat fusion protein, *trans*-activation is severely diminished, suggesting that TAR does more than just recruit Tat to the vicinity of the HIV-1 promoter but may recruit, for example, a coactivator. In addition, unlike RNA-targeted Tat *trans*-activation, DNA-targeted Tat *trans*-activation results in enhancement of the short transcripts, suggesting that short transcript repression requires Tat bound to TAR RNA. This is consistent with the observation that *trans*-activation of the HIV-1 promoter by GAL4-VP16 and GAL4-CTF fusion proteins also results in enhancement of both short and long transcripts.

To compare Tat and other *trans*-activators with respect to their sensitivity to mutations in TBP, we have used the altered specificity TBP assay first developed in yeast by K. Struhl and colleagues (Harvard University). Our results indicate that Tat responds to mutations in TBP similarly to CTF but differently from VP16.

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In Press

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TELOMERASE COMPONENTS AND REGULATION

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Chromosome stability is essential for cell viability. Eukaryotes have linear chromosomes, and the telomeres that cap the ends protect chromosomes from degradation and recombination. In the 1930s, Muller and McClintock recognized that broken chromosomes that lacked telomeres were unstable. Subsequently, experiments in protozoa, yeast, and mammalian cells demonstrated the essential nature of telomeres for chromosome structure and function. The recent finding that telomeres shorten with age in normal human somatic cells but are stabilized in tumor cells has suggested that regulation of telomere length may have a role in cellular senescence and immortalization.

Telomerase is the RNP polymerase that is essential for maintaining telomere length in many eukaryotes. To understand both the telomerase enzyme mechanism and the role of telomerase in cell immortalization, we have concentrated on cloning the protein and RNA components of the enzyme. In the past

year, we have collaborated with Geron Corporation in cloning the RNA component of human telomerase. We have cloned the mouse homolog of this gene and showed that it is essential for telomerase function. The identification of these genes allows the study of both the regulation and function of mammalian telomerase. We have also cloned the protein component of *Tetrahymena* telomerase. These proteins represent the first telomerase protein components isolated from any organism. The fact that the sequences are not homologous to those of any other proteins in the database suggests that telomerase represents a new class of DNA polymerases.

Cloning Human Telomerase RNA

A. Avilion [in collaboration with Calvin Harley, Bryant Villeponteau, Junli Feng, and Walter Funk of Geron Corporation]

The potential role of telomerase activity in cellular immortalization and cancer focused attention on cloning the mammalian telomerase components. Strategies were designed to clone the human RNA component using approaches that would amplify the RNAs containing potential template sequences. Specialized cDNA libraries representing small RNAs were subjected to several rounds of selection for RNAs containing the potential template sequence CUAACCCUA. A number of candidate RNAs containing this sequence were characterized to determine whether the RNA copurified with telomerase activity and whether oligonucleotides complementary to the template region would inhibit activity. The genomic locus for the best candidate RNA was cloned, and the template region was altered to generate telomerases that would synthesize either TTTGGG or TTGGGG repeats. Using both transient transfection and stable transformants, the mutant telomerase activity in extracts from these cells was detected.

The human RNA component (hTR) is much larger than the ciliate telomerase RNAs. The RNA is approximately 550 nucleotides in length, and it appears not to be a polymerase III transcript because it contains internal tracts of T residues. The RNA is not polyadenylated, and preliminary results suggest that its transcription is inhibited by low levels of a amanatin, suggesting that it is transcribed by the modified form of RNA polymerase II which transcribes many of the U-snRNA genes in mammalian cells.

Cloning Mouse Telomerase RNA

M. Blasco

The mouse telomerase RNA gene was cloned from a genomic library using the human genes as a probe. Surprisingly, the mouse telomerase RNA (mTR) has only 58% similarity to the human RNA gene. In contrast, other small RNAs are highly conserved between human and mouse. The low degree of homology extends through the template region of the human and mouse RNAs. Interestingly, the mouse template region is shorter than for the human RNA. This shortened template may have a role in the decreased processivity of the mouse telomerase relative to the human enzyme. The probability of dissociation may be greater for the mouse than the human telomerase because of the lesser degree of complementarity between the template and the telomere primer. Mutational analysis of the human and mouse RNAs will allow a greater understanding of the functional consequences of the sequence divergence between these species.

Expression of Human and Mouse RNAs

M. Blasco, A. Avilion

To study the regulation of the human and mouse telomerase RNAs, we followed RNA levels in tissues and cell lines where telomerase activity levels vary. Northern analysis of 16 different human tissues showed as expected that testis and ovary expressed the highest level of hTR; however, surprisingly, a number of other tissues also expressed significant levels of RNA (Fig. 1, top). Of the tissues in which the RNA is detected, it has been shown that normal kidney, prostate, and adult liver lack detectable telomerase activity. This suggests that telomerase RNA may be present but inactive in a number of human tissues.

To examine the regulation of the telomerase RNA in mice, we probed Northern blots containing RNA from various tissues from both newborn and adult mice. All of the tissues tested (intestine, brain, kidney, lung, and liver) in the newborn mice had detectable levels of telomerase RNA. However, telomerase

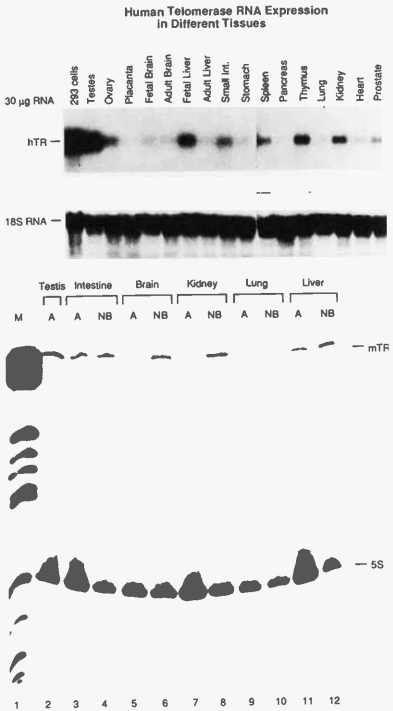

FIGURE 1 (*Top*) Expression of telomerase RNA in human tissues. A Northern blot of human RNA (30 μg) from different tissues was probed with hTR and with 18S RNA as a loading control. Telomerase RNA is expressed in all tissues at various levels, with the highest level of the RNA being in the testes and ovary. Immortalized 293 cells have twofold more telomerase RNA than testes on a per microgram basis. (*Bottom*) Expression of telomerase RNA in mouse tissues. Northern blots of newborn and adult tissues were probed with the mouse telomerase RNA (mTR) and 5S RNA as a loading control. Total RNA (25 μg) was loaded from each tissue: testis (adult only), intestine, brain, kidney, lung, liver. (NB) Newborn samples; (A) adult tissues. This mouse RNA was kindly provided by Dr. Ron DePinho (Albert Einstein College of Medicine).

RNA was only detected in testes, intestine, lung, and liver of the adult mice. The RNA levels were significantly lower in the adult tissues than in newborn tissues (Fig. 1, bottom). The detection of telomerase RNA in adult mouse tissues correlates well with the expression of telomerase activity in adult mice. Unlike human somatic tissues, which do not express detectable telomerase, activity is detected in mouse liver, spleen, and kidney but not in mouse brain. The increased RNA levels in newborns suggest that there is developmental regulation of telomerase.

It is striking that the RNA levels in various tissues are similar in mouse and humans, yet the telomerase activity does not correlate with RNA levels in humans. This suggests that there is additional negative regulation of telomerase in human cells that is not present in the mouse. This difference in telomerase regulation may help explain the long-standing question of why mouse cells spontaneously immortalize in culture and human cells do not. If telomerase reactivation is required for immortalization and human cells have an extra level of repression relative to mouse, it may be easier to activate telomerase in mouse cells. Understanding why differences in hTR levels do not reflect a difference in telomerase activity in these tissues may be an important lead in targeting telomerase for anti-cancer therapies.

Cloning Telomerase Proteins

K. Collins [in collaboration with R. Kobayashi, Cold Spring Harbor Laboratory]

To understand both the biochemistry and regulation of telomerase, we cloned the telomerase protein components from highly purified telomerase fractions. Two polypeptides of 80 kD and 95 kD were identified that reproducibly copurified with telomerase activity (Fig. 2). In collaboration with Ryuji Kobayashi of the CSHL Protein Chemistry Facility, we obtained 7 peptides from p80 and for 25 peptides from p95. Degenerate oligonucleotides were designed and used to obtain polymerase chain reaction (PCR) products from both genes. The full-length gene sequence was then obtained using a combination of cDNA libraries, genomic libraries, and PCR. Genomic Southern blots probed with the PCR products for either p80 or p95 protein showed that both genes are single copy in the *Tetrahymena* genome. Northern analysis showed a band of about 2.9 kb for p95 and a band of 2.5 kb for the p80 mRNA. All of the original peptides obtained by direct protein sequencing were found in the sequence of the open reading frame of the cloned p80

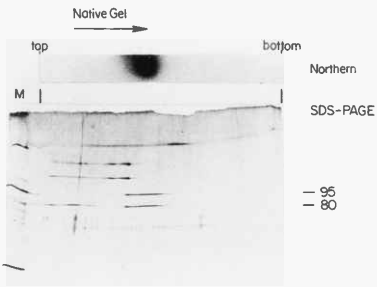

FIGURE 2 p80 and p95 copurify with telomerase. Telomerase was purified over five columns, and then a glycerol gradient was loaded onto a native gel in three duplicate lanes. The positions where telomerase migrated in the native gel were determined by Northern blotting of one lane to detect telomerase RNA and by assaying telomerase activity from different positions of the gel in the second lane. The protein components were identified by SDS-PAGE analysis of the third lane. Two proteins (p80 and p95) comigrated with activity and with telomerase RNA in the first-dimension gel, suggesting that these are telomerase components.

and p95 genes. Neither of the proteins has homology with counterparts in the protein Genbank.

To determine if p80 and p95 were components of telomerase, we generated antibodies against these two proteins. Synthetic peptides were synthesized utilizing sequences from each protein. Antisera were affinity-purified and used in immunoprecipitation experiments. Telomerase activity was specifically immunoprecipitated using an antibody directed against p80; the activity was not precipitated if the peptide specific for this antibody was first added to the reaction to block antibody binding. An unrelated affinity-purified antibody that did not recognize either p80 or p95 also did not immunoprecipitate telomerase activity. This experiment indicated that p80 is a functional component of telomerase. Western analysis of the pellet fraction showed that p95 coprecipitated with p80 protein in fractions that had activity. In addition, the p80 and p95 proteins copurified with each other over all columns tested and comigrated in a native gel and in a glycerol gradient even after RNase treatment. These data suggested that p80 and p95 bind to each other directly and are required for telomerase activity. Based on the mass of telomerase, the active enzyme is likely to consist of one subunit each of the telomerase RNA, p80, and p95.

The sequences of p80 and p95 did not show significant homology with other proteins in available databases. The p80 protein contains a zinc finger that may be involved in RNA or DNA binding. A region in the p95 protein can be aligned with the active sites from two different classes of polymerases: RNA-dependent RNA polymerases and DNA polymerases α and β. Sequences from the four conserved regions in polymerases, the box A, B, C, and D regions, align with several RNA-dependent RNA polymerases. The lack of any other sequence homology with known polymerases together with these motif regions suggests that telomerase represents a new class of polymerase. RNA-dependent RNA polymerases are thought to be one of the earliest known polymerases; if telomerase is indeed most closely related to this class of polymerase, it suggests that telomerase may be an ancestral form of polymerase.

Telomerase in the Cell Cycle

K. Buchkovich, A. Avilion, L. Mantell, M. Blasco

Telomerase activity is not detected in many human tissues. Since telomeres shorten only during replication, it was hypothesized that the telomerase may only be active in actively dividing cells. To address this, we assayed telomerase in actively growing cells, quiescent arrested cells, and at various stages in the cell cycle. *Xenopus* S-phase extracts and M-phase extracts had similar levels of telomerase activity. Human HeLa cells and mouse 3T3 cells synchronized at S phase with hydroxyurea or at M phase with nocodazole also have similar levels of telomerase activity at both cell cycle phases. Consistent with this lack of regulation, in quiescent mouse cells arrested by either serum deprivation or growth to confluence, telomerase activity was at levels similar to those in log phase growing cells. Finally, we found that the level of the mouse telomerase RNA was not altered throughout the cell cycle, indicating that the RNA levels paralleled the telomerase activity. Although telomerase is clearly present at all stages of the cell cycle, it is not yet clear when the enzyme functions to elongate telomeres.

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PLANT GENETICS

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Transposon Tagging and Genetic Analysis of the Maize *Indeterminate* gene

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Maize plants that are homozygous for the *indeterminate* (*id1*) mutation have a prolonged vegetative phase of development that results in an increase in the number of leaves and an extremely late flowering phenotype or no flowering. Mutant plants that do flower often exhibit floral reversion accompanied by morphological aberrations; i.e., the female flower (ear) forms as a vegetative branch and the male flower (tassel) exhibits a proliferous structure of seedlings emerging from the floral spikelets. Therefore, the *Id1* gene product is required for the transition from vegetative to reproductive growth, as well as for the maintenance of the floral state.

We previously isolated a new *indeterminate* mutation from a *Ds2* transposon mutagenesis screen (see 1993 Annual Report) and have shown that it is allelic to two known *id1* mutations, *id1-R* (the original *id1* mutant to be identified) and *id1-Compeigne* (courtesy of Ben Burr, Brookhaven National Laboratory). We designate this new allele as *id1-m1*. The *id1-m1* allele was crossed into several different backgrounds, and molecular analysis was performed on these plants to determine if a *Ds2* element segregated with the *id1-m1* allele. A *Ds2*-specific probe (a gift from Sarah Hake, University of California, Berkeley) was used to analyze several generations of *id1-m1* segregating families by Southern blotting. In more than 120 progeny analyzed, a 4.2-kb *Sac*I fragment was found that was always present in plants that carried the *id1-m1* allele and was absent in plants that did not carry it.

CLONING OF *id1-m1*

The 4.2-kb fragment was cloned into a pUC-based plasmid, pLITMUS29, and the position of the *Ds2* insertion was determined (Fig. 1). When DNA flanking the *Ds2* element was used to reprobe the original Southern blots, a single 4.2-kb *Sac*I fragment was found in *id1-m1* homozygous plants and a 2.9-kb *Sac*I fragment was found in plants that did not have the *id1-m1* allele; heterozygous plants had both bands. Interestingly, when flanking DNA was used to probe *Sac*I-cut DNA from *id1-R* plants, no band was visible. This suggests that the *id1-R* allele might be a deletion of part or all of the *Id1* gene. Furthermore, the *id1-Compeigne* allele, when probed with the flanking DNA, exhibited a pattern which suggests that an insertion of approximately 3 kb exists in the left flank region. Taken together, these results strongly suggest that the *id1-m1* mutation is the result of a *Ds2* insertion.

FIGURE 1 Position of the *Ds2* insertion.

FLOWERING TIME OF *id1-m1* IS REGULATED BY *Ac*

Genetic experiments were performed to provide further proof that the *id1-m1* mutation is the result of a *Ds2* transposon insertion and to get an idea of how the *Id1* gene product regulates the transition from vegetative to reproductive growth. A line was created in which the *id1-m1* allele was linked to the *bz2-m2* allele by the selection of crossovers between these two loci. The genetic distance between them was found to be approximately 1.4 cM. With the *id1-m1* allele closely linked to the *bz2-m2* allele, anthocyanin sectors in the aleurone were used as a marker for *Ac* activity. The *Ac* element used in these experiments is located on chromosome 9 (Kelly Dawe, pers. comm.) and therefore segregates independently of the *id1-m1 bz2-m2* loci. We wanted to determine if the *Ac* activity that causes a variegated phenotype in the aleurone by somatic excision of the *Ds2* element from the *bz2-m2* allele has a similar effect on the *id1-m1* phenotype; i.e., does somatic excision of the *Ds2* element from the *id1-m1* allele restore *Id1* function such that the flowering time for the *id1-m1* mutants is reduced in lines carrying an *Ac* element?

To compare flowering time of *id1* mutants in the presence and absence of *Ac* activity, spotted and bronze kernels from each ear of ten different families that were the result of the self of plants with the genotype *id1-m1bz2-m2/Id1bz2;Ac/–* were planted in the 1994 summer field. (Heterozygous *id1-m1/Id1* plants were used because *id1* mutants normally cannot be crossed.) As expected, 1/3 of the spotted kernels from these ears segregated *id* mutant plants. The other 2/3 had normal flowering times and should be heterozygous plants, i.e., *id1-m1bz2-m2/Id1bz2*. The bronze kernels of the selfed progeny of *id1-m1bz2-m2/Id1bz2, Ac/–* heterozygous plants represent 7/16 of the total kernels on each ear. Of these *bz* kernels, 1/4 (or 4/16) should be homozygous for the tester allele, *Id1bz2/Id1bz2*. The other 3/16 represent kernels that carry the *id1-m1bz2-m2* allele but have no *Ac* element and therefore are not spotted. Of these kernels, 1/3 should also be homozygous for *id1-m1bz2-m2* and therefore express the *indeterminate* mutant phenotype. Therefore, of the bronze kernels planted, 1/7 should segregate *id1* mutants. Overall, the experiment involved comparing the flowering time of *id1* plants grown from spotted kernels (*+Ac*) with that of *id1* plants grown from bronze kernels (*–Ac*). These were compared to the flowering time of normal siblings found in both populations.

All kernels were planted in the third week of May, 1994; Figure 2 shows the results of the experiment. Normal plants from both spotted and bronze kernels usually made between 11 and 13 leaves, and all shed pollen sometime between the third week of July and the first week of August when they were approximately 9 to 11 weeks old. From the 10 rows of spotted kernels planted, 47 clearly *indeterminate* plants were segregating; i.e., none of these had emerging ears or tassels by the time their normal siblings had shed out, and they all had more than 13 visible leaves. The 10 rows of bronze kernels yielded 17 *indeterminate* plants. These also showed no signs of flowering by the time their normal sibs had shed out, and they appeared to grow more slowly than the spotted kernel *id* plants; i.e., they had fewer leaves and they were shorter. By the first week of September, at about 15 weeks after planting, some of the spotted kernel *id* plants had tassels emerging and several had leafy ear shoots, whereas the bronze kernel *id* plants showed no signs of flowering. In mid-September, three of the spotted kernel *id* plants

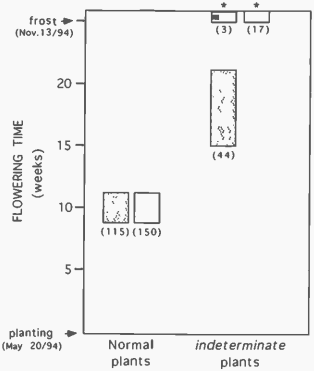

FIGURE 2 *Ac* effect on *id1*⁺ flowering time. (*Speckled boxes*) Spotted kernels (+AC); (*open boxes*) bronze kernels (–Ac); (*) no flowers.

were shedding pollen and the ears were silking; these were either selfed or crossed to each other. These plants had between 15 and 17 leaves. Approximately half of the *id1* mutant plants from spotted kernels were showing tassels at this time, whereas the bronze kernel *id1* mutants continued to produce leaves but no flowers.

By mid-October, when the plants were about 21 weeks old, all of the spotted kernel *id* plants, with the exception of three plants in one family (see below), had either shed out or had tassels emerging, and all had from 17 to more than 20 visible leaves. Most of these did not make ears, and the few ears that did emerge had marked vegetative characteristics. All of the *id* plants from bronze kernels, however, continued to produce leaves, and they had neither tassels nor ears. These plants continued to grow vegetatively and make approximately 18 to 25 leaves until the first killing frost on November 13, 1994 when they were 25 weeks old. Several of these plants were dissected and found to have tassel primordia ranging from 0.5 to 1.0 cm in length. By comparison, several *id1-R* plants that were planted at the same time in a nearby field were dissected and found to have similar size tassel primordia; none of these *id* plants produced ears.

Since spotted kernels indicate the presence of *Ac*, it appears that *id1-m1* plants that had an *Ac* element in the background flowered significantly earlier than plants from the same family that did not have an *Ac* element. In fact, the latter plants did not flower at all, and they made only tiny tassel primordia by the time they were 6 months old at season's end. This is what one would expect if the *id1-m1* allele had a *Ds2* element inserted into it which responded to *Ac* transposase; i.e., *Ac* induces the somatic excision of the *Ds2* element, occasionally producing sectors of normal tissue that can synthesize the functional *Id1* gene product.

It is interesting to note that mosaic plants were not seen in the *id1-m1* plants that flowered earlier; i.e., sectors of normal tissue juxtaposed to mutant tissue were not apparent. Instead, the plants had an intermediate phenotype. This suggests that the *Id1* gene product does not act cell autonomously and that it is a diffusible substance or that it mediates the production of a diffusible product. Furthermore, the progeny that were obtained from the rare crosses of *id1-m1* homozygous mutant plants all exhibited the mutant phenotype. This shows that the tassels and ears of these plants were not derived from sectors of normal tissue. Rather, they are derived from mutant tissue that flowered earlier by virtue of the presence of normal *Id1* gene product that was provided by alleles that had somatically lost the *Ds2* element and restored partial function. Clonal analysis experiments are currently under way to confirm these observations which suggest that Id acts non-cell autonomously.

ISOLATION OF STABLE NULL DERIVATIVES

Finally, as mentioned above, of the 47 *id* plants derived from spotted kernels, all except 3 flowered before the end of the season. These three plants originated from the same family and, in general, resembled *id1-m1* plants derived from bronze kernels in terms of growth rate, leaf number, and inability to flower. DNA was prepared from each of these *id* plants and Southern blotting was performed using *Ds2*-flanking DNA as a probe. Unlike all *id1-m1* plants examined so far, which contain a single 4.2-kb *Sac*I fragment, all three of these plants had a 2.9-kb *Sac*I band; i.e., similar in size to the *Sac*I band found in plants with a normal *Id1* allele. Primers flanking the *Ds2* insertion of *id1-m1* alleles were used to amplify and clone this region from one of the three mutants. Sequence analysis revealed that this plant differed from normal plants in this region by having a 5-bp insertion. This sequence appears to be the remnants of a target-site duplication, initially caused by the *Ds2* insertion. In effect, a stable revertant allele of *id1* was created by the imprecise excision of *Ds2*. This inserted DNA causes a frameshift in the putative open reading frame of the Id protein (see below). As observed, this new *indeterminate* allele (termed *id1-X*) should not respond to the presence of an *Ac* element. This finding provides additional evidence that the earlier flowering phenotype of *Ac*-containing *id1-m1* plants is the result of somatic excision of the *Ds2* element during development.

THE Id CLONE INCLUDES AN ORF THAT HAS HOMOLOGY WITH ANIMAL TRANSCRIPTION FACTORS

Sequence analysis of the regions immediately flanking the *Ds2* element revealed that the transposon is inserted into a putative open reading frame (ORF) of at least 84 amino acids. Preliminary RNA blot analysis using a DNA fragment containing part of the ORF region as a probe showed that it hybridized to a relatively abundant transcript of approximately 2.0–2.2 kb in poly(A)$^+$ RNA from shoot apex tissue and, to a lesser extent, in mature leaf tissue. Very little

hybridization was detected in seedling RNA, and no transcript was detected in RNA from roots.

The 165-bp ORF fragment was used to screen two maize cDNA libraries; one from immature ear (a gift from Bruce Veit and Sarah Hake) and the other from seedlings (a gift from Alice Barkan). A total of 12 cDNA clones were isolated: 3 from the immature ear library and 9 from the other library. Restriction analysis of the clones from the immature ear library showed that they were identical, and sequence analysis of their 3′ ends revealed that they had no poly(A) tails. Sequence analysis of the 3′ ends of the nine cDNA clones from the seedling library revealed two classes of cDNAs. However, when each of these three cDNAs were made into probes and rehybridized to *Sac*I-cut genomic DNA blots, they each showed a distinct pattern of bands, and they did not hybridize to the 4.2-kb or 2.9-kb bands that the Ds2 flanking probes revealed. Therefore, these clones represent three different types of cDNAs that are distinct from the Id1-coding sequence, yet perhaps closely related, at least in the 165-bp ORF region. This suggests that a family of *Id1*-related genes exists in maize. RT-PCR is currently being used to isolate the *Id1* cDNA clone.

Comparison of the *Id1* genomic ORF sequence with known sequences in the database showed that they have similarities to "zinc-finger" DNA-binding proteins from several species, including humans, *Drosophila*, and *Xenopus* (Fig. 3). This is suggestive evidence that if the ORF is a part of the *Id1*-coding sequence, the Id1 protein might function as an important regulator of the vegetative to reproductive transition in maize.

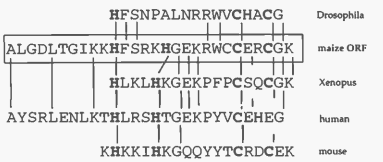

FIGURE 3 Comparison of *Id1* genomic ORF sequence with known sequences in the database.

Genetic Analysis of Embryogenesis in *Arabidopsis* Using Gene-Trap and Enhancer-Trap Transposon Tagging

S. Woody, V. Sundaresan

Although there exist well-characterized animal models such as *Drosophila*, mouse, and *Xenopus*, in which embryo development and pattern formation are understood in considerable molecular detail, comparably little is known about the mechanisms of embryogenesis used by higher plants. Due to fundamental differences between plant and animal development (e.g., immobility of plant cells and the absence of maternal contributions), the animal paradigms may not be directly applicable to those of plants. Thus, an understanding of the genetic and molecular aspects of plant embryogenesis is crucial to our understanding of the diversity of mechanisms that underlie embryonic development in general.

We are using the gene-trap/enhancer-trap transposon-tagging system described previously in the 1992–1994 Annual Reports to identify and characterize embryo-specific gene expression in the model plant system *Arabidopsis*. Plant lines mutagenized using an *Ac/Ds* transposon-tagging system are being screened for transposon-induced mutations that prevent or otherwise disrupt embryogenesis. In this system, plants homozygous at the *DsG* or *DsE* starter locus are crossed with lines homozygous for a derivative of *Ac*; the *Ac* element employed is incapable of transposition, but it is able to supply transposase, the enzyme required for *Ds* transposition. A selection scheme based on the presence of selectable markers associated with the *Ac* and *Ds* elements enables us to identify transposant lines, in which the *Ds* element has re-inserted at sites distant from the starter locus.

Insertion of *Ds* into coding or regulatory sequences important for embryogenesis may result in an embryo-defective (*emb*) phenotype when homozygous. Although mutations in a large number of "housekeeping" genes (e.g., genes required for DNA replication, protein synthesis, or metabolic functions) are predicted to result in the *emb* phenotype, the presence of the GUS reporter gene in the *Ds* elements allows us to ascertain the tissue specificity of gene expression using a simple staining procedure. Lines that display an *emb* phenotype and whose staining patterns indicate embryo-specific gene expression are likely to carry *Ds*-induced mutations in loci whose products or activities are essential for development of the embryo as it progresses through its various differentiated states. In addition, transposant lines heterozygous for either *DsG* or *DsE* are being

screened for insertions that result in embryo-specific GUS expression. This screen may allow identification of duplicated or functionally redundant genes that might have gone undetected in previous mutagenesis schemes. We are most interested in lines in which GUS expression is restricted to particular embryonic cell types or is induced only at particular developmental stages; such lines will constitute a collection of marker lines that will be useful in determining hierarchical relationships among regulatory genes that control embryogenesis.

Experiments are in progress to generate 4000–5000 new transposant lines. Multiple pairwise crosses between *Ac* and *DsG* or *DsE* starter lines have been completed to produce seed representing approximately 20,000 F_1 lines. In the past 9 months, seeds from about 5000 individually grown F_1 plants have been collected; with the recent expansion of greenhouse facilities, we have nearly 4000 F_1 lines growing at any given time. The F_2 seeds are currently being screened at a rate of 400 families per week, thereby generating new transposant lines at a rate of about 100 per week.

In addition, working in collaboration with the Martienssen, Ma, and Grossniklaus labs here at the Laboratory, we have recently conducted a pilot screen of approximately 700 previously generated transposant lines carrying *DsG* or *DsE* elements. Seeds corresponding to the F_3 generation, in which the *Ds* elements should be segregating, were allowed to germinate on agar plates. Seedlings were examined for mutant phenotypes indicative of insertions that disrupt pattern formation in the embryo and stained to determine GUS expression patterns. In a separate phase of the screen, the same lines were sown in soil, allowed to flower, and thus set seed. Inflorescences containing embryos at early stages of development (0–4 days after self-fertilization) were harvested and stained for GUS expression. Finally, the phenotypic screen for *emb* mutations, identified by the presence of aborted seeds in nearly mature siliques, is currently in progress.

In this preliminary screen, we have identified four lines in which GUS expression segregates among developing seeds and appears to be embryo-specific. One of these lines is shown in Figure 4 in which GUS expression is evident throughout the embryo but appears to be stronger in the basal domain. Since this screen was conducted in such a way as to maximize the likelihood of detecting GUS expression, diffusion of the products of the GUS reaction prior to formation of the indigo precipitate limited the spatial resolution of the screen. We are currently using modification of the GUS staining protocol to identify more precisely the cell types responsible for GUS expression in these lines.

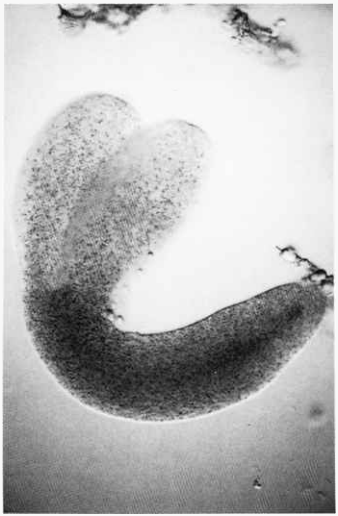

FIGURE 4 GUS expression is evident throughout the embryo, but appears to be stronger in the basal domain.

Gene-Trap and Enhancer-Trap Reporter Gene Expression Patterns in the *Arabidopsis* Leaf and Shoot

P. Springer, Q. Gu, R. Martienssen

In vascular plants, the apical meristem represents the ultimate source of all the postembryonic tissues of the shoot. The meristem comprises a small dome of cells at the tip of the seedling axis and gives rise to axillary buds, resulting in branching, as well as leaves and other lateral appendages. Leaves are typically initiated as small groups of founder cells on the

flanks of the meristem, which rapidly divide and expand to form a peg-like structure. Leaf primordia are elaborated by specification of three major axes and sequential differentiation of leaf cell types. Leaves are widely regarded as the "ground state" lateral organ, as they are interchangeable with other types of lateral organs (cotyledons, bracts, and floral organs) by homeotic mutation or environmental signaling. Leaf development is strongly influenced by the apex from which it arises, and positional and inductive signals between apex and primordium are paramount in the early stages of leaf determination. We are using our gene-trap and enhancer-trap transposon mutagenesis system to identify genes involved in pattern formation in the developing leaf and shoot apical meristem in *Arabidopsis*.

Approximately 1000 F_3 transposant lines were stained for reporter gene expression in a pilot screen; 9–10-day-old seedlings with 5–6 leaves were stained by incubating overnight in X-gluc, a chromogenic

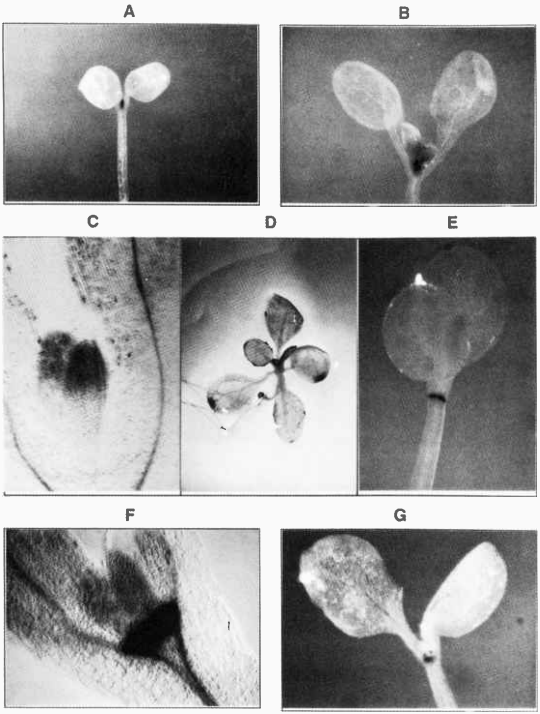

FIGURE 5 Patterns of reporter gene expression in seedlings from enhancer and gene trap lines. Seedlings from eight independent lines were stained in X-gluc and cleared in ethanol (*A–H*). Staining is shown in leaf primordia (*A*), proximal (*C*), distal (*D*), and marginal (*E*) domains in the leaf and various domains within the shoot apex (*F, G, H*).

substrate for β-glucuronidase (GUS). About 40% of enhancer-trap lines and 20% of gene-trap lines displayed reporter gene expression in the seedling. Of these, only 6% of gene traps and 15% of enhancer-trap lines had GUS activity in the leaf, and only about 1–4% were specific to leaves and leaf primordia. Only 2–3% of enhancer-trap lines and less than 1% of gene-trap lines were specifically stained in the shoot apex.

Patterns of reporter gene expression varied widely in different lines. Some tissues, such as stipules and lateral root primordia, were stained in a large proportion of the lines and may reflect artifactual or deregulated reporter gene expression. Many patterns stained one organ predominantly, notably the cotyledon, the root/hypocotyl system, or the leaves and leaf primordia. Tissue- and cell-type-specific expression was observed in some lines: In one line, expression in trichomes and root epidermis might reflect common epidermal patterning mechanisms; in another line, expression was restricted to guard cells. Of particular interest, however, were patterns restricted to domains of the leaf and of the apical meristem that define pattern elements of the primordium. For example, several enhancer-trap lines stained early leaf primordia in the distal domain, coincident with zones of cellular differentiation. In another line, reporter gene expression in 3-day-old seedlings seemed to be restricted to a domain of cells within the shoot apical meristem. Several examples of these patterns are shown in Figure 5.

Mutant phenotypes have only been characterized in the F_3 generation from some of these lines, but so far, about 1–2% have seedling pigment phenotypes (albino, *fusca*, etc.), whereas only 0.5% have potential leaf shape or meristem mutants. About 3% of the F_3 lines exhibited seedling defects ranging from rootless seedlings to those with aberrantly initiated leaf primordia or distorted trichomes. Of these, about one- third exhibited reporter gene expression in the seedling, and two-thirds did not. Segregation ratios indicated that in some cases, seedling defects might be caused by physiological germination abnormalities. Embryonic lethals and semisterile gametophyte lethals have not yet been scored for the whole collection. However, as in *Drosophila*, we anticipate that many of the genes involved in patterning of secondary fields (such as the leaf) will also be involved in other stages of development and may lead to early lethal phenotypes. One such gene has been identified and characterized from our gene-trap screen (see below).

Structure and Function of the *prolifera* Gene in *Arabidopsis*

P. Springer, J. Montagu, H. Cross (URP program),
V. Sundaresan, R. Martienssen [in collaboration
with W.R. McCombie, Cold Spring Harbor Laboratory]

Last year, we reported on the cloning and partial sequence analysis of an MCM2-3-5-like gene from *Arabidopsis* that was identified using a gene-trap transposon insertion (transposant line GT148). The gene-trap reporter gene was expressed in proliferating cells in each plant tissue examined, including primary and lateral root meristems, heart stage embryos, leaf primordia, floral buds, developing carpels, and ovule primordia. In the leaf, the pattern of expression became restricted to the proximal mitotic domain as the leaf expanded, and no expression was detected in the mature organ. For this reason, we have named this gene *PROLIFERA*. A partial *Prolifera* cDNA clone was obtained via RACE PCR (polymerase chain reaction). Further clones were recovered from a flower cDNA library (from D. Weigel, Salk Institute), and last year, we reported that database searches revealed that these clones had strong homology with the MCM3 family of proteins (which includes MCM2, MCM3, and CDC46/MCM5 in *Saccharomyces cerevisiae*, CDC21 of *Schizosaccharomyces pombe*, and the mammalian P1 protein). The 5′ end of the cDNA has now been amplified, and the complete cDNA sequence has been deter-mined, along with a partial genomic map of the insertion allele, *prl-1*. The full-length cDNA sequence shows extensive homology with the *CDC47* gene from *S. cerevisiae* (S. Dalton, Roche Institute, pers. comm.), which was first identified from the European yeast genome sequencing effort (open reading frame YBR1441). The homology extends beyond the highly conserved MCM domain, which is 40–70% identical between members. In yeast, conditional mutants in these genes arrest in S phase, and localization of the corresponding proteins changes during the cell cycle: They are nuclearly localized in G_1 but disappear from the nucleus shortly after the G_1-S boundary, returning only during mitosis. These proteins may be components of "Licensing Factor" (Tye, *Trends Cell Biol. 4:* 160 [1994]), which has been proposed to regulate initiation of DNA replication via chromatin access (Blow and Laskey, *Nature 332:* 546 [1988]). The *DsG* insertion in *prolifera* lies in an 85-bp intron toward the 3′ end of the *prl* gene and results in a translational fusion with the *GUS* reporter gene. Un-

der conditions that limit diffusion of the chromogenic product, staining reveals that the gene fusion appears to be nuclearly localized in some cells, especially in the root (not shown).

Disruption of *PROLIFERA* results in reduced fertility: heterozygous *prl*/+ plants are semisterile, having reduced seed set. This is due to a partial (50%) reduction in transmission of the mutant allele through the female gametophyte, resulting from premature arrest of megagametogenesis. Early embryo lethals are also observed in self-pollinated siliques, suggesting that the few *prl*/*prl* homozygotes that result from fertilization of *prl* embryo sacs abort early during embryogenesis. When *Ac* transposase is reintroduced by crosses, the semisterile phenotype becomes unstable: whereas most siliques are semisterile, occasional siliques are fully fertile, suggesting that the mutation has reverted to wild type in these flowers. Amplification of revertant alleles via PCR has revealed that *DsG* has left the locus in these siliques, resulting in restoration of the wild-type phenotype.

In addition to generating revertants, we are using *Ac* transposase to remobilize the *Ds* element at the *prl* locus in order to generate (1) new insertion/deletion alleles of *prl* that may be less leaky than *prl-1* and (2) closely linked insertions into nearby genes. We will identify these insertions by site-selected mutagenesis using PCR. We have recently developed a method for this procedure in maize (see below), which should be applicable to *Arabidopsis*.

Site-selected Transposon Mutagenesis at the *hcf106* Locus in Maize

L. Das, R. Martienssen

The *High chlorophyll fluorescence106* (*Hcf106*) gene in maize is required for chloroplast development, and the *hcf106-mum1* allele is caused by the insertion of a Robertson's *Mutator Mu1* element into the promoter of the gene. Seedlings homozygous for *hcf106-mum1* are pale green and die 3 weeks after germination, but only in the presence of active autonomous *Mu* regulatory transposons (*Mutator* activity) elsewhere in the genome. When *Mutator* activity is lost, the mutant phenotype is suppressed, and homozygous plants have an almost wild-type phenotype. We have been attempting to isolate derivative alleles in which part of the *hcf106* gene has been deleted, so stabilizing the mutant phenotype in a non-*Mutator* background. Deletions adjacent to *Mu* elements are known to arise at a high frequency in *Mutator* lines.

To isolate new *hcf106* alleles, we have developed a method for site-selected transposon mutagenesis in maize. This procedure, first described for *Caenorhabditis elegans* and *Drosophila*, involves using the PCR to screen pools of individuals for insertions and deletions in genes of known sequence. As described last year, two-dimensional pools of seedlings segregating for the progenitor allele *hcf106-mum1* were screened by PCR for deletions associated with Robertson's *Mutator*; 1500 seedlings from the cross *hcf106*/+ x +/+ were germinated in *x-y* grids and DNA was prepared from pools of 32 seedlings from each row (*x*) and column (*y*) of the grids. The DNA was digested with an enzyme that cuts uniquely in the *hcf106* gene at the initiator ATG codon. DNA from the *hcf106* locus was then amplified using a primer from the transposon and a primer from the second exon of the *hcf106* gene (Fig. 6a). Digestion ensured that only alleles that had lost the initiator ATG codon via deletion would be amplified in subsequent PCR. As we reported last year, three seedlings were identified by cross-referencing *x* and *y* pools that had PCR products of the predicted size, and an example is shown in Figure 6b. The structure and genetic properties of the three new *hcf106* alleles have now been determined (Fig. 6c).

One of the three new alleles, *hcf106-mum3*, is a 244-bp deletion of sequences flanking the *Mu1* element. This deletion removes the first exon, and the first two ATG codons. As predicted, progeny tests have shown that this allele is no longer suppressible: In the absence of *Mutator* activity, plants heterozygous for *hcf106-mum3* give rise to 1/4 mutant progeny following outcrossing and self-pollination, regardless of the presence of *Mu* activity, as monitored by *Mu* element methylation, or phenotypic suppression at other loci. The other two alleles, *hcf106-mum2* and *hcf106-mum4*, were not simple deletions of this type. Instead, they are new insertions of *Mu* elements at the *hcf106* locus. *hcf106-mum2* results from the insertion of a *Mu1* element into the first intron of the *hcf106-mum1* allele, whereas *hcf106-mum4* results from the insertion of a *dMuDR* element at the same location in the + allele. Remarkably, all three new alleles have breakpoints at the same nucleotide in the first intron of the gene. This probably reflects the fact that the target size for recovered events was very small (360 bp between the *Taq*I site and the primer). One of these insertions

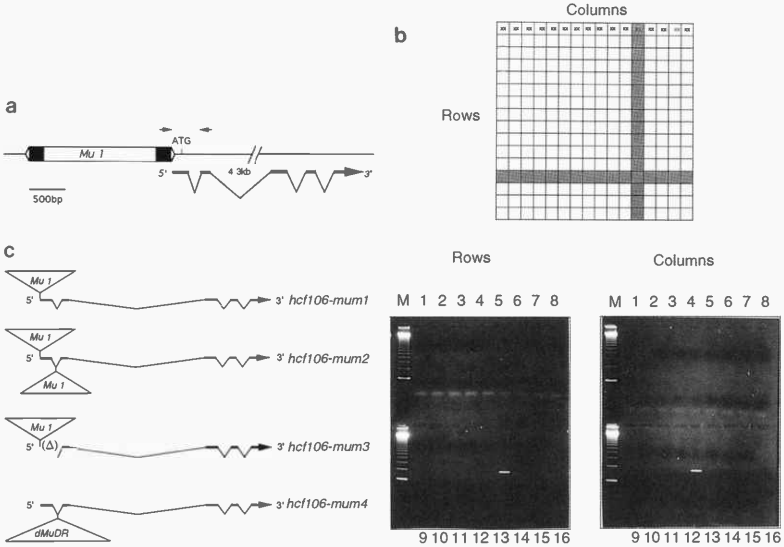

FIGURE 6 Site-selected transposon mutagenesis at the *hcf106* locus in maize. (*a*) Map of the *hcf106-mum1* allele. The *Mu1* element is inserted in the promoter. The locations of the initiator ATG and the primers described in the text (arrows) are shown. The 4.3-kb second intron is shown not to scale (*broken line*). (*b*) PCRs with pooled samples. Seeds were germinated in a 16 x 16 grid, with two seedlings at each position in the grid as shown (x). DNA was extracted from pools of 32 seedlings from each row and each column and amplified using primers from the *Mu1* element and the second exon of the gene as described in the text. Each lane of each gel was loaded with PCR products from individual rows and columns. M is a molecular-weight marker lane. The same derivative allele was amplified in one row and one column, and thus identified by a unique address. (*c*) Schematic maps of progenitor *hcf106-mum1* and derivative *hcf106-mum2*, *hcf106-mum3*, and *hcf106-mum4* alleles. Exon sequences are represented by horizontal lines, and introns are shown as dips. The deletion in *hcf106-mum3* is shown as a triangle.

(*hcf106-mum2*) also gave a lethal phenotype in the absence of *Mu* activity, whereas the other (*hcf106-mum4*) gave only a weak, viable pale green phenotype in this background. The affect of *Mu* activity on this allele has not been determined.

Although the sample size is small, our results suggest that this procedure can be used to rapidly identify transposon insertions into known genes in a single generation. Null derivative alleles can then be isolated in a second generation using the insertion as a starting point. This two-step procedure represents a powerful and simple way to "knock out" maize genes identified by sequence alone, by using only a few thousand progeny from a simple cross.

Role of Hcf106 in Chloroplast Development

A.M. Settles, A. Yonetani, R. Martienssen [in collaboration with R. Voelker and A. Barkan, University of Oregon; and with R. Derby and D. Spector, Cold Spring Harbor Laboratory]

High chlorophyll fluorescent (*hcf*) mutants of higher plants and algae represent a class of nuclear genes required for the development of photosynthetically active chloroplasts. *hcf106* is an example of such a mutation in maize: In mutant seedlings, chloroplast thylakoid membranes have lost their characteristic

lateral heterogeneity and fail to accumulate a subset of membrane protein complexes. This suggests that the *Hcf106* gene product has a role in membrane organization and thylakoid protein uptake or assembly. Three thylakoid protein uptake pathways have been defined by in vitro studies in higher plant chloroplasts (Cline et al., *EMBO J. 12:* 4105 [1993]), and each is required for uptake of a subset of thylakoid lumenal proteins. *hcf106* thylakoid membranes accumulate reduced levels of some of these proteins. For example, they accumulate low levels of the 23-kD subunit of the oxygen-evolving complex but normal levels of plastocyanin (A.Yonetani and R. Martienssen, unpubl.). Using antibodies raised against recombinant lumenal proteins, R. Voelker and A. Barkan have shown that labeled precursors to OE23 accumulate in *hcf106* mutant seedlings. Furthermore, labeled precursors to OE16, another protein taken up by the same pathway, also accumulate, whereas other lumenal proteins are found at normal levels. These results suggest that *hcf106* mutants may be blocked in this pathway.

By using our deletion and insertion derivative alleles, we have found that substantial amounts of mature lumenal OE23 and OE16 proteins are found in *hcf106* null mutants. This implies either that the pathway is leaky or that *hcf106* activity is partially redundant. The latter possibility is supported by the fact that a duplicate gene closely related to *hcf106* has been found in maize (unpubl.). However, this gene is mutant in the genetic backgrounds we are using. To test the former possibility, we are constructing double mutants with other *hcf* mutations. In particular, *hcf137* (*tha1*) has been found to be defective in the other lumenal protein import pathway (R. Voelker and A. Barkan, pers. comm.). *hcf106 hcf137* double mutants should be very informative in this context.

We have cloned the *Hcf106* gene by transposon tagging, and antisera have been raised against the gene product. Hcf106 is a chloroplast membrane protein, and immunolocalization by light and electron microscopy suggests that much of the protein is found in thylakoid grana, which do not form in mutant chloroplasts. The mature protein sequence has a putative membrane spanning domain close to the amino terminus, and a carboxy-terminal acidic domain. The Hcf106 protein is protease sensitive in intact thylakoids and lysed plastids, suggesting that the carboxy-terminal domain protrudes outward into the stroma. Hcf106 behaves as an integral membrane protein after washing with alkali and sodium bromide. It may therefore be a component of the thylakoid uptake apparatus.

Molecular Genetic Characterization of *Iojap* in Maize

C.-D. Han, R. Martienssen

Plants homozygous for the *iojap* (*ij*) mutation have patterned white stripes on their leaves (Jenkins, *J. Hered. 15:* 467 [1924]) and give rise to albino seedlings in their maternal progeny when pollinated by wild-type plants (Rhoades, *Cold Spring Harbor Symp. Quant. Biol. 11:* 202 [1946]). The degree and extent of striping and the maternal transmission of the *ij*-affected plastids are largely dependent on genetic background (Jenkins, *J. Hered. 15:* 467 [1924]; Coe et al., *Am. J. Bot. 75:* 634 [1988]). These properties have made *iojap* a genetic paradigm for cytoplasmic inheritance and nuclear-organellar interactions in plants, and many models have been proposed for *Ij* function.

On the basis of rRNA profiles of mutant and wild-type plants, Walbot and Coe (*Proc. Natl. Acad. Sci. 76:* 2760 [1979]) proposed that *iojap* plants suffered a programmed loss of plastid ribosomes that could account for the maternal transmission of *ij*-affected plastids. Some ribosomal proteins are encoded by plastid genes, so that loss of plastid translation due to homozygous nuclear mutation could lead to the permanent loss of plastid-encoded ribosomal proteins. Restoration of the nuclear components of the plastid translational apparatus in heterozygous maternal progeny would not be expected to restore plastid ribosomal function if the plastid-encoded components were no longer present. Heritable ribosome-deficient plastids would result. Last year, we reported that the Ij protein was associated with 50S plastid ribosomal subunits and that this association was EDTA-sensitive. Association with 50S ribosomal subunits suggests a role for the Ij protein in plastid translation and supports the model proposed by Walbot and Coe.

This model has now been tested genetically by examining Ij-ribosome association in ribosome deficient plastids. The cold-sensitive mutant *virescent 16* becomes chlorotic and fails to accumulate plastid ribosomes when grown at 16°C. In preliminary experiments, Ij protein was found at normal levels in *v16* mutants grown in the cold, but it was no longer associated with 50S complexes on polysome gradi-

ents. In contrast, ribosomes from wild-type plants grown at 16°C or from mutant plants grown at 24°C have normal levels of ribosome-associated lj protein. This confirms that lj is associated with plastid ribosomal proteins.

P Alleles and Expression of Flavonoid Biosynthetic Genes

E. Grotewold

The *P* gene encodes a Myb-domain protein required for normal flavonoid pigmentation in some maize floral tissues. The pigment specified by *P* has been called "phlobaphene," and this pigment accumulation is most dramatic in pericarp and cob glumes. We have shown that P achieves the regulation of pigment accumulation by directly activating a subset of flavonoid biosynthetic genes (Grotewold et al. 1994).

Our previous studies on the *P* gene have concentrated on the analysis of the *P-rr* allele, which specifies red pigmentation in the pericarp and cob glumes (Fig. 7). Genetic analysis as well as saturation mutagenesis of the *P-rr* allele suggested that a single *P* component is responsible for both pericarp and cob pigmentation. Yet, the *P-wr* and *P-rw* alleles show independent pigmentation in the pericarp and the cob (Fig. 7). Biochemical experiments indicate that the nonpigmented *P-wr* pericarps lack flavonoids in general, not just the phlobaphene pigment (Styles and

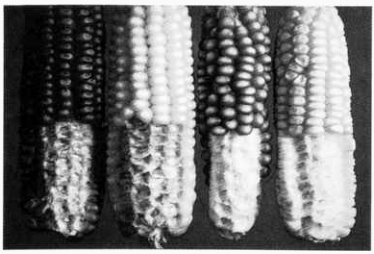

FIGURE 7 Pericarp and cob pigmentation in maize ears with different *P* alleles. From left to right: *P-rr* (red pericarp and red cob glumes), *P-wr* (white pericarp and red cob glumes), *P-rw* (red pericarp and white cob glumes), and *P-ww* (white pericarp and white cob glumes). (Taken by Dr. Thomas Peterson.)

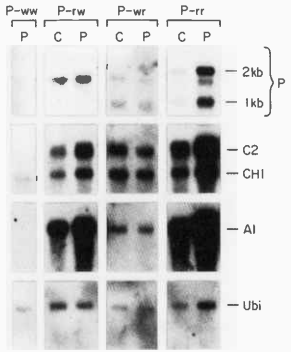

FIGURE 8 Northern blots of pericarp (P) or cob glumes (C) poly(A)⁺ RNA from ears with different *P* alleles. Northern blots were probed with a *P*-specific probe (P), with probes corresponding to three *P*-regulated genes (*C2*, *CHI1*, and *A1*), and with a ubiquitin (Ubi) probe as loading control.

Ceska, *Maydica 34:* 227 [1989]). We decided to investigate this phenomena with the idea that it could provide important information on the mechanisms regulating tissue-specific P expression or function.

To carry out these studies, poly(A)⁺ RNA was isolated from *P-rr*, *P-wr*, and *P-rw* pericarps and cob glumes. Northern blots were hybridized with probes specific for the *P* gene transcripts and for the genes regulated in the pericarp by P in the *P-rr* allele (Grotewold et al. 1994). As had been found previously (P. Athma and T. Peterson, unpubl.), the nonpigmented *P-wr* pericarps accumulate P transcripts at levels similar to those of the pigmented cob glumes (Fig. 8). Yet, instead of the 2-kb and 1-kb transcripts normally found in *P-rr* pericarps or cob glumes, *P-wr* transcripts are 1.8 kb and 1 kb long. A similar result was observed in the nonpigmented *P-rw* cob glumes: A single 1.8-kb transcript accumulates in the nonpigmented cob glumes at levels comparable to those found in the pigmented pericarps (Fig. 8). Since P expression could not account for the different pigment distribution found in the *P-wr* and *P-rw* alleles, we decided to investigate if the P regulatory function was affected in the nonpigmented tissues of these other *P* alleles. We had previously shown that *C2*, *CHI1*, and *A1* transcript accumulation in pericarps is completely dependent on the presence of a functional *P* gene. When probes corresponding to these P-

regulated genes were used to probe the same Northern blots containing pericarp and cob poly(A)$^+$ RNAs from the different *P* alleles, transcripts for these three P-regulated genes could be detected in both pigmented and nonpigmented tissues (Fig. 8).

These results have several important implications. First, they show that expression of the P-regulated genes is not sufficient for the accumulation of the P-specified pigment. Second, they indicate that accumulation of the P-specified pigment requires other, yet unidentified (enzymatic?), steps. These steps are probably regulated differentially in the pericarp and the cob glumes by the different *P* alleles.

Analysis of BMS Transgenic Cell Lines for P and R+C1

E. Grotewold [in collaboration with Ben Bowen, at Pioneer Hi-Bred International, Johnston, Iowa]

Our studies have shown that in *P-rr* pericarps (Fig. 7), a functional *P* gene is required for the accumulation of transcripts from the *C2*, *CHI1*, and *A1* genes. Using transient expression experiments, we showed that the *A1* promoter can be activated by a cloned *P* cDNA (corresponding to the 2-kb *P* mRNA; Grotewold et al., *Proc. Natl. Acad. Sci. 88:* 4587 [1991]). In addition, we demonstrated that the *A1* gene contains P-binding sites that are sufficient for the regulation by P (Grotewold et al. 1994). However, when this *P* cDNA (35S::P, 35S is a plant constitutive promoter derived from CaMV) was introduced into *P-ww* pericarps (Fig. 7) using the particle gun, no synthesis of the *P*-specified pigment was observed. Pericarps derived from the same ears, however, efficiently produced purple anthocyanin sectors when bombarded with 35S::R+C1, the regulators of anthocyanin biosynthesis. (Anthocyanins are also flavonoid-derived pigments, yet they derive from 3-hydroxy flavonoids, not from 3-deoxy flavonoids as the *P*-specified pigments, the phlobaphenes.) These results could mean that the cloned *P* cDNA was not sufficient to activate all the *P*-regulated genes or that the bombarded pericarps were not able to form the red phlobaphene pigment from the colorless precursors.

To distinguish between these two possibilities, we introduced 35S::P cDNA into BMS (Black Mexican Sweet) cells and selected for stably transformed lines using an herbicide resistance gene as a selectable marker (in collaboration with Dr. Ben Bowen, at Pioneer Hi-Bred International, Johnston, Iowa). As a control, similar lines were generated with 35S::R+35S::C1 (35S::R+C1). BMS cells do not normally express any of these regulators. The 35S::P transgenic cell lines did not show any evident pigmentation, whereas the cells transgenic for 35S::R+C1 showed the purple color characteristic of anthocyanin accumulation. Some of the 35S::P transgenic cell lines were analyzed for flavonoid accumulation; in most of the lines, variable amounts of a compound behaving on TLC as flavan-4-ol could be detected (Bowen and Butler, pers. comm.). The accumulation of flavonoids in these cell lines is suggestive that P is activating all the structural genes of the pathway. Northern blot analysis (Fig. 9) showed

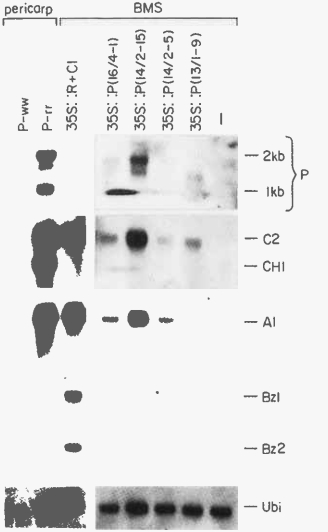

FIGURE 9 Northern blots of pericarp or BMS cells poly(A)$^+$ RNA. Four BMS cell lines transgenic for 35S::P and one transgenic for 35S::R+C1 were used for this study. Northern blots were hybridized with probes specific for the *P* gene (P), with probes corresponding to three *P*-regulated genes (*C2*, *CHI1*, and *A1*), with two genes specifically regulated by R+C1 (Bz1 and Bz2), and with a ubiquitin (Ubi) probe as loading control.

that this is in fact the case: 35S::P is able to activate the *C2* and *A1* genes. 35S::R+C1 activates *C2* and *A1*, in addition to all of the *C1*-specific genes (*A2*, *Bz1*, and *Bz2*) (Fig. 9).

Expression of *CHI1*, which encodes chalcone flavanone isomerase (Grotewold and Peterson 1994), could not be detected in either the 35S::P or 35S::R+C1 lines (Fig. 9). This was unexpected, since it is believed that in both types of cell lines, the isomerization of chalcone must take place in order to accumulate the flavonoids present in the 35S::P lines or the anthocyanins present in the 35S::R+C1 lines. Preliminary studies indicate that BMS cells do have *CHI* genes (not shown), suggesting that for some unknown reason, P and R+C1 are not able to activate *CHI* in these cells. These results suggest that *CHI* is likely regulated by a mechanism different from that of *C2* and *A1*, which probably requires specific factors expressed in the tissues where P and R+C1 are normally expressed, yet absent in these BMS cells.

This lack of *CHI* activation, however, has interesting consequences. *CHI* mutants have never been found in maize. Although in other systems it has been proposed that the isomerization of chalcone to flavonone could be spontaneous, this has never been proven in maize. Our results indicate that this can be the case and may explain why *CHI* mutants have never been observed in maize.

Myb Homologs in *Arabidopsis* Flowers

C. Roberts (URP program), E. Grotewold

Myb homologous proteins are characterized by a conserved amino-terminal region involved in DNA binding, called the Myb domain. The Myb domain is formed by two or three repeats each containing three α-helixes. Each Myb repeat has a helix-turn-helix structure formed by the second and third helixes of each repeat, similar to the structures found in homeodomain proteins or the λ repressor. The third helix of each repeat is involved in DNA contact; hence, among different Myb-domain proteins, conservation is the highest in this region.

Our previous studies have been focused on the study of two Myb-domain proteins, P and C1, that regulate maize flavonoid biosynthesis (Grotewold et al. 1994). In plants, Myb-domain proteins have been shown to be involved in cellular processes other than the regulation of flavonoid accumulation. Our interest was to determine if Myb-domain proteins are involved in normal flower development. Given the information available about the homeotic genes that regulate flower development and morphogenesis in the dicot *Arabidopsis thaliana*, we decided to use *Arabidopsis* as the system to study the possibility that Myb-domain proteins are involved in a regulatory hierarchy required for normal flower development.

We started these studies by cloning Myb homologous genes from *Arabidopsis* flowers. For this purpose, we designed degenerate primers corresponding to the conserved third helices of the first and second Myb repeats. We isolated poly(A)$^+$ RNA from wild-type *Arabidopsis* flowers and synthesized a first-strand cDNA, primed with oligo(dT). Using this set of primers and polymerase chain reaction (PCR) amplification, we obtained products approximately 190 bp long, very close in size to what was expected from the position of the primers in the sequence of the Myb domain. These PCR products were cloned and grouped according to their patterns of cross-hybridization; 23 different clones were grouped into five classes, according to this classification. About three members of each class were sequenced to confirm their homology with Myb-like genes. Once this was confirmed, some of these clones were used as probes to screen 500,000 clones from an *Arabidopsis* flower-specific cDNA library and to pull out 35 cDNA clones corresponding to four of the five classes previously identified. Partial sequence information of 9 cDNA clones suggest that 8 of them correspond to different genes.

Northern hybridization of flower and cauline leaf RNAs indicates that two of these clones are not flower-specific. Future studies will involve the complete sequencing of these clones and the analysis of their expression in different *Arabidopsis* tissues. In addition, we want to determine if mutations in homeotic genes that affect *Arabidopsis* flower development affect the expression of any of these Myb genes.

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ARABIDOPSIS FLOWER DEVELOPMENT AND G PROTEIN FUNCTION

H. Ma C. Flanagan, Y. Hu, H. Huang, D. Kostic, Y. Mizukami, P. Rubinelli, L. Stricker, T. Su, H. Tu, C. Weiss, Y. Zhang

We are interested in understanding at the molecular level morphogenesis, cell-cell interaction, and cellular differentiation during development, using the small plant *Arabidopsis thaliana*. Recent studies in *Arabidopsis*, the snapdragon, and other plants (Coen and Meyerowitz, *Nature 353*: 31 [1991]; Ma 1994) have led to considerable progress in the understanding of the molecular mechanisms controlling flower development. Genetic analyses have identified a number of *Arabidopsis* floral homeotic genes; mutations in one of these genes, *AGAMOUS* (*AG*), cause double-flower phenotypes, where the stamens are converted to extra petals and the ovary is replaced by a new flower. *AG* is a member of the MADS-box gene family (Schwarz-Sommer et al., *Science 250:* 931 [1990]) and likely encodes a transcription factor. We continue to study *AG* and other MADS-box genes *AGL1*, *AGL2*, and *AGL3*.

Given the complexity of flower development, it is likely that many genes important for flower development remain to be discovered. We have recently isolated a new mutant, *fon1* (*f*loral *o*rgan *n*umber), which has an increased number of reproductive organs. We have performed initial phenotypic characterization and have begun the cloning of the *FON1* gene. Furthermore, to identify new genes required for flower development, we have collaborated with Drs. V. Sundaresan and R. Martienssen to establish an enhancer trap/gene trap transposon insertional mutagenesis system (see 1993 Annual Report from Drs. V. Sundaresan and R. Martienssen). During 1994, we were joined by the newly arrived Cold Spring Harbor Fellow Dr. Ueli Grossniklaus in our efforts to generate a large number of independent insertional lines. In an alternative approach to isolate new genes that function during flower development, we have begun a new project aimed at identifying genes specifically expressed in carpels or stamens.

Plant development is highly plastic, modulated by a variety of external signals and signals from other plant cells. Little is known about cell-cell interaction during development, and the mechanisms by which plant development is regulated by environmental signals is also poorly understood, if at all. In animals and simple eukaryotes, G proteins are known to have important roles in signal transduction, including pathways that are important for regulating development. It is possible that G proteins also have a role in regulating plant development. We continue to study *GPA1*, encoding a G protein α subunit (Ma et al., *Proc. Natl. Acad. Sci. 87:* 3821 [1990]). In addition, we have isolated a new *Arabidopsis* gene that encodes a G protein β subunit (Weiss et al. 1994).

In 1994, in addition to the people previously in-

troduced, Yue Zhang and Hua Tu from the Molecular and Cellular Biology Program SUNY Stony Brook conducted laboratory rotation research projects with Hai Huang; Leigh Stricker, who was one of ten finalists for the Partner for the Future program in 1992 and is now an undergraduate student at Boston College, spent the summer working with Hai Huang on the molecular analysis of the *FON1* gene; Thomas Su, an undergraduate at UCLA, was 1994's URP in our lab, and he worked on AGL2 DNA-binding experiments.

Expression of the *AGL1* Gene in Wild-type *Arabidopsis* Flowers

C. Flanagan, Y. Hu, H. Ma

AGL1 is one of the *Arabidopsis* MADS-box genes cloned by their homology with *AG,* and it is preferentially expressed in flowers. To obtain clues about the function of *AGL1,* we have extensively characterized, using RNA in situ hybridization, its spatial and temporal expression patterns within the flower. Our results indicate that *AGL1* expression is restricted to only one type of floral organ, the gynoecium composed of two fused carpels. This is the first known example of a MADS-box gene that is expressed in only one of the four floral organs; others are expressed in two (e.g., *AG*) or four (e.g., *AGL2*) types of organs. *AGL1* expression arises late in flower development, when the carpels have begun to elongate. *AGL1* expression is clearly visible at stage 8 (of 12 floral stages), and it is limited to the tops of carpels. During stage 10, *AGL1* expression is also seen in the developing ovule primordia. In mature stage-12 flowers, *AGL1* expression is primarily in the ovules, along the edges of the septum, and as verticle stripes along the margins of each carpel. Some expression can also be seen in the transmitting tissue of the gynoecium and in the nectaries. Within the ovules of mature flowers, *AGL1* is highly expressed in the endothelium and inner integument but is reduced in the outer integument. *AGL1* expression is also seen along the edges of the funiculus, in continuum with the edges of the septum of the gynoecium. At a slightly later stage, *AGL1* expression in the endothelium is particularly pronounced. These results suggest that *AGL1* is probably involved in late carpel development, particularly in ovule development. That expression is high at the top of the developing carpels, in the carpel margins (near the placenta), and along the edges of the septum and funiculi suggests that *AGL1* may be involved in carpel fusion and/or in providing nutrients to the developing ovules.

In Vitro DNA-binding Analysis of a Plant MADS Domain Protein: AGL2

H. Huang, Y. Zhang, T. Su, H. Ma

Our previous results indicated that the MADS-domain protein AGL2 is a DNA-binding protein and its binding consensus sequence resembles the target sequences (CArG box) of SRF and MCM1. Comparison of plant MADS-domain proteins, including AGL2, indicates that AGL2 can be divided into four regions: the MADS domain (M) at the amino terminus, the K domain (K) near the center, an interdomain region (I), and the carboxy-terminal region; the M, I, K, and C regions contain 56, 35, 65, and 90 amino acids, respectively. AGL2 is thought to bind DNA as a dimer. To test this, and to define the regions required for DNA-binding and/or dimerization, we made four AGL2 deletions from the carboxyl terminus using restriction sites. These truncated AGL2 proteins, AGL2Δ70, AGL2Δ77, AGL2Δ98, and AGL2Δ158, have 70, 77, 98, and 158 amino acids, respectively, starting from the presumptive translation initiation codon. The first two contain M and a portion of I, the third one has both M and I, and a small portion of K, and the last one contains all of the AGL2 except C. Our results demonstrated that all of the truncated AGL2 proteins except AGL2Δ70 can bind to oligo(A) (containing a sequence CCATTAATGG to which AGL2 binds). This result indicates that the first 77-amino-acid residues are sufficient for DNA binding.

To test whether AGL2 binds DNA as a dimer, we tested DNA binding with pairs of two different truncated AGL2 proteins. We found for each of two such pairs (AGL2Δ77/AGL2Δ158 and AGL2Δ98/AGL2Δ158) that in addition to the band expected for each truncated protein, one band, and only one band, of intermediate mobility was observed, suggesting the binding of the heterodimer, but not higher multimers. Interestingly, the AGL2Δ70/AGL2Δ158 heterodimer could also bind DNA, although the AGL2Δ70 homodimer failed to bind. This suggests that the portions of I and K that are absent in AGL2Δ70 (but present in AGL2Δ98) are important for dimerization and/or

protein-DNA complex formation/stability. This hypothesis is supported by the fact that other MADS domain proteins (*Arabidopsis* AP3 and PI and *Antirrhinum* DEFA and GLO) require I and at least a portion of K to bind DNA.

Arabidopsis has numerous MADS-domain proteins, some of which are expressed in overlapping regions of the plant. Therefore, heterodimers between different MADS-domain proteins may form and may have physiological significance. This is known to be the case for AP3 and PI. Since AG and AGL2 are both expressed in the center of the floral meristem, and later in reproductive organs, we tested the dimerization between a truncated AG protein (lacking its carboxy-terminal region) and each of the smaller truncated AGL2s. Like AGL2Δ158, this truncated AG formed heterodimers with AGL2Δ70, AGL2Δ77, and AGL2Δ98, and the heterodimers bound to oligo(A).

Characterization of the Floral Organ Number Mutant *fon1*

H. Huang, Y. Hu, H. Tu, L. Stricker, H. Ma

We previously reported the isolation of an *Arabidopsis* floral organ number mutant, *fon1*, among the T-DNA insertional lines that we generated (H. Huang and H. Ma, 1993 Annual Report). The *fon1* mutation is recessive to wild type, and mutant plants have normal vegetative development. However, the mutant produces flowers with increased numbers of the reproductive organs: stamens and carpels, of which there are six and two, respectively, in wild-type flowers. In *fon1* plants, the early-appearing flowers have more severe phenotypes, with eight to ten stamens and four carpels, whereas later flowers tend to have only six or seven stamens, and three or four carpels. In addition, carpel development in *fon1* plants is often affected, such that the carpels fail to fuse on one side, resulting in curled pistils that are sterile or minimally fertile. Because the increase in stamen and carpel numbers of the *fon1* mutant is similiar to that of previously identified floral mutants *clv1* and *clv2*, we crossed homozygous *fon1* with *clv1* or *clv2* plants to test if *fon1* can complement *clv* mutants. The F_1 progeny of both *fon1* × *clv1* and *fon1* × *clv2* produced normal flowers with normal numbers of floral organs, indicating that *fon1* is in a gene different from that of *CLV1* and *CLV2*. The *fon1 clv1* double mutant (F_2 segregation: 73 wild type, 14 *fon1*, 19 *clv1*, and 6 double mutant) and *fon1 clv2* double mutant (F_2 segregation: 126 wild type, 37 *fon1*, 33 *clv2* and 4 double mutant) showed very similar phenotypes, with up to 20 stamens and 8 or more fused carpels, more severe than the single mutants. We have also performed crosses of *fon1* with several other floral mutants, and the double mutants are being characterized.

Cosegregation studies indicated that the *fon1* mutation is tightly linked to the T-DNA insertion: DNA blot hybridization showed that 153 *fon1* plants from segregating F_2 and F_3 progeny of a cross between *fon1* and wild type contained the same T-DNA bands. Using a T-DNA fragment as a probe, we have isolated a junction fragment that contains both T-DNA and plant sequences. RNA analysis with this junction fragment as a probe identified a 2.7-kb transcript that is expressed in both flowers and vegetative tissues from wild type but not in tissues from the *fon1* mutant. We are in the process of isolating the *FON1* gene.

Generation and Preliminary Characterization of Enhancer/Gene Trap Transposants

H. Ma, C. Flanagan, Y. Hu

To be able to identify new genes important for *Arabidopsis* development, we have collaborated with Drs. Sundaresan and Martienssen to establish an enhancer/gene trap system for *Arabidopsis* using the maize *Ac/Ds* transposable elements (Martienssen et al., 1992 Annual Report; Sundaresan et al., 1993 Annual Report; Ma and Hu, 1992 Annual Report; Sundaresan et al., in prep.). As part of the joint effort between four laboratories at CSHL (Sundaresan, Martienssen, Ma, and Grossniklaus) to generate a large number of the enhancer/gene trap transposon insertional lines (transposants), we have performed a number of crosses between homozygous *Ac*- and *Ds*-carrying plants. Thus far, we have obtained a total of more than 45,000 F_1 seeds from crosses between *Ac* and *Ds* lines. About 8000 of the F_1 seeds have been planted. The F_2 seeds from a single F_1 plant form an F_2 family, and more than 2000 F_2 families have been

plated on agar medium to select for transposants carrying a *Ds* element at a new locus. Thus far, we have generated about 1000 transposants.

Our interest in flower development has prompted us to investigate the expression patterns of the GUS reporter gene, carried within the *Ds* elements, in 468 F_2 transposant lines. We have examined the staining patterns in the inflorescences, individual mature flowers, seed pods, and cauline leaves (as a control for vegetative tissue). Overall, nearly 50% of the lines have a staining pattern in these tissues. Of the 374 gene trap lines we tested, 159 (or 43%) stained positively for GUS; of 94 enhancer trap lines, 83 (88%) had staining patterns.

These results are consistent with our expectations, since more patterns are expected in the enhancer trap lines. Of those lines with staining patterns, 67 (81%) of the enhancer trap lines and 86 (54%) of the gene trap lines had staining exclusively within the flower. A large majority of these stained anthers and/or pollen grains, which is consistent with findings of other investigators that a large number of genes are expressed in anthers and/or pollen (e.g., in tobacco, 25,000 transcripts are expressed in the anthers, 11,000 of which are specific; and in maize 20,000 transcripts are found in pollen, 7,000 of which are specific; Scott et al., *Plant Science 80:* 167 [1991]). Because the anther/pollen staining occurs at such a high frequency, we do need to be cautious in interpreting the staining patterns; further analysis is needed before we are certain that they reflect expression of endogenous genes.

The other patterns are quite unique and cover a broad spectrum of floral parts and stages of flower development. Some interesting examples of patterns among the gene trap lines are in the carpels and seed pods only, in the style region of the gynoecium, in young buds only, and in developing petals (but not mature ones). Among the enhancer trap lines, interesting patterns include stamens only, the gynoecium of mature flowers, and in young buds, becoming restricted to anthers, carpels, and petals in intermediate flowers and later to filaments and the style, transmitting tissue and stigmatic tissue in the gynoecium of mature flowers. These many patterns strongly suggest that a wide variety of gene and enhancer trap insertions are being obtained by this mutagenesis system, which will be extremely useful in our efforts to study and understand flower development in *Arabidopsis*.

Analysis of Multiple Functions of the *Arabidopsis* MADS-box Gene *AG* in Sexual Reproduction

Y. Mizukami, H. Ma

AG EXPRESSION IN THE SEED IS NEGATIVELY REGULATED BY AP2 DURING SEED DEVELOPMENT

We have continued our analyses of the *Arabidopsis* floral organ identity gene *AG*, by characterizing transgenic plants with a fusion of the CaMV 35S promoter and *AG* cDNA (Mizukami and Ma, *Cell 71:* 119 [1992]).

Since *AP2* negatively affects *AG* function in specifying organ identity during early flower development, it is possible that there is also an antagonism between *AG* and *AP2* functions during ovule development. To test this possibility, we compared the *AG* expression pattern in wild-type ovules and seeds to that in the *ap2* mutant. We found that there is a possible negative regulation of *AG* expression by *AP2* in a specific group of cells, the chalazal cells, of developing seeds. During early stages of ovule development, *AG* is expressed in the chalazal region to which the nucellus is attached. As ovules develop, *AG* expression remains high in the endothelium, but it diminishes in other regions including the chalazal cells. *AG* expression is very weak in the wild-type chalazal cells at full maturity of the ovule, and it is no longer detected in developing seeds with a heart-stage embryo. However, in mature *ap2* ovules and developing *ap2* seeds, *AG* expression remains high in the chalazal cells at least until the embryo reaches the late torpedo stage. Recently, it was observed that *AP2* expression remains relatively high in the chalazal cells of wild-type seeds at this stage (K.D. Jofuku and J.K. Okamuro, pers. comm.).

These observations suggest that the *AG* mRNA level may be negatively controlled by *AP2* after the maturation or fertilization of ovules. In addition, a structural difference at the chalazal region was also seen between wild type and *ap2*. Furthermore, *AG* expression under the control of the CaMV 35S promoter in transgenic plants resulted in *ap2*-like seeds. Together, these results strongly suggest that heterochronic or ectopic *AG* expression in the chalazal cells may cause aberrant development of *ap2* mutant and 35S-*AG* transgenic seeds and that negative regulation of *AG* expression by AP2 may be required for normal seed development.

AG MAY BE INVOLVED IN THE MAINTENANCE OF FLORAL MERISTEM IDENTITY

We reported previously that 35S-*AG* transgenic plants flower much earlier than wild-type plants and that the apex terminates with a cluster of sessile flowers (Mizukami and Ma, 1993 Annual Report), similar to those of the *Arabidopsis* early flowering mutant *terminal flower* (*tfl1*); thus, *AG* may be involved in the regulation of the fate of meristems. To examine this possibility, we generated double mutants between *ag* and *tfl1* mutant plants, and 35S-*AG* transgenic lines in a *tfl1* mutant background. We found that ectopic *AG* expression in wild-type and *tfl1* mutant plants accelerates flowering under both short-day (SD) and continuous light (CL) photoperiods, whereas flowering in *tfl1* mutant plants under SD photoperiods is much later than under the CL photoperiods. Furthermore, *ag-1 tfl1-1* double-mutant plants flower later than *tfl1-1* mutant plants in CL photoperiods. These results together indicate that ectopic *AG* expression and the *ag* mutation are epistatic to *tfl1*, suggesting that the effect of the *tfl1* mutation on maintaining floral induction is largely through *AG*. It is known that the *AG* mRNA is only detectable in floral buds. Furthermore, the *ag-1* mutation alone does not affect flowering time. Therefore, although it is not likely that *AG* is involved in the primary apical meristem transition from the vegetative to the reproductive phase, *AG* probably does function to enhance flowering after the transition. On the other hand, the apical meristem of *ag-1 tfl1-1* double-mutant plants grown under CL photoperiods produces a terminal *ag-1* flower at the apex; however, it partially reverts to an inflorescence, producing axillary, secondary *ag-1* flowers. Since similar reversion of flowers to inflorescences was seen in axillary *ag-1* mutant flowers under SD photoperiods, *AG* probably inhibits reversion of established floral meristems to inflorescence meristems, therefore maintaining floral meristem identity under SD photoperiods as well as its determinacy under both photoperiods.

Isolation of Organ-specific cDNAs by Subtractive Hybridizations

P. Rubinelli, H. Ma

Last year, we described subtractive hybridizations between cDNAs from wild-type floral mRNAs and cDNAs from that of *ag* mutant flowers, which lack stamens and carpels, using a method developed by Wang and Brown (*Proc. Natl. Acad. Sci. 88:* 11505 [1991]). We have characterized a number of cDNA clones resulting from the subtractions by Southern analyses with both wild-type and *ag* mutant floral cDNAs as probes. On the basis of the hybridization patterns, the clones were divided into three classes. The first class hybridized with both wild-type and *ag* probes and are likely to be not specific to reproductive organs. The second class hybridized weakly to the wild-type cDNA probe but not detectably to the *ag* probe. These clones were therefore potentially specifically expressed in the reproductive organs. The third class hybridized to neither probe, suggesting a lower level of expression relative to the clones of the first and second classes. To further analyze the organ specificity of the clones of the second and third classes, RNA dot-blot or gel-blot hybridizations were performed with RNAs from leaf, wild-type floral buds, *ag* mutant floral buds, and *apetala3* (*ap3*) mutant floral buds, which lack stamens but have carpels, using individual class-2 or class-3 clones as probes. Of 21 clones tested, 18 hybridized only with wild-type floral RNA; 13 of these putatively stamen-specific clones were sequenced. Many of these (8) showed significant sequence homology with known enzymes or structural proteins. Two clones showed no significant homology with known proteins and have relatively low expressions.

To identify carpel-specific cDNAs, we performed a second series of subtractive hybridizations, between *ap3* floral cDNA and *ag* floral cDNA, since *ap3* floral buds have no stamens. A total of 168 clones were analyzed and 20 showed either no hybridization to either *ap3* or *ag* cDNA probes or weak hybridization to the *ap3* probe only. Of these 20, only 4 clones showed putative carpel specificity on RNA dot blots. Three of these clones showed strong sequence homology with the same drought-induced proteinase inhibitor from *Brassica napus*. In situ hybridization has confirmed the carpel-specific expression, which is confined to late-stage carpels beginning just prior to anthesis and localized to the transmitting tract.

It is likely that the stamen- and carpel-specific genes perform functions that are needed during the growth and differentiation of these organs. In addition, these stamen- and carpel-specific cDNAs should be useful as molecular markers of specific cell or tissue types in *Arabidopsis* floral organ mutants. The

promoter regions of these genes can be used to analyze organ-specific *cis*-regulatory elements that confer developmentally regulated cell or tissue specificity; they can also be used to direct tissue- or cell-specific expression of other genes to study the function of the expressed genes.

Characterization of Plant G Proteins

C. Weiss, H. Ma

SUBCELLULAR LOCALIZATION

We are characterizing the subcellular localization of GPα1 using fractionation methods and immunofluorescence. The subcellular fractionation suggests that GPα1 is not present in the nucleus or in chloroplasts. It is associated with the microsomes and not found with soluble proteins. Isolation of endo- and plasma membranes indicates a relatively good correlation between GPα1 and both the plasma membrane and the endoplasmic reticulum (ER) compartment. Similarly, immunofluorescence experiments suggest that GPα1 is associated with the plasma membrane and the ER, as it colocalizes to some extent with ConA, which stains the plasma membrane and the ER in plant cells. These results suggest that the *Arabidopsis* G protein α-subunit GPα1 might be involved in the transmission of extracellular signals across the plasma membrane, similar to G proteins in mammalian cells and also that it might be involved in regulating lipid and protein synthesis occurring in the ER.

BIOCHEMICAL CHARACTERIZATION

Although the amino acid sequence of GPα1 suggests that it is a GTP-binding protein similar to mammalian heterotrimeric G protein α subunits, no biochemical function was ascribed to GPα1. Some heterotrimeric G proteins are ADP-ribosylated by the cholera or pertussis bacterial toxins. We expressed GPα1 in insect cells using the bacculovirus expression system. The cell extracts were tested for ADP-ribosylation by cholera toxin. In cells expressing GPα1, an ADP-ribosylated product of the size of the overexpressed GPα1 protein (45 kD) was observed when the cell extracts were supplemented with plant cell extracts. This product could be immunoprecipitated with antibodies directed against GPα1. The ADP-ribosylation result strongly suggests that the biochemical function of GPα1 is indeed related to animal G protein α subunits.

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STRUCTURE AND COMPUTATION

Scientists in this section are engaged in basic research and methodology development in gene discovery and in gene and protein functional analysis. The program is comprehensive and has major programs both in the experimental and computational aspects of gene discovery and in the analysis and prediction of gene and protein structure and function. The main scientific thrust and overall goals of this section are to develop and apply methods that

- establish chromosomal position of heritable disease genes by linkage and segregation analysis of affected families
- automate high-speed DNA sequencing over long contiguous regions of genomic DNA
- automate the detection of segments of genomic DNA sequences that have specific biologic content
- predict gene function by homology/analogy to existing sequences
- isolate and characterize minuscule amounts of proteins for cloning genes directly
- use X-ray diffraction patterns with crystalline proteins to determine their three-dimensional structure with the goal of understanding their function

COMPUTATION

T. Marr W. Chang, S. Cozza, D. Cuddihy, M. Hiller, R. Koskela, W. Li, S. Lincoln, M. Mallison, C. Reed, J. Salit, A. Tracy

In addition to the basic research in genome informatics done in this program, we have had a major software development project for several years leading to a computer program that we call Genome Topographer (GT). GT has been designed to be a general-purpose scientific computing system used to study complex problems in human genetics and disease, such as cancer, diabetes, psychiatric disorders, and infectious diseases. A major design goal with GT was to build a computer system that could support such studies from start to finish, i.e., from epidemiologic and genetic analyses to functional analysis of candidate genes.

We now have established much of the basic infrastructure of this computer system, and it is now being used for part of its intended purposes. For example, GT is being used as an integral part of the Dana Foundation Consortium on the Genetic Basis of Manic Depressive Disorder project described elsewhere herein. With the basic software infrastructure in place, we have begun to add some of the sophisticated analysis programs that have been developed in the Computation Program for sequence analysis (e.g., see W. Chang's report) and for genetic linkage analysis.

GT enables users to do the following: (1) Query, gather, and systematically organize data from all of the major publicly available genome databases containing genetic and physical mapping data, including the nascent data required to construct and interpret the maps (e.g., pedigree and genotyping data), and DNA and protein sequences. GT can also be used to store relevant data as gleaned from the scientific literature or from patient interviews, for example, using the powerful editing and viewing tools found in GT. (2) Customize and integrate GT, using network-based collaboration tools, into their own local laboratory operations, including direct interfacing into laboratory instruments for automated data gathering. (3) Manipulate, perform sophisticated analyses, and visualize all data stored in the database in informative ways. Taken together, these features allow users to

construct, with relative ease, on-line databases of the primary data needed to study a genetic disease from the stage of family collection and diagnostic ascertainment through cloning and functional analysis of candidate genes, including mutational analysis and screening for biochemical interactions with candidate molecules.

Software Developments Resulting from the Dana Foundation Manic Depressive Disorder Consortium

R. Koskela

The major role of CSH in this consortium includes overall project management, data merging, data analysis, and error checking. One of the major challenges associated with this project is that genotyping data (using three different experimental approaches) are being generated at each of the three sites involved in the project (see description below). Another challenge is keeping the variety of data up to date and consistent across the three sites. The variety of data includes (1) family information and associated clinical and diagnostic data, (2) DNA samples from the families, and (3) genotypes of hundreds of individuals using hundreds of polymorphic markers. We also have had to develop methods and software for automated error checking, including allele calling and consistency checking. We have also developed methods for transforming the different allele measurement systems into a standard form and for transforming our standard form into formats needed to perform a variety of sophisticated genetic analyses.

We have used the experiences gained in working on this project to design and develop general methods and computer representations for implementation in the GT system. Therefore, the software in the GT system will be generally applicable to the study of complex diseases, including, for example, cancer, heart disease, and infectious diseases.

Sequence Analyst

W. Chang, D. Cuddihy, T. Marr

Sequence Analyst is a powerful computer program for sequence analysis that has two completely novel aspects: the search methodology and the visualization methodology. Both are the direct results of our experience and research in biological sequence analysis. We released version 1 in March, 1995, and are incorporating it into the Genome Topographer.

The goal of sequence analysis is to aid the interpretation of biological data and to suggest further experiments. The fundamental problem of protein sequence comparison is to judge whether two or more sequences share structural and/or functional features, based on similarities observed in their amino acid sequences. Current understanding of the underlying processes of structure and function is not sufficient for a completely rigorous solution, but two developments in particular have combined to produce methods that are reasonably rigorous and successful: assigning a score to a single aligned pair of residues, according to chemical properties or statistical analysis of allowed mutations in known homologous sequences (Dayhoff et al., Atlas of Protein Sequence and Structure, vol. 5 [suppl. 3] 345–352 [1979]) and the alignment of regions by dynamic programming (Smith and Waterman, *J. Mol. Biol. 147:* 195 [1981]; Gotoh, *J. Mol. Biol. 162:* 705 [1982]). There is an important distinction between algorithms and heuristics. Algorithms such as Smith-Waterman and Gotoh solve rigorously a clearly defined problem, namely, finding the highest scoring local alignment between two sequences. Heuristics, on the other hand, do not typically provide performance guarantees (controls). Precisely because we do not fully understand the biology, we should try to eliminate variables and unknowns that are introduced by heuristics. For example, BLAST (Altschul et al., *J. Mol. Biol. 215:* 403 [1990]) and FASTA (Pearson, *Genomics 11:* 635 [1991]) work well when sequences are highly similar, but both will introduce poorly understood artifacts (false positives and negatives) when searching for distant alignments. Sequence Analyst is an algorithm that solves what we feel is the "right" problem: find every (sufficiently long) alignment that has the proper evolutionary distance as defined by the scoring function. We describe below the PAM model of protein evolution because it is the basis of protein similarity search and provides the rationale for our new search method.

The widely used PAM scoring matrices (Dayhoff et al. need reference) were calculated on the basis of 1600 accepted point mutations in 71 groups of closely related proteins. Qualitatively, each matrix reflects the intrinsic chemical classification of the 20 amino acids. Quantitatively, this model of amino acid substitution assumes that further nonlethal mutations fol-

low the same rate and distribution, extrapolated to distantly related proteins. PAM stands for Point Accepted Mutation where "point" means percent. Thus,

1 PAM = The evolutionary process of mutating one percent of residues
90 PAM 50% identity over putative domain (usually significant)
120 PAM 33% identity (sometimes significant)
250 PAM 20% identity (rarely significant)

Generally, 50% identity over a putative domain (not the entire sequences) is considered extremely significant; in the PAM scale, this corresponds to about 90 PAMs. Occasionally, a particular 33% identity (120 PAMs) alignment is claimed to be significant. The key distinction is that xPAMs is an evolutionary distance, whereas PAMx is a (related) scoring matrix used to find xPAM alignments. More precisely, PAMx scores measure the likelihood that the aligned portions of two sequences are xPAMs apart evolutionarily (Altschul, *J. Mol. Biol. 219:* 555 [1991]). The mathematics is quite simple and intuitive:

> Amino acid substitution matrices are devices for distinguishing a target frequency of aligned pairs from a background frequency of random pairs. Let p(x) denote the frequency of amino acid x. Then p(x)p(y) is the frequency of observing the pairing (x,y) by chance. Let q(x,y) denote the frequency of observing the pairing (x,y) among alignments that are the target of matrix S. Then q(x,y)/p(x)p(y) gives the ODDS that the observed pairing (x,y) comes from the target as opposed to chance. The score of pairing (x,y) has the form of a log-odds score:
>
> $$S(x,y) = \log q(x,y)/p(x)p(y)$$
>
> The score of an aligned block is the sum of the individual pair scores. (Taking logarithm simplifies calculations, by converting multiplication of odds to addition of log-odds.) Because this is an equation, the q(x,y) target frequencies can be calculated from the matrix S, and vice versa.

Indeed, PAMx (scoring matrix) detects xPAM (evolutionary distance) optimally. But what does it mean to score a yPAM alignment using PAMx, if y is very different from x? The score would not be useful, because it only gives the likelihood that the yPAM alignment is xPAM, not the likelihood that it is "good." One has to search databases with multiple PAMs and reject those alignments that do not fit the particular matrix used. The selection criterion for PAMx has two components: An alignment has to score at least 30 "bits" because there are many chance alignments with that score; furthermore, the UNIT SCORE of an alignment (average score per position) should be close to the expected unit score of an xPAM (target) alignment, which can be calculated:

PAM distance	Percent identity	Expected unit score (bits)	Minimum length to yield 30 bits
40	70	2.3	14
80	55	1.4	21
120	33	1.0	31
250	20	.36	83

One would like to search separately at each PAM (40, 80, 120; with various gap penalties) for all sufficiently long alignments with a given unit score; unfortunately no previous method will work with a unit score constraint. There are artifacts such as short, high unit score alignments and long, low unit score alignments that will cause dynamic programming (Smith-Waterman-Gotoh, which finds the highest scoring alignment without regard to unit score) to miss real targets; it is too late to comb through the output after a search is done. Examples of artifacts:

```
Scoring matrix: PAM40  Gap-Open Penalty: 22  Gap-Extension Penalty: 4
Similarity Score : 19  Match Percentage : 100%

P53  42  DLMLS
         |||||
BRC1 749 DLMLS

P53                                                              285
  EEENLRKKGEPHHELPPGSTKRALPNNTSSSPQPKKKPLDGEYFTLQIRG
  |:: :|  |::  ::  ::  ::|:|  |
BRC1                                                             418
  EVDEYSGSSEKIDLLASDPHEALICKSDRVHSKSVESNIEGQIFGKTYRK

P53                                                              335
  RERFEMFRELNEALELKDAQAGKEPGGSRAHSSHLKSKKGQSTSRH
  : : : | | :  : :  |:  | | |: :: |
BRC1                                                             468
  KASLPNLSHVTENLIIXAFVSEPQIIQERPLTNKLKRKRRPTSGLH

Scoring matrix: PAM120  Gap-Open Penalty: 22  Gap-Extension Penalty: 4
Similarity Score : 16.5  Match Percentage : 33%

 P53  263 NLLGRNSFEVRVCACPGRDRR
          :| : | | |   | |::
 BRC1 1727 KMLNEHDFEVRGDVVNGRNHQ
```

This last alignment has the right unit score for PAM120 but is too short.

We have succeeded in developing an algorithm that is rigorous and very fast up to PAM120 (Chang and Marr 1994). In fact, the time needed to do all three PAMs (40, 80, 120) is comparable to a single Smith-Waterman-Gotoh (best uniprocessor implementation), about 15 minutes for a 400-residue query sequence. It is fast because each separate search

focuses on a narrow and well-characterized class of alignments. Sequence Analyst spends most of its time at PAM120, because distant alignments are more difficult to discover. (If a program takes the same amount of time to find close and distant alignments, then it must be wasting its effort.) Even on relatively inexpensive workstations (as opposed to massively parallel computers with thousands of processors), it is now quite feasible to search routinely at multiple PAMs and various gap penalties. Compared to Smith-Waterman-Gotoh, the only loss seems to be the artifacts. It also works very well for DNA or cDNA search at the 15% error level (allowing gaps). This algorithm incorporates a key subroutine called "maximal unit score targets" (MUST) and extends previous work by W. Chang on string matching (Chang and Lawler, *Algorithmica 12:* 327 [1994]; Chang and Lampe, *Proc. Symp. Combinatorial Pattern Matching,* Springer-Verlag Lecture Notes in Computer Science 644:172-181 [1992]). Here is a brief outline of the algorithm:

- Calculate and tabulate 3.2 million pentamer scores—best match between each pentamer and the query sequence (allowing gaps).
- This gives upper bounds on real alignment scores for each pentamer.
- Given a database sequence, look up scores of pentamers and solve rigorously the one-dimensional problem, i.e., find all sufficiently long regions with a given unit score (upper bound).
- Apply dynamic programming only to those regions (5–25% depending on PAM).
- This works if the target unit score is greater than the pentamer noise.
- Query sequence may have to be partitioned into overlapping blocks of size 40–200 depending on the PAM.

Finally, we will say a few words about the user interface. A similarity search of a protein sequence against a large database can produce a massive amount of data that must be scrutinized for useful information. Therefore, one needs both a rigorous analytical engine that produces fewer artifacts and a graphical user interface that is concise and effective. It should make it possible for one to scan the data in a few minutes, with just a small number of keystrokes or mouse-clicks. Related alignments should appear together on screen. Version 1 of Sequence Analyst provides a simple, clean interface called Graph that is based on character graphics (Unix curses); it works well through both network and dial-up modem connections. Version 2 will be an integral part of Genome Topographer and will have more informative graphics as well as mouse drag-and-drop, etc. The architecture of Sequence Analyst is modular—one can feed to Graph a BLAST output file (through a filter that also removes redundancies, a serious problem with BLAST). It will become fully object-oriented inside Genome Topographer. The same interface and visualization will work for a protein or DNA database search (many-to-one alignment), multiple alignment, degenerate PCR (multiple protein fragments against DNA or cDNA databases), DNA sequencing contigs, and physical maps of clone libraries such as our map of fission yeast (Mizukami et al., *Cell 73:* 121 [1993]). Sequence Analyst is currently the test case for several extensions to GT, interfacing external analytical tools and building experiment-protocol classes.

Dana Foundation Consortium on the Genetic Basis of Manic Depressive Disorder

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The Dana Foundation Consortium on the Genetic Basis of Manic Depressive Disorder (MDD) is an exciting new multi-institutional project dedicated to finding genes involved in susceptibility to MDD. This is the first major program at CSHL involving clinicians, computers scientists, human geneticists, and quantitative biologists.

MDD is a serious psychiatric disorder, with significant evidence of a major hereditary component, which is characterized by extraordinary differences in an affected person's mood. The mood swings experienced by a person with one form of the disease can range from extreme elation and unrealistic self-confidence in one's abilities, or mania, at one time to extreme dispair and depression at another time. These patients suffer from the bipolar affective disorder

type of MDD. Many people suffer from another form of this disorder wherein they cycle from periods of feeling "normal" to periods of depression, without the "highs." These patients suffer from the recurrent unipolar depression form of the disorder. Typically, these feelings cycle during an affected person's lifetime, sometimes weekly and sometimes at longer periods of time. Untreated, the affects of the disease usually become more intense and unpleasant, leading to an extraordinary statistic that 60–80% of all suicides of adolescents and adults suffered from these disorders. Estimates of the overall incidence of these disorders put at risk about 2–5% of the population. The costs of the illness to individuals who have it, to their family members, and to society cannot be overstated; it is closely associated not only with suicide, but also with violence, alcohol, and substance abuse, greatly elevated medical costs, and lost productivity.

The Dana Foundation Consortium draws upon unique skills at three institutions: informatics and quantitative analysis and Cold Spring Harbor, psychiatric diagnostics and clinical psychiatry at Johns Hopkins, and human genetics and laboratory analysis at Stanford. The consortium is using a genomic approach to the problem of finding genes involved in MDD, made possible by the Human Genome Program. We are involved in a genome-wide scan for susceptibility genes in the DNAs from approximately 50 families with individuals suffering from MDD.

Full time roles of people at CSHL include overall project management, data analysis and error checking, and genetic analysis system requirements (R. Koskela, described in Marr Lab section) and development of automated, high-speed genotyping capabilities (H. Feilotter, described below).

Genotyping Laboratory at Cold Spring Harbor

H. Feilotter

During 1994, Cold Spring Harbor initiated a genotyping effort to complement the genotyping being done by both of the collaborating groups. Genotyping is a method used to follow the transmission of chromosomes through generations within families. Specific chromosomal fragments can by identified by the use of highly polymorphic markers, which can be used to differentiate between maternal and paternal chromosomal segments. Linkage between any marker and the disease phenotype is detected as the co-segregation of the disease phenotype with a particular chromosomal fragment within a family more often than expected by chance alone. The markers used for these studies are short tandem repeats that are densely scattered throughout the human genome and that exhibit a high degree of polymorphism because of the addition or subtraction of repeat units within the genome. Maps showing the localization of specific markers to the chromosomes and information about the surrounding sequence for use in PCRs are publicly available and are widely used as a resource for genotyping studies.

Making use of these maps and the DNA from the 31 families that have been ascertained by the clinical team at Johns Hopkins Hospital, genotyping at Cold Spring Harbor is being designed to complement the genome-wide scan being carried out by the collaborating groups at Johns Hopkins and Stanford Universities. The genotyping technology being implemented here is fluorescent-based semi-automated genotyping. It makes use of four single-isomer fluorescein dyes that can be coupled to primers. The labeled primers corresponding to specific markers are used in PCRs to amplify the specific polymorphic region from DNA from individuals in the study. The products are separated on acrylamide gels using an ABI 373 PRISM DNA Sequencer and detected as fluorescent signals after excitation by a laser. Information is automatically collected and stored, minimizing the error caused by human transfer of data. The availability of multiple color dyes allows the use of one as a size standard in each lane, increasing the accuracy of allele sizing. The other three colors can be used to visualize different PCR products in the same size range in the same lane. In addition, products of different sizes can be labeled with the same color and visualized in the same lane. This combination allows as many as 12 different markers to be analyzed in a single lane, greatly increasing throughput compared to more traditional genotyping technologies. Sizing of the alleles from each individual is done rapidly and with high accuracy through the use of the interactive software programs GENESCAN 672 and Genotyper DNA Fragment Analysis Software (Applied Biosystems).

The genotyping effort initiated here has two major goals. The first is to provide a rapid and efficient method for completing the genome-wide scan to 10-

cM resolution. The second is to apply this technology to screen more markers in any areas where the preliminary screen has indicated putative linkage to increase the resolution.

Maps showing the position of all markers currently used in the study have been constructed using the Genome Topographer software (in collaboration with T. Marr's laboratory) to provide a clear representation of the degree of genome coverage already achieved. A total of 34 gaps greater than 20 cM remain. Initial genotyping will be aimed at filling these gaps. Once the gaps are filled, the next stage of the genotyping project will focus on achieving more dense coverage in regions of putative linkage.

Currently, the genotyping operation has been initiated in collaboration with the laboratory of Dr. R. McCombie, who has provided the 373 Sequencers for use. Initial genotyping has been carried out with a set of 15 markers that were developed at Johns Hopkins Hospital by Dr. R. Levitt. Initial estimates of through-put and accuracy based on preliminary results suggest that this system will support the production of more than 1300 genotypes per week with very high reproducibility.

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GENOME SEQUENCE ANALYSIS

W.R. McCombie N. Kaplan A. Johnson
M. Lohdi A. Melnikov

The long-term objective of our lab is to apply powerful new technologies to analyze the structure and function of eukaryotic genomes. This program is divided into technology development and sequence analysis components. The technology development effort functions to minimize the cost and maximize the speed of genome sequencing. The sequence analysis effort applies the best available technology at any given time toward determining the structure of genomic regions of interest, particularly that of the model organism *Schizosaccharomyces pombe.* We have also this year begun sequencing from the plant model organism *Arabidopsis* and from the human genome.

Technology Development

M. Lohdi, A. Johnson, N. Kaplan, A. Melnikov, W.R. McCombie

Typical DNA sequencing reactions are primed with an oligonucleotide of 17–24 bases in length. Oligonucleotides of this length are usually present only once in a genome, so when a primer is synthesized for a sequencing reaction, it is only used once for sequencing that region and has no other use. The typical oligonucleotide synthesizer makes enough material to prime hundreds of reactions and thus much money is wasted when primer walking is

used for sequencing. Moreover, the problems of coordinating oligonucleotide synthesis and sequencing reactions make managing a sequencing project using this strategy very difficult once a scale-up in the number of reactions is done. One way to avoid many of these problems is to use three adjacent hexamers that comprise an 18-mer as a primer, rather than the 16-mer itself. Since a given hexamer is present many times in the genome, each can be used as part of different strings and can hence be used more than once. Moreover, since there are only 4096 possible hexamers, they can be synthesized in advance and the correct set of three chosen to make any primer.

This was originally developed for manual, radioactive sequencing by Kieleczawa et al. (*Science 258:* 1787 [1992]). We have been attempting to develop the use of these hexamer strings on ABI sequencers. In the previous year, we showed considerable progress and were able to achieve modest read-lengths using hexamer primers on single-stranded templates. The signal from these reactions, however, was very weak at the lower limit of what is detectable with the ABI 373 DNA sequencers that we use. To improve signal strength and hence the quality of the sequence data, we began trying to carry out cycle-sequencing reactions. We have been unable to use thermostable DNA polymerases with hexamers because of a requirement for an extension reaction at 4°C. None of the thermostable enzymes have activity at this temperature. We chose to use a thermolabile enzyme and replenish the enzyme after each cycle. This may limit the number of cycles that could be carried out, so we chose to do only three cycles to determine the effect.

Reactions were carried out as originally determined, except that at the end of the 37°C extension, they were heated to 95°C for 2 minutes and cooled to 4°C for 10 minutes and additional Sequenase was added. The samples were then incubated at 37°C for 10 minutes, followed by 2 minutes at 95°C. The samples were once again collected and another cycle was carried out. After a total of three cycles, the samples were prepared for gel analysis and loaded on a sequencing gel. Typical reactions yielded about twice the original strength found with a single reaction cycle. This greatly improved the sequence quality. Whereas 400-base read-lengths were considered good with a single cycle, with multiple cycles, reads of 450–500 bp are common. In some cases, even longer reads were obtained with cycle sequencing in this manner.

We now believe that we have demonstrated that hexamer strings will at least be a useful adjunct to current sequencing technology. It will be very useful for primer walking to sequence small regions of DNA as well as for at least limited gap filling in sequencing larger regions of DNA. We are continuing to test and improve this methodology, and we foresee it having a more important role in ongoing sequencing projects in our lab and others in the upcoming year.

Genome Sequence Analysis

N. Kaplan, M. Lohdi, A. Johnson, A Melnikov,
W.R. McCombie

One of the challenges to large-scale sequencing is scaling up the handling of data and samples. We discussed the establishment of our data handling system in last year's Annual Report. This provides a firm foundation for handling the DNA-sequencing data. This year, we emphasize the scale-up of the sequencing itself by integrating automation into the sequencing process. Two primary instruments were integrated to allow our scale-up of sequencing to begin. We were able to successfully scale-up to running two to three gels per day as a result of this effort.

One instrument is an ABI catalyst that carries out DNA-sequencing reactions on either double- or single-stranded templates. Personnel were trained to operate the instrument, and lab sample-handling procedures were modified to integrate the ABI catalyst into our sequencing stream. As a result, we typically use the catalyst for between 48 and 72 sequencing reactions per day.

The second instrument is a robot that carries out double-stranded DNA template preparation. This is a prototype instrument on loan to us from Qiagen. We were one of the U.S. test sites for this device. Our lab procedures have once again been modified to make the most use of this device, and we have worked with the manufacturer to solve problems with the instrument. This device can now readily provide 96–192 templates per day with minimal human intervention. Since we sequence each of these templates twice, this is enough for 192–384 sequencing reactions per day.

The sequencers themselves have also been upgraded. We changed from 24-cm gels to 34-cm gels for standard runs and now have the capability of running 48-cm gels as well. These have substantially im-

TABLE 1 *S. pombe* Contig Status

Cosmid	Sequence reads	Contig size	(number)	(largest)
PGAA	1012	38,335	(1)	(38,335)
PGAB	532	43,789	(1)	(43,789)
PGAC	618	43,701	(5)	(34,737)
PGAD	486	40,863	(18)	(5,664)
PGAE	574	44,724	(6)	(14,509)

proved sequence read-lengths. The net result of all of these changes is that we now can obtain 450–550 bases routinely off double-stranded templates produced by a robot. This compares to 350–400 that we were getting per read previously, which is a drastic improvement in sequence throughput. This capability has been applied to sequencing a region of the fission yeast genome.

We began last year to sequence three overlapping cosmids from the genome of *S. pombe*. The current status of all of the *S. pombe* cosmids currently in progress is shown in Table 1. This year, we completed a 31-kb contig from the first of these cosmids, PGAA, as well as the shotgun phase of sequencing on the two other cosmids being sequenced. We have been carrying out gap filling and conflict resolution on these additional cosmids, PGAB and PGAC. The contig from cosmid PGAA has a region of about 31 kb, which has been completed. The remaining part of this cosmid, which is proximal to the telomere, has some problems in the assembly and is still being analyzed. Cosmid PGAB has a single large contig, which is partially a cosmid cloning vector. PGAB is missing what is probably a small region at one of the cosmid insert junctions. In addition, a large number of regions of single-stranded coverage and ambiguities in PGAB need to be resolved. Cosmid PGAC has five major contigs; most of this sequence is, however, present in one 34-kb contig. Some conflict resolution and joining of the remaining contigs remain with PGAC.

We have started sequencing two additional cosmids, PGAD and PGAE. An initial shotgun phase on these cosmids has been completed and a preliminary assembly has been carried out on each. The results indicate that additional shotgun reactions should be carried out on PGAD and PGAE and these will be done in the upcoming year.

We have begun an analysis of the sequence thus far obtained. The initial phase has been to carry out database searches using the *S. pombe* DNA sequence as a query to search public databases. Blastx searches, which translate the *S. pombe* sequence to protein sequence and then search protein databases were carried out. The results showing some possible matches are shown in Table 2. These are genes, the

TABLE 2 Putative *S. pombe* Genes Found by Genomic Sequencing

Amino acid permease
Alanine racemase
Agglutinin attachment subunit
MDR
Aminotriazole resistance
AMP deaminase
β-glucosidase
Hypothetical 33.7-kD protein
Oligo-1,6-glucosidase
High-affinity glucose transporter
Ferric reductase
Isocitrate lyase
Allantoin permease
Nitrogen assimilation tf
Alcohol dehydrogenase
Guanylate kinase
Adenosine deaminase
Hypothetical protein 4B0218
Ubiquitin-conjugating enzyme
Asparaginyl tRNA synthetase
S. cerevisiae chromosome IX protein
DNA-binding protein REB1
cdc20
DTFIIf30
Fructose-1,6 biphosphatase
Sulfated surface glycoprotein
Replication protein A
Membrane-associated protein
Elongation factor EF-G
Neutral trehelase
Mitosis inhibitor protein kinase
Hypothetical 23.3-kD protein
Poly(A)-binding protein
23-kD cortical cytoskeletal-associated protein
p60-related protein
Sorbitol dehydrogenase
α-tubulin
Hypothetical 40.2-kD protein K12H
YBL0520 gene product (egf-1 receptor)
VSP-3 gene product
Ribosomal protein L37a
Myosin heavy chain (NuMA protein)

putative homologs of which were found by our sequencing of the *S. pombe* genome. We are continuing analysis of this region and will intensify our analyses. Once this is completed, an accurate sequence for this region will be obtained. This work has already resulted in more than 30 new genes being found from *S. pombe*.

As a further test of the sequencing capability, we chose to sequence a human cosmid obtained from Mike Wigler and Nikolai Lisitsyn (CSHL). A total of 895 sequence reads were carried out. These were assembled with large contigs following assembly. Gap filling was carried out and the project currently has a small number of gaps of approximately known size and a number of ambiguities that need to be resolved. Initial analysis of this region of the human genome indicates that it is gene-poor. No clear homologies with other genes were found. In addition, Grail analysis for potential coding regions has not been helpful in finding obvious coding regions with similarities to database entries. We will continue analysis of this region, particularly in light of the rapidly expanding public cDNA databases.

We began an effort in collaboration with R. Martienssen and V. Sundaresan here at the Laboratory to sequence sites of insertion of transposable elements in *Arabidopsis thaliana*. Transposed lines were isolated by the Martienssen and Sundaresan labs and cDNA clones containing the transposon were obtained by P. Springer. Several clones of different lengths were sequenced, and the data were used for searching public databases. This revealed that these clones have considerable similarity to the highly conserved MCM2 gene. We sequenced additional overlapping partial cDNA clones provided by Dr. Springer. This provided most of the sequence of the gene. We chose primers and walked through regions of inadequate coverage until the sequence of the entire coding region was obtained.

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PROTEIN CHEMISTRY

R. Kobayashi H. Cai
N. Poppito
B. Schwender

Two new people joined our group this year. Hui-Zhi Cai is a post-doc who earned her Ph.D in chemistry at the University of Basel, Switzerland. She will be working primarily on methods development. Nora Poppito had been working with Dan Marshak on peptide synthesis and transferred to my group to assist us with protein sequencing. Brian Schwender continued work with yeast protein database construction in two-dimensional gels within the QUEST project but left at the end of the year when the project come to an end. Brian is now at Massachusetts General Hospital.

Our task at Cold Spring Harbor Laboratory is to sequence proteins, and thus a major part of the research is in collaboration with many other scientists here at the Laboratory. In addition, we have been trying to develop a new method for micro-characterization of protein, especially a protein-sequencing method.

Yeast Protein Database Construction

B.J. Schwender, R. Kobayashi [in collaboration with J.I. Garrels, B. Futcher, G.I. Latter, Cold Spring Harbor Laboratory]

Yeast *Saccharomyces cerevisiae* protein database construction in two-dimensional (2D) gels as part of the Quest project has continued this year. We have been identifying protein spots on the 2D gels by analyzing their internal amino acid sequences. In this manner, we can determine from the sequence relatively abundant proteins; however, some of the proteins turned out to be unknown. By the end of the year, we identified 38 proteins including 5 unknown proteins.

Protein Sequencing

N. Poppito, R. Kobayashi

The collaboration with other scientists at Cold Spring Harbor Laboratory is a major activity in our laboratory. In April, Nora Poppito started working in the protein-sequencing section of the protein chemistry core facility. This summer, the Association of Biomolecular Resource Facilities (ABRF) sponsored a comparison of the three, different, most widely used methods for internal sequence analysis by five different facilities at Harvard University, Yale University, Rockefeller University, Wistar Institute, and Cold Spring Harbor Laboratory. These methods are in-gel digestion, blotting to PVDF or NC membranes after gel electrophoresis, and enzymatic digestion. Our in-gel method proved to be the most sensitive in terms of peptide recovery. Since internal sequence analysis of protein has an essential role in the strategy of cloning a particular gene, this technique has been extensively used at Cold Spring Harbor since 1992 and has been very successful. Much attention has been paid to telomerase in cancer research. This year, protein components of *Tetrahymena* telomerase have been sequenced in collaboration with Dr. Carol Greider's lab. Cold Spring Harbor Laboratory is the first to sequence the protein components and clone the gene of this important enzyme.

Methodological Study of Protein Sequence Analysis

H. Cai, R. Kobayashi

This project was initiated to develop a more sensitive method than Edman degradation by utilizing highly sensitive chemiluminescence detection. Chemiluminescence is a luminescence induced by chemical reaction. To use the chemiluminescence detection system, chemiluminergic (fluorescence) compounds must be introduced into sequencing products. We finally synthesized a fluorescent isothiocyanate to replace phenylisothiocyanate which is used in Edman degradation. We are currently examining this new reagent to determine whether it can be used for protein sequencing.

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MACROMOLECULAR CRYSTALLOGRAPHY

X. Cheng O. Antar, C. Casciato, J. Horton, Y. Liu, T. Malone, K. McCloy, M. O'Gara, R. Xu

The goal of our group is to determine the structures of a number of biologically important proteins to atomic resolution to understand better their functions in cell processes. Our studies are designed to benefit from and to complement the genetic and biochemical studies by other groups at Cold Spring Harbor Laboratory. During the year of 1994, several new projects have been initiated that resulted from the interactions between the members of our group and other members of the Laboratory or outside of the Laboratory. Below are short summaries of accomplishments we made during the past year.

Cell Cycle Protein Regulators

J. Horton, R. Xu, O. Antar, T. Malone [in collaboration with G. Hannon, H. Zhang, D. Beach, S. Waga, and B. Stillman, Cold Spring Harbor Laboratory]

We continue our quest to understand the underlying molecular mechanisms of regulation and activity of proteins that control the proliferation and differentiation of eukaryotic cells. Unto this end, we have chosen to concentrate our efforts on determining the three-dimensional structures of several cyclin-depen-

dent-kinase inhibitors (CDIs). CDIs appear to be potent regulators of cell proliferation: When their mediation is absent or hindered, neogenesis ensues.

Before crystallization and structural determination can occur, large amounts (tens of milligrams) of pure, homogeneous protein are needed. This first step is often a deterrent in structure analysis, and it has been particularly troublesome in our study of CDIs. Last year, several recombinant systems for high expression of p16, a CDI whose activity appears to be absent in many human tumors, were evaluated. Each expression system yielded no or little protein. This year, in collaboration with G. Hannon in David Beach's laboratory, good expression of a GST-p16 fusion protein was obtained that contains a thrombin cleavage site. After digesting the fusion protein, and after a few additional purification steps, more than 5 mg of very pure p16 per liter of *Escherichia coli* culture was attained. This expression has finally allowed us to do an extensive screening for crystallization conditions of p16. Although thin, needle-like crystals have been observed under certain conditions, they are not suitable for X-ray diffraction analysis. We are following several leads to grow larger p16 crystals under similar conditions.

Additionally, expression systems that would allow us to obtain large, soluble amounts of two other CDIs, p21 and p27, have been explored. A new construct (by S. Waga in Bruce Stillman's laboratory) has been prepared that appears to have increased p21 expression levels to an appropriate amount for purification and crystal screening. A GST-p27 fusion protein has also been expressed to a high level (by H. Zhang in David Beach's laboratory). A fast-screen search for crystals of this protein has also been initiated.

The DNA-binding Domain of MBP1, a Eukaryotic Transcription Factor

R. Xu, T. Malone [in collaboration with C. Koch and K. Nasmyth, Institute of Molecular Pathology, Vienna, Austria]

In eukaryotic organisms, genes involved in DNA replication are often subject to some form of cell cycle control. In the yeast *Saccharomyces cerevisiae*, most genes involved in DNA replication are transcribed transiently during late G_1 as cells become committed to enter a new cell cycle at Start. Their promoters all contain one or more versions of an 8-base-pair motif (ACGCGTNA) containing an *Mlu*I restriction enzyme site (bold-face) and called the *Mlu*I cell cycle box (MCB). A transcription factor, called MCB-binding factor (MBF), is composed of two proteins, Swi6 and Mbp1. MBF activates the transcription of genes encoding S-phase proteins by binding their MCB via Mbp1.

Mbp1 is a 120-kD protein and its amino-terminal 124 amino acids bind MCB elements (Koch et al., *Science 261:* 1551 [1993]). The sequence of this DNA-binding domain is not related to previously described DNA-binding motifs. To facilitate structural study of the DNA-binding domain of Mbp1, termed Mbp1Δ(1-124), an overexpression strain was constructed by C. Koch and K. Nasmyth. A 16-residue peptide (MARRASVGTDHHHHHH) was fused amino-terminal to Mbp1Δ(1-124), which allows affinity purification on immobilized nickel columns via the six histidine residues.

Initial conditions for crystallization were searched using a sparse matrix screening method by the hanging-drop vapor diffusion technique. Single crystals were grown at 16°C, and they reached their maximum size of approximately 0.2–0.3-mm long after MONTHS. Luckily, the crystals diffract X-rays to high resolution beyond 2.0 Å. Crystals were of a tetragonal space group and had unit-cell dimensions of $a = b = 43.8$ Å, $c = 124.9$ Å. From the molecular mass of polyhistidine-tagged MBP1Δ(1-124) and unit cell parameters, we calculated the crystal's packing density (1.85 Å^3/dalton) and the solvent content (34%). After a failure in isolating conventional isomorphous heavy atom derivatives, we attempted to apply the phasing method of multiwavelength anomalous diffraction (MAD). The method requires the use of a synchrotron radiation source to select a wavelength associated with a particular resonant electronic transition. The experiment also requires anomalous scattering centers. The polyhistidine-tagged Mbp1Δ(1-124) contains three methionines at positions −16, 1, and 26. We replaced the methionines in the protein with selenomethionine by overexpressing the protein in minimal medium that supplies selenomethionine to obtain the Se-containing proteins (and crystals) for MAD analysis (Hendrickson et al., *EMBO J. 9:* 1665 [1990]). This technique was pioneered by J. Horton of this group during his graduate study in Wayne Hendrickson's laboratory (Columbia University). Peaks in Figure 1 correspond to Se sites in one unit cell.

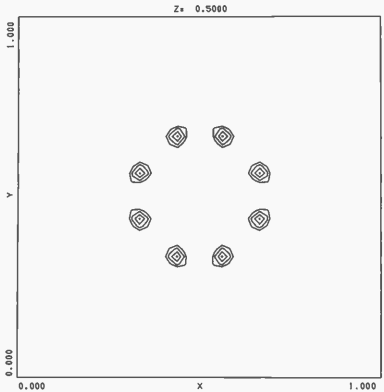

FIGURE 1 The Harker section from an isomorphous Patterson map. The map was computed from differences between Se-met data measured at the edge wavelength (0.979 Å) of Se K-edge and remote wavelength (0.950 Å) at beamline X12C of National Synchrotron Light Source, Brookhaven National Laboratory. The map was contoured in increments of 1.0 σ, starting at 2.5 σ.

A three-dimensional crystal structure of VP16 will not only shed light on the mechanism of VP16 function, but also facilitate further biochemical characterization of the protein.

A construct encoding a GST fusion with the central region (amino acids 48–412) of VP16 that is sufficient for VP16-induced complex formation but lacks the activation domain has been made by C. Huang in Winship Herr's laboratory. We carried out large-scale production of the protein for crystallography. The fusion protein was purified on a glutathione-agarose column, and VP16 was released from the fusion by thrombin digestion at the junction between the GST and VP16 sequences. Thrombin and VP16 were then separated by Q-Sepharose column chromatography. Purified VP16 was crystallized by the hanging-drop method into rod-like crystals with an average dimension of 0.2 x 0.2 x 1.0 mm^3 at 16°C (Fig. 2).

Preliminary results indicate that the VP16 protein crystallizes into an orthorhombic space group with unit cell dimensions of a = 60 Å, b = 77 Å, c = 83 Å. The crystals diffract X-rays beyond 3 Å. Conventional screening of heavy atom derivatives and X-ray data collections are under way.

Viral Protein VP16

Y. Liu, K. McCloy [in collaboration with C. Huang and W. Herr, Cold Spring Harbor Laboratory]

The herpes simplex virus (HSV)-encoded protein VP16 is not only an essential structural component of the HSV viron, but also the activator of HSV immediate-early (IE) gene expression. VP16 is one of the most potent transcriptional activators identified in eukaryotes. It forms a multiprotein complex with cellular proteins Oct-1 and HCF to activate viral IE gene transcription by binding to TAATGARAT elements in the IE promoters. This binding enables the activation domain of VP16 to interact with the basal transcriptional machinery and activate transcription of the viral IE genes. Therefore, structural studies of VP16 will lead to an understanding of multiprotein-DNA interactions involved in transcriptional regulation. Since transcriptional regulation is intimately related to cell growth, cell cycle control, and oncogenesis, VP16 has been a subject of intensive studies.

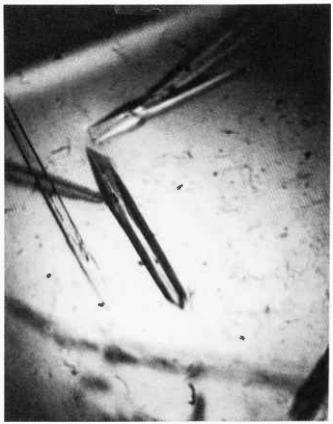

FIGURE 2 Crystals of VP16. Demensions: 0.48 x 0.07 mm.

Casein Kinase I

R. Xu, T. Malone [in collaboration with J. Kuret, Molecular Geriatrics Corp.]

Casein kinase I (CKI) is a serine/threonine-specific protein kinase that is ubiquitous in eukaryotic organisms. Last year, we reported the structure of the catalytic domain of CKI from *Schizosaccharomyces pombe* in complex with MgATP at 2.0 Å resolution. The crystals were formed in a trigonal space group ($P3_221$). This year, we solved the same binary complex in an orthorhombic space group ($P2_12_12_1$) by molecular replacement.

Casein Kinase II

J. Horton [in collaboration with D. Marshak, Cold Spring Harbor Laboratory]

Casein kinase II (CKII) is involved in the phosphorylation cascade originating with the cdc2 kinase, one of the most important macromolecular control sites of the cell cycle, and participates in mitogenic signaling, particularly in neural development. Last year, the growth of three different crystal forms of the regulatory subunit (CKIIβ) of the CKII kinase was reported: One is of the full-length protein (residues 1–215), one of a truncated form (residues 1–189), and another contains a mixture of both full-length and truncated proteins. The truncation appears to occur as the result of some unknown protease, and most protein preparations yield a mixture of the two forms that does not produce good crystals. An investigation is under way so that we can reproducibly obtain either the full-length or truncated form during expression and/or protein purification.

To achieve good expression of CKIIα, the catalytic subunit, site-specific mutagenesis at the active site of CKIIα is being done in further collaboration with Dan Marshak's laboratory. Active CKIIα, when expressed in *E. coli*, has a deleterious effect on cell growth. It is for this reason that good expression of an inactive mutant of this kinase is being undertaken. CKIIα has an active site similar to that of other kinases whose three-dimensional structures have been solved, and the mutation should not affect the rest of the structure. We hope to get crystals of CKIIα and reconstituted whole CKII to understand how protein-protein interactions of the subunits affect this kinase's activity and regulation.

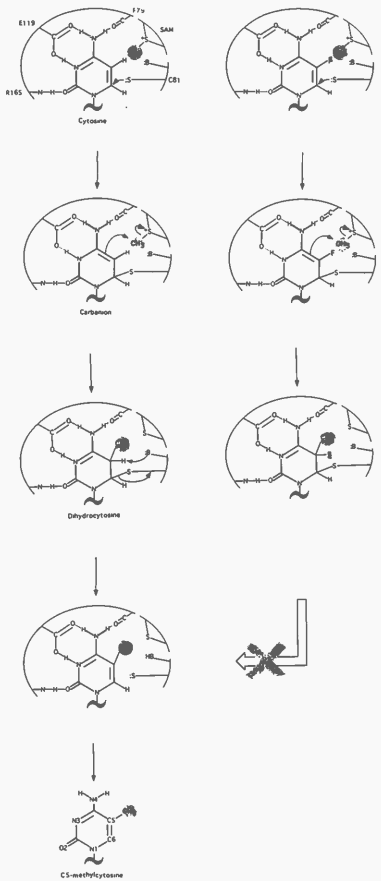

FIGURE 3 Reaction pathway for C5-cytosine methyltransferases based on the mechanism proposed by Wu and Santi (*J. Biol. Chem.* 262: 4778 [1987]), modified by Baker et al. (*Gene 74:* 207 [1988]) and Chen et al. (*J. Am. Chem. Soc. 115:* 5318 [1993]), and confirmed by the crystallographic work on M·*Hha*I. The residues are inferred from the M·*Hha*I structure. A base (:B) that abstracts a proton from carbon-5 is needed for the β-elimination step. A water molecule bound to the side chains of Asn-304 and Gln-82 may serve as the required general base catalyst.

DNA Modification by *Hha*I Methyltransferase

M. O'Gara, K. McCloy [in collaboration with R.J. Roberts, New England Biolabs, Inc.]

In 1993, we solved and published the structure of the M·*Hha*I-AdoMet complex (Cheng et al., *Cell 74:* 299 [1993]). From this binary structure determined at 2.5 Å resolution, we learned that the M·*Hha*I folds into two broad domains: a catalytic domain that contains catalytic and cofactor-binding regions and a DNA-recognition domain. Later, we solved and published the ternary structure for a chemically trapped covalent reaction intermediate between the M·*Hha*I, a duplex 13-mer DNA oligonucleotide containing methylated 5-fluorocytosine at its target, and reaction product AdoHcy (Klimasauskas et al. 1994). This structure determined at 2.8 Å resolution reveals the three-dimensional basis for the functions of amino acids implicated in DNA-sequence specificity, catalysis, and cofactor binding. The DNA is bound in a cleft between the two domains. The structure is the first to demonstrate "an amazing distortion in DNA induced by a methyltransferase," i.e., the substrate nucleotide is completely flipped out of the helix during the modification reaction. The structure also provides a clear example of induced-fit for both protein and the DNA in the sequence-specific substrate binding by M·*Hha*I.

The above ternary structure represents one step of the reaction pathway (Fig. 3, step 3). During the past year, we crystallized, collected, and solved structures of M·*Hha*I with cognate DNA (Fig. 3, step 1) and methylated DNA (Fig. 3, step 4). The latter two structures reveal the role of two conserved residues, Gln-82 and Asn-304 in M·*Hha*I, in the proton elimination step of C5-cytosine methylation. Thus, all of the amino acids implicated in catalysis have been identified from the M·*Hha*I structures.

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NEUROSCIENCE

Neuroscience research at CSHL matured this year to yield significant results. Technological advances and biological discoveries have advanced our understanding of several molecular mechanisms underlying development of behavioral plasticity. As a result, we are a few steps closer to exposing various factors that contribute to cognitive dysfunction. Holly Cline, Roberto Malinow, and their co-workers, neurobiology newcomers in 1994, have developed a technique for delivering foreign proteins to vertebrate neurons. Combining this technique with in vivo imaging, the Cline lab has shown that expression of an altered calcium/calmodulin kinase (CAMKII) produces shorter neurons with fewer branches in the developing visual system. Using similar virus-delivered constructs in electrophysiological experiments, the Malinow lab has produced clear evidence that CAMKII functions during long-term potentiation—a form of synaptic plasticity in vertebrate brains strongly correlated with learning.

At a cellular level of analysis, Hiro Nawa and co-workers have shown that seizure induces persistent expression of a growth factor (BDNF) lasting for several days. This observation implies lasting effects from such neural trauma. Grisha Enikolopov and colleagues have revealed for the first time a clear cellular function for nitric oxide (NO) in the brain. Nerve growth factor (NGF) produces an increase in NO, which then promotes the terminal differentiation of new neurons by inhibiting cell proliferation. Interfering with this process can lead to premature neuronal death.

At a physiological level of analysis, Yi Zhong has shown that the Ras/Raf pathway is activated during synaptic transmission and is required to function along with the cAMP pathway to modulate potassium channels. This discovery has served to blur our conceptual distinctions between "development" and "physiology" in neuronal function. We, in fact, may be studying the same molecular processes only manifested at different times in the life of the neuron.

Finally, Tim Tully and co-workers have revealed a critical function for the transcription factor CREB during the formation of long-term memory (LTM). In essence, CREB appears to act as a "molecular switch." Turning the CREB switch off in transgenic fruit flies suppresses the formation of LTM, although learning and short-term memory remain normal. Turning the CREB switch on, in contrast, enhances the formation of LTM. Consistent with these fruit fly results, Alcino Silva and colleagues have shown impaired long-term memory, but normal short-term memory, in mutant mice carrying a gene knockout of CREB. Taken together, these behavioral analyses of fruit flies and mice suggest the existence of an evolutionarily conserved molecular mechanism underlying the formation of long-term memory.

MOLECULAR NEUROBIOLOGY OF LEARNING AND MEMORY IN *DROSOPHILA*

T. Tully J. Yin, J. DeZazzo, H. Tobin, J. Christensen, H. Zhou, I. Sanchez (URP), C. Jones, G. Bolwig, R. Mihalek, M. Del Vecchio, J. Wallach (M.I.T.), M. Regulski, Z. Asztalos, S. Pinto, D. Wood, N. Arora (Eton College)

Our demonstration last year that a fruit fly homolog (*dCREB2*) of the vertebrate CREB transcription factor functions specifically in the formation of long-term memory (LTM) led us to conceptualize a functional model, in which the opposing functions of CREB (family member) activators and repressors defined two specific behavioral parameters—the number of training sessions and the rest interval be

tween them—required to form maximal LTM. This "C" model yielded three general predictions: (1) No number of massed training sessions (no rest interval between them) will ever induce LTM. (2) Overexpression of CREB repressor will block the LTM normally induced by ten spaced training sessions. (This is the result we reported last year.) (3) Overexpression of CREB activator will *enhance* LTM by reducing the required number of training sessions or the rest interval between them or both.

This year, we have confirmed predictions 1 and 2. Since work in other laboratories already has implicated CREB in LTM formation in mollusks and in mice, our demonstration of enhanced LTM formation by overexpression of a CREB activator is likely to be an evolutionarily conserved function. Thus, CREB becomes the first bona fide mechanistic component of LTM formation against which drug therapies can be developed. Such "cognitive enhancers" promise to ameliorate the memory dysfunction associated with learning disabilities, Alzheimer's disease, and the normal aging process (senile dementia).

composed solely of LTM, which shows no decay from 1 to 7 days after spaced training (Tully et al. 1994).

In the absence of heat shock, one, two, or ten massed training sessions failed to produce any LTM in wild-type flies or in *hsp-dCREB2-a* transgenic flies from two independently derived lines (Fig. 1). Ten spaced training sessions, however, produced maximal levels of LTM in all three lines. These data indicate that memory formation was normal in transgenic flies when the *hsp-dCREB2-a* transgene was not induced.

When flies were trained 3 hours after heat shock, ten spaced training sessions still produced maximal LTM in wild-type and transgenic flies. Moreover, one, two, or ten massed training sessions failed to produce any LTM in wild-type flies. In contrast, one, two, or ten massed training sessions produced maximal LTM in transgenic flies. These data indicate that (1) heat shock itself did not produce any nonspecific effects, (2) memory formation after spaced training still was normal in transgenic flies, and (3) LTM formation was *enhanced* in transgenic flies after heat-shock induction of the *hsp-dCREB2-a* transgene.

dCREB2 as a Modulator of Long-term Memory

T. Tully, J. Yin, M. Del Vecchio, H. Zhou, H. Tobin
[in collaboration with J. Wallach, visiting from the Massachusetts Institute of Technology]

Genetic dissection of long-lasting, consolidated memory has yielded a clear characterization of LTM in fruit flies (Tully et al. 1994). As a result, we have been able to show a specific disruption of LTM by induced expression of a repressor isoform of *dCREB2* (Yin et al. 1994). The *dCREB2* gene is alternatively spliced into both repressor and activator isoforms (J. Yin et al., submitted), and we now have generated transgenic *hsp-dCREB2-a* flies carrying an inducible activator CREB isoform (*dCREB2-a*). Using a procedure identical to that of Yin et al. (1994), we quantified 7-day memory in wild-type or *hsp-dCREB2-a* transgenic flies with or without heat-shock induction of the *dCREB2-a* transgene. Seven-day memory retention is produced only after spaced training in wild-type flies and is completely blocked in *hsp-dCREB2-b* transgenic flies with induced expression of the *dCREB2-b* repressor isoform (Tully et al. 1994; Yin et al. 1994). Thus, 7-day memory is

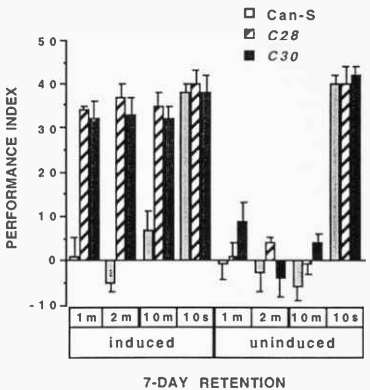

FIGURE 1 Induced expression of a transgene carrying a *dCREB2* activator isoform enhances long-term memory. Seven-day retention in wild-type (Can-S) or uninduced transgenic flies (*C28* or *C30*) is produced normally by spaced (10 sec) but not massed (1, 2, or 10 min) training. Maximal LTM is produced after just one training session (1 min), however, in induced transgenic flies. This effect is the functional equivalent of a "photographic" memory.

The observation that maximal LTM was formed after just one training session is particularly revealing. The usual requirement for multiple training sessions to form LTM was no longer necessary. Thus, induced overexpression of a CREB activator has produced in otherwise normal flies the functional equivalent of a photographic memory.

This enhanced memory effect appeared to be specific to LTM. Untrained transgenic (*C28*) flies' abilities to smell odors (olfactory acuity) or sense and react to electric shock (shock reactivity) were normal 3 hours (equivalent to the time of odor-shock training) or 7 days (equivalent to the time of conditioned odor avoidance testing) after heat-shock induction of *hsp-dCREB2-a* (Fig. 1). In addition, "learning"—determined by measuring conditioned odor avoidance immediately after one training session—was normal in transgenic (*C28*) flies 3 hours (PI + SEM = 83 + 1 for Can-S or 83 + 2 for *C28*; N = 6 PIs per group) or 7 days (PI + SEM = 83 + 2 for Can-S or 84 + 2 for *C28*; N = 6 PIs per group) after heat-shock induction of *hsp-dCREB2-a*. Thus, enhanced memory formation in *hsp-dCREB2-a* transgenic flies was specific to the induction of LTM.

Mammalian CREB must be phosphorylated at Ser-133 to function as a PKA-responsive transcriptional activator. In *Drosophila*, the *dCREB2-a* activator isoform contains a consensus PKA phosphorylation sequence surrounding Ser-231 (J. Yin et al., submitted). Transgenic flies, carrying a mutant activator with an alanine substituted for Ser-231, were generated to test the hypothesis that enhanced LTM formation depended on phosphorylation of the activator transgene. Wild-type (Can-S) flies, flies from two independently derived *hsp-dCREB2-pka* transgenic lines (*1-2* and *2-26*), and flies from an *hsp-dCREB2-a* transgenic line (*C28*) were subjected to one training session 3 hours after heat shock. Subsequent 7-day memory was maximal in transgenic *C28* flies carrying the induced CREB activator isoform but was not detected in wild-type flies (as expected) or in transgenic *1-2* or *2-26* flies carrying the induced CREB mutant activator (data not shown). For flies carrying both the wild-type and mutant CREB activator isoforms, Western blot analysis revealed similar induced levels of expression after heat shock (data not shown). These data indicate that enhanced formation of LTM depends on phosphorylation of the CREB activator transgene.

Taken together, results from these experiments and our previous work (Yin et al. 1994) support a model based on the notion that opposing functions of CREB activators and repressors act as an LTM modulator to determine the parameters of extended training (number of training sessions and rest interval between them) required to form LTM. In its simplest form, this model supposes that associative learning (training) functionally induces both CREB activator and repressor isoforms. Immediately after training, enough CREB repressor exists to block the ability of CREB activator to induce downstream events. Then, CREB repressor isoforms functionally inactivate faster than CREB activator isoforms. In this manner, the net amount of functional activator (C = activators-repressors) increases during a rest interval and then accumulates over multiple spaced training sessions to induce further the downstream targets involved with the formation of LTM.

Conceptually, C defines a quantal amount of CREB activator available after a single training session. For olfactory learning in wild-type flies, this quantal amount presumably is small—even after relatively long rest intervals. As a result, multiple training sessions are required to sum quanta and thereby to produce maximal LTM. This perspective yields an enlightening interpretation of results from transgenic flies with induced expression of *hsp-dCREB2-a:* The quantal size of C after one training session was large enough to induce maximal LTM.

Molecular Cloning of a Ca^{++}-dependent Nitric Oxide Synthase

M. Regulski, T. Tully

In recent years, a novel type of intercellular messenger, nitric oxide (NO) gas, has been shown to be involved with several aspects of developmental plasticity, long-term potentiation, and learning. These reports have been controversial, however, because of the variable and nonspecific effects of pharmacological agonists and antagonists of the enzyme that synthesizes NO, NOS. In an effort to clarify some of these issues, we have cloned a *Drosophila* homolog (*dNOS*) of the vertebrate NOSs.

The *dNOS* message contains one long open reading frame (ORF) of 4350 bp encoding a protein of 1350 amino acids. This conceptual *Drosophila* protein shows a significant degree of amino acid se-

quence homology with the vertebrate NOS proteins: It is 38% identical to rat brain NOS, 36% identical to bovine endothelial NOS, and 28% identical to mouse macrophage NOS. The *Drosophila* sequence contains all the known functional domains of vertebrate NOS: heme, calmodulin, FMN, FAD, and NADPH binding. In the NADPH-binding domain, all amino acids that have been shown to be contact points with the dinucleotide in crystals of ferrodoxin $NADP^+$ reductase are perfectly conserved in the putative *Drosophila* protein. Moreover, the region between the heme- and calmodulin-binding sites is 70% identical between rat and *Drosophila* proteins and is likely involved with binding of arginine and possibly cofactor tetrahydrobiopterine.

Extracts made from *dNOS*-transfected human embryonic kidney 293 cells showed significant NO synthase activity as measured by the standard L-arginine to L-citrulline conversion assay (0.1276 ± 0.002 pmolc/mg/min). (In a parallel experiment, specific activity of the rat neuronal NOS expressed from the same vector in 293 cells was 3.0 ± 0.02 pmole/mg/min, $N = 4$.) DNOS activity was dependent on exogenous Ca^{++}/calmodulin and on NADPH, two cofactors necessary for activity of constitutive mammalian NOSs. DNOS activity was reduced 90% by the Ca^{++} chelator EGTA (data not shown). In addition, 500 mM *N*-(6-aminohexyl)-1-naphthalenesulfonamide (W5), a calmodulin antagonist, diminished DNOS activity to 18% (0.0222 ± 0.001 pmolc/mg/min, $N = 2$). DNOS activity also was blocked by inhibitors of mammalian nitric oxide synthases. N^G-nitro-L-arginine methyl ester (L-NAME) reduced DNOS activity 84%, and 100 mM N^G-monomethyl-L-arginine acetate produced a complete block (data not shown). These enzymatic data demonstrate that DNOS is a Ca^{++}/calmodulin-dependent nitric oxide synthase.

Molecular Genetics of *latheo, linotte, nalyot,* and *amnesiac*

T. Tully, R. Mihalek, S. Pinto, J. Christensen, I. Sanchez, D. Wood, G. Bolwig, J. DeZazzo

We continue to work on these learning and memory genes to understand better their cellular functions and distributions in time and space. In the last year, we have rescued the lethality associated with *latheo* null mutants and have fully rescued the learning and memory deficits associated with the *linotte* mutant by inducing the expression of each gene's corresponding wild-type transcripts in transgenic flies. These results provide conclusive evidence that we have cloned the appropriate transcripts. The *nalyot* transcript has been cloned (partially), sequenced, and identified. It is a helix-turn-helix (HTH) transcription factor called *Adf-1*, which originally was identified as a binding protein in the promoter region of the *Alcohol dehydrogenase* (*Adh*) gene. It also is known to regulate the *Dopa decarboxylase* (*Ddc*) gene, temperature-sensitive alleles of which produce deficits in olfactory learning. We also have discovered a P-element insertion into cytological region 19A, which fails to complement the memory defect of *amnesiac* mutants. This has allowed the resurrection of our attempts to clone the gene. Finally, we have begun neuroanatomical work on brain slices of various mutants. To date, we have established that *linotte* and *nalyot* mutants have normal (gross) adult brain structures. In the upcoming year, work on each of these genes will likely yield significant new molecular information.

Behavioral Properties of Learning and Memory in Normal and Mutant Flies

T. Tully, N. Arora, Z. Asztalos, C. Jones

Our behavioral work continues to yield important "genetic dissections" of learning and memory. We nearly have finished behavioral experiments characterizing olfactory habituation of the jump response in normal and mutant flies. Development of such a non-associative olfactory learning task will allow us to assess whether similar molecular-genetic processes underlie associative and nonassociative forms of learning and memory. We also have confirmed an observation from Dr. M. Heisenberg's lab (University of Würzburg, Germany) that first-instar larvae fed hydroxyurea during a specific 1.5-hour period fail to develop mushroom body structures in the adult brain and fail to show any olfactory associative learning. We extended these results, however, by showing that habituation of the olfactory jump response was normal in mushroom body ablated flies. These data indicate an anatomical dissection of associate and nonassociative learning in fruit flies.

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SIGNAL TRANSDUCTION UNDERLYING NEUROTRANSMISSION

Y. Zhong N.J.D. Wright J. Shanley
H. Zhou C. Kim

We are interested in understanding signal transduction mechanisms underlying neurotransmission and how these mechanisms are involved with or contribute to the cellular basis of learning and memory. The approach we are taking is to first analyze neurotransmission in *Drosophila* learning and memory mutants. Further pharmacological and genetic manipulation may then allow us to elucidate the signal transduction mechanism disrupted by mutations and its role in neurotransmission. On the basis of such an understanding, we may be able to manipulate and mutate other components in the signal transduction pathway. The effects of these genetic alterations on learning and memory can then be tested. Thus, these combined genetic, electrophysiological, and pharmacological studies may lead to a better understanding of cellular mechanisms of *Drosophila* learning and memory.

Currently, we are focusing on neurotransmission coupled by heterotrimeric G proteins. There are probably more than 1000 types of such receptors that have been analyzed up to now. Upon ligand binding, the transmitter receptors interact with heterotrimeric G proteins, allowing G_α and $G_{\beta\gamma}$ subunits to disengage. The G_α subunit is known to modulate the activity of adenylyl cyclase or phospholipase C, resulting in synthesis of second messengers, cAMP, or diacylglycerol and IP3, respectively. These second messengers, in turn, lead to modulation of ion channel activity. In addition, a recent report (Crespo et al., *Nature 369:* 418 [1994]) has indicated that the $G_{\beta\gamma}$ subunit complex activated via muscarinic receptors can stimulate the small guanine-nucleotide-binding protein Ras, leading to activation of mitogen-activated protein kinase (MAPK). This result has raised the possibility that the Ras/MAPK pathway might provide another second-messenger cascade for neurotransmission. We have developed both neuromuscular and central nervous system preparations for examining the roles of these signal transduction mechanisms in *Drosophila* neurotransmission.

Coactivation of cAMP and Ras/Raf Pathways in Peptidergic Transmission

Y. Zhong

We have previously shown that a pituitary adenylyl cyclase-activating polypeptide (PACAP38)-like neuropeptide functions as a neurotransmitter at the larval neuromuscular junction by Western blot, immunohistochemical, and electrophysiological analyses. Either high-frequency stimulation of motor axons or application of PACAP38 induces a similar biphasic muscle response: an inward synaptic current fol-

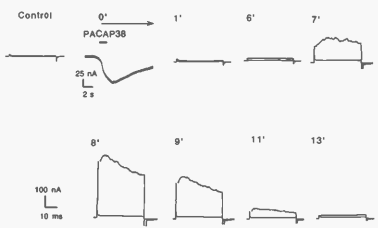

FIGURE 1 PACAP38-induced responses in larval body-wall muscles recorded by voltage clamp. The muscle membrane is held at −80 mV. At 0′ (0 minute), a pulse of 4 μM PACAP38 (as indicated by the bar) is focally applied to the neuromuscular junction, which induces an inward current as the early response. This early inward current lasts for many seconds. The muscle K^+ currents are also monitored before the application and each minute after the application. The command voltages are stepped from −80 to −50 and 0 mV, respectively. Only the leakage current is elicited by the voltage step to −50 mV, but voltage-dependent currents are activated by depolarization to 0 mV. The amplitude of voltage-activated K^+ currents remains relatively stable during the period prior to the application and 6 minutes after application. But a dramatic increase, as the late response, is seen at 7 minutes (7′) after the application, which peaks at 8 minutes and returns to the control level at about 13 minutes.

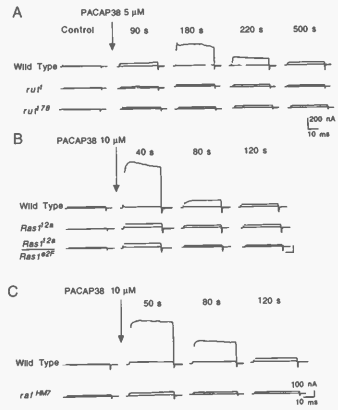

FIGURE 2 Diminished PACAP38 response in *rut*, *Ras1*, and *raf* mutants. (*A*) PACAP38-induced enhancement of voltage-activated K^+ currents is abolished in *rut* mutants. The arrow indicates focal application of PACAP38 (5 μM). (*B*) Enhancement of K^+ currents induced by 10 μM PACAP38 is eliminated in *Ras1* mutants. N = 14, 8, and 10 for wild-type, $Ras1^{12a}/Ras1^{12a}$, and $Ras1^{12a}/Ras1^{e2F}$ larvae. (*C*) Enhancement of voltage-activated K^+ currents induced by PACAP38 (10 μM) is eliminated in *raf* mutants. N = 14 and 10 for wild-type and raf^{HM7} larvae. "Control" current traces were recorded before application of PACAP38. The time in seconds after pressure-ejection of PACAP38 is indicated at the top of the current traces. Data were recorded with the bath saline containing 1 mM Ca^{++}.

lowed by more than 100-fold enhancement of voltage-activated K^+ currents (Fig. 1). For the sake of clarity, we will focus on the mechanisms underlying the enhancement of K^+ currents. Since the vertebrate PACAP38 receptors are known to activate adenylyl cyclase, the effects of *rutabaga* (*rut*) mutations on this PACAP38 response were examined.

The *rut* mutants have been isolated on the basis of poor performance in an associative learning test. The gene encodes a Ca^{++}/CaM-sensitive adenylyl cyclase. The PACAP38-induced enhancement of K^+ currents was eliminated in two *rut* mutants examined (Fig. 2A), suggesting that the cAMP cascade is necessary for this neuropeptide response. However, application of a membrane-permeable cAMP analog was not sufficient to produce the PACAP38-like response. Further analysis revealed that the PACAP38-like response was also defective in *ras1* and *raf* mutants (Fig. 2B,C). Finally, activation of both the cAMP and Ras/Raf pathways was sufficient to induce PACAP38-like enhancement of K^+ currents. Therefore, coactivation of the cAMP and Ras/Raf pathways is suggested to mediate PACAP38-like neuropeptide transmission at the *Drosophila* neuromuscular junction (Fig. 3). These findings establish a role for the Ras/Raf pathway as another signal transduction mechanism, in addition to the cAMP and phosphoinositide pathways, in neurotransmission and neuromodulation.

Cloning the Gene Encoding the PACAP38-like Neuropeptide

H. Zhou [in collaboration with J. Yin and T. Tully, Cold Spring Harbor Laboratory]

As discussed above, the PACAP38-like neuropeptide functions as a transmitter and this peptide-induced transmission is defective in the learning and memory mutant *rut*. Since the PACAP38-like neuropeptide also appears to act in the *Drosophila* central nervous

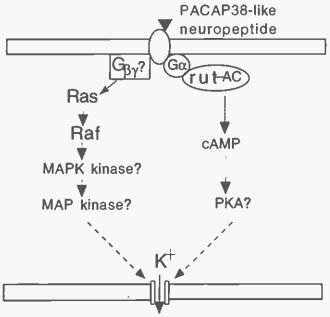

FIGURE 3 A coincident signal transduction model for modulation of K^+ currents induced by a PACAP38-like neuropeptide. Question marks indicate molecules whose function has not yet been tested. Dashed arrows indicate where the cAMP and Ras/Raf pathways may converge; whether such convergence is on channels directly or via an intermediate target remains to be determined.

system, it is possible that this peptide transmitter may be involved in learning or memory. To test this possibility, we will clone the gene encoding this peptide and generate mutants or transgenic flies, which will then allow us to test learning and memory of those flies with an altered expression of this peptide.

We have been able to recover a polymerase chain reaction (PCR) product with primers derived from the amino- and carboxy-terminal amino acid sequences of vertebrate PACAP38 from both *Drosophila* genomic DNA and mRNA. The deduced amino acid sequence of the PCR product matches well in all critical charged residues, which are critical for the function of the peptide. Twelve clones that hybridize with the PCR product have been pulled out of the larval cDNA library. We are now sequencing these clones to determine whether they encode the PACAP38-like neuropeptide.

Neurotransmission in the *Drosophila* Central Nervous System

N. Wright

To understand cellular mechanisms of learning and memory, we will first study the *Drosophila* central nervous system. However, the small-sized *Drosophila* brain and neurons made this task very difficult. As a first step, we have developed a preparation of cultured neurons for studying signal transduction mechanisms of neurotransmission. Neurotransmitters are delivered to patched neurons in culture by a micropipette via either pressure ejection or iontophoresis. The neuronal responses to perfused transmitters are recorded by the patch-clamp method. We have examined a number of transmitters including serotonin, PACAP38, and AmP peptides. AmP is a neuropeptide that may be encoded by the *amnesiac* (*amn*) gene (cloned by M. Feany and W. Quinn at the Massachusetts Institute of Technology). The *amn* mutants disrupt middle-term memory without apparently affecting learning and short-term memory. These preliminary analyses have led to the study of a new type of neurotransmission, as well as underlying signal transduction pathways.

It appears that serotonin, AmP, and PACAP38 can induce similar responses: an outward synaptic current and modulation of voltage-activated K^+ currents (Fig. 4), when applied to patched CNS neurons. We are focusing on the outward synaptic current. Closer examination has revealed that this outward synaptic current is a result of closing "leakage" current channels. This leakage current has a reversal potential of 0 to +10 mV. These channels remain open all the time but are closed in response to application of serotonin, AmP, or PACAP38. The leakage current may have

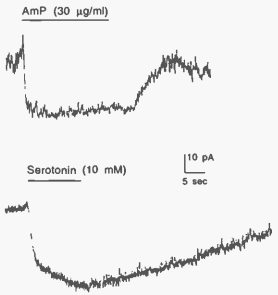

FIGURE 4 AmP neuropeptide- and serotonin-induced responses in cultured *Drosophila* central nervous system neurons. Neurons were clamped at −80 mV. Transmitters were delivered at the periods and concentrations as indicated.

an important modulatory function since it is recorded from all central nervous system neurons and appears to mediate actions of a number of neurotransmitters. We are characterizing the channels gating this leakage current and chasing the gene that possibly codes for these channels. The purpose of these analyses is to lay a foundation for determining how neurotransmission mediated by this leakage current is affected in learning and memory mutants and whether this neurotransmission mechanism can be further manipulated for testing its role in learning and memory.

Plasticity of Nerve Terminal Arborization

C. Kim, J. Shanley, Y. Zhong

In addition to modulation of ion channel activity, signal transduction activated by neurotransmitters may also lead to regulation of gene expression, which may produce a long-term change in nerve terminal arborization or neural connectivity. Such neural plasticity has been hypothesized as a mechanism underlying formation of long-term memory. We have used motor nerve terminal arborization in *Drosophila* as a model to dissect the molecular mechanisms underlying activity-dependent neural plasticity.

It was previously shown that increased neural activity leads to enhanced motor nerve terminal arborization. This activity-dependent plasticity is probably mediated, at least in part, by the cAMP cascade. This is supported by observations that the enhancement of nerve terminal arborization is partially blocked in *rut* mutants that lack Ca^{++}/CaM-activated adenylyl cyclase activity and that the arborization is enhanced in *dunce* mutants, resulting probably from higher cAMP due to disrupted phosphodiesterase activity by the mutations. We hypothesize that neural activity leads to synthesis of cAMP, which then influences nerve terminal arborization by activating transcription factors, cAMP-response-element binding proteins (CREBs), to regulate gene expression. Jerry Yin and Tim Tully here at the Laboratory have made transgenic flies carrying an inducible CREB activator or CREB blocker. We are using these transgenic flies to test whether CREB is required for neural plasticity. Preliminary results are very encouraging, but more extensive analysis is needed for making any conclusions.

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DEVELOPMENT AND MODIFICATION OF NEURONAL CIRCUITS

H. Cline J. Edwards, I. Miloslavskaya, S. Witte, G.-Y. Wu, D.-J. Zou

Neural circuits in the brain can be modified by changing the inputs and electrical activity in the circuit; however, the mechanisms underlying these modifications are only partially understood. One possibility is that neurons add or retract branches in response to different patterns of input activity. Addition or retraction of branches would also mean that the synaptic connections on the branches are added or retracted. To test this hypothesis and to determine how activity-dependent changes in neuronal connections might occur, we take time-lapse images of neurons in the visual system of albino *Xenopus* tadpoles under conditions that modify synaptic activity. This system is particularly well suited for these ex-

periments because the neuronal connections in the visual system are sensitive to the changes in visual stimulation or synaptic activity. In addition, the heads of albino *Xenopus* are virtually transparent so that the central nervous system can be observed in the intact animal.

Single neurons in intact living animals are labeled with fluorescent dyes. We then use in vivo time-lapse confocal microscopy to study the dynamic rearrangements of either the presynaptic retinal axons or the postsynaptic tectal neurons as they elaborate the axonal or dendritic processes or "arbor" within the central nervous system. The morphology of the neurons can then be observed by placing the anesthetized animal on the stage of the microscope. High-resolution images of the neuronal morphology are collected with a laser scanning confocal microscope. This system offers the unique opportunity to observe neurons growing in their normal complex environment.

Observations of single axons taken over a 3-day period show large-scale rearrangements in the shape of the axon arbors. Time-lapse images of growing axons taken at 30-minute or 2-hour intervals show that the net increase in axon branch number that occurs over several days is the result of dynamic addition and retraction of branches, rather than a straightforward addition of branches and the persistence of those new branches. In fact, 90% of the branches that are added to the growing axon are subsequently retracted. During 8 hours of observation, a modest increase is seen in the total number of new branches that reflects the slight imbalance in branch additions versus retractions that occurs at each observation. The modest changes in total branch number seen during 8 hours accumulate to result in the more obvious changes seen over a period of days. The portion of the axon that persists throughout the 8-hour observation, or the "core" structure of the axon, is surprisingly small, whereas the portion that changes from one observation to the next can be 50% of total branch length. Prior to these in vivo observations, we had not recognized the dynamic capacity of neurons to change their structure over such a short time course of hours.

Dynamic Rearrangement of Axon Branches Is Activity-dependent

S. Witte, H. Cline

We tested whether one of the factors controlling the dynamic rates of branch rearrangements in retinal axons is synaptic activity. In support of this hypothesis, we have found that blocking the NMDA type of neurotransmitter receptors on the postsynaptic neurons over 24 hours causes the presynaptic axons to grow larger than normal. Such a change in neuronal morphology could result from a relative

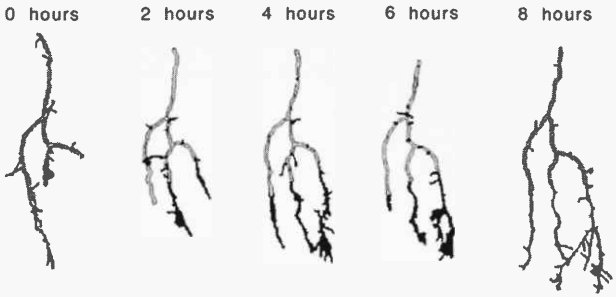

FIGURE 1 Reconstruction of an axon imaged at 2-hr intervals over 8 hr. The portion of the axons that is unchanged over the 8 hr is gray. The portion of the axon that changes from one observation to the next is black. This includes branches that are added or retracted and the portion of branches that elongate or shorten.

decrease in branch retractions or an increase in branch additions. Further observations taken at shorter intervals indicated that blocking the NMDA receptors increased the relative number of branch additions, and this accounts for the net increase in the number of branches in the neuron observed over 24 hours. In addition, blocking the postsynaptic NMDA receptors increased the absolute rates of branch additions and retractions in the presynaptic neurons so that the neuronal structure was more dynamic than normal.

In a separate set of experiments, we have increased NMDA receptor activity in the postsynaptic neurons by synchronizing visual activity with strobe illumination. We predicted that strobe illumination would stabilize neuronal structure and increase the lifetimes of axon branches; however, the results indicate that the influence of synaptic activity on neuronal structure is more complex than our simple hypothesis predicted. Strobe illumination did increase in the lifetimes of axon branches and thereby stabilize neuronal structure, but the increased stability was transient. Early in the course of the 8-hour experiment, branches were stabilized, but later in the experiment they were not. We also observed a change in the distribution of branch additions on the axon. The transient stabilization of branches is best explained by a model in which the probability of synaptic modifications reflects two major variables: (1) the recent history of electrical activity in the neuron and (2) the relative strength of the synaptic stimulation. Early in the experiment, the summed electrical activity in the neuron is low and the relative strength of the input is high, so many synapses and their branches are stabilized. Later in the experiment, when the neurons have experienced 6 hours of synchronized inputs, the summed electrical activity of the neuron is higher. Even though the strength of the inputs is the same, their strength relative to the history of activity in the neuron is now low. Consequently, synapses and branches are not stabilized. The ability to vary the threshold for synaptic modifications could provide the neuron control over the proportion of its contacts which are stabilized.

Taken together, these studies indicate that synaptic activity can clearly modify neuronal structure. Specifically, decreasing activity of the NMDA-type receptor destabilizes the neuronal structure and can cause a net growth, whereas increasing NMDA receptor activity transiently stabilizes neuronal structure and decreases local sprouting in the axon.

Regulation of Neuronal Growth by CaMKII

D.-J. Zou, I. Miloslavskaya, H. Cline

Traditionally, experiments probing the roles of protein kinases have used pharmacological tools to manipulate kinase activity. In previous experiments, we have found that drugs that decreased protein kinase activity in the entire optic tectum resulted in modified growth of the retinal ganglion cell axons; however, these experiments could not resolve between different calcium-sensitive protein kinases, nor could we determine whether the result was due to decreased kinase activity in the retinal axons, tectal cells, or both.

To address both the problem of pharmacological specificity and the need to alter kinase activity in either the pre- or postsynaptic cell population, we have used vaccinia virus as a vector to introduce the gene for the constitutively active truncated calcium-calmodulin kinase II (tCaMKII) into frog neurons. A major advantage of vaccinia virus is that synthesis of foreign proteins does not require insertion of the viral genome into the host genome. Therefore, vaccinia virus can be used to express foreign proteins in postmitotic neurons. Another essential feature of vaccinia virus is that all synthesis of the viral proteins is cytoplasmic and requires the host-cell protein synthesis machinery. In the case of the visual system, this means that the central nervous system can be infected by injection of the virus into the brain ventricle, but the retinal axons will not synthesize foreign protein because they lack polyribosomes. Finally, infections of *Xenopus* larvae with high-titer virus result in intense expression of ectopic protein in the majority of cells within the central nervous system. Therefore, vaccinia virus can be used to express ectopic protein specifically in tectal neurons without expression in the presynaptic retinal ganglion cells.

We have used vaccinia virus to deliver tCaMKII into tectal neurons. Truncated CaMKII, consisting of amino acids 1–290, lacks the regulatory domain of the protein and is constitutively active. Infection of tectal neurons with tCaMKII alters the elaboration of the retinal axon arbor, whereas infection with a control virus, carrying the gene for β-galactosidase (β-gal) does not. In these experiments, single retinal axons were imaged and then either the β-gal virus or the virus containing the genes for β-gal and tCaMKII (tCaMKII virus) was injected in the brain ventricle.

Three days later, the same axon was found in the optic tectum, and confocal optical sections were collected through the axon.

Over a period of 3 days, retinal axons typically increase their branch number and total branch length. Axons from animals infected with the β-gal virus (β-gal axons) are comparable to untreated controls. Axons whose postsynaptic partners express tCaMKII (tCaMKII axons) show a significant decrease in branch number, total branch length, and arbor complexity during the same 3 days. Close examination of the axon arbors reveals that there are relatively fewer short branches in the tCaMKII axons, although some axon branches have persisted and continued to extend in length.

These data indicate that increasing CaMKII activity in the postsynaptic tectal neurons decreases the complexity of the presynaptic retinal axons. There are two possible mechanisms by which tCaMKII in tectal neurons might cause such a change in axonal morphology. One is that CaMKII phosphorylation of cytoskeletal proteins prevents the addition of new branches to tectal neurons. The axons become simpler because the tectal cells have failed to sprout as many branches. Another possibility is that CaMKII activity decreases the stability of some synapses and causes the retraction of preexisting branches in both tectal neurons and retinal axons. The tCaMKII axons show a net loss of branch tips. This observation favors the hypothesis that some synapses are destabilized and branches retract. Taking images at the 2-hour intervals used in the experiments described above will demonstrate whether the stability of branches has changed in tCaMKII axons or whether the rates of branch additions are decreased.

Regulation of Tectal Cell Development

G.-Y. Wu, H. Cline

Studies on the factors controlling the morphological development of tectal neurons have been limited by the technical difficulty of reproducibly labeling single tectal cells early in their process of differentiation. We have found that iontophoresis of DiI is an excellent method to visualize single tectal neurons in the living animal. To study the development of tectal cell morphology, we labeled cells in the ventricular layer of the optic tectum. Most of these cells have completed their final round of cell division or divide one more time before the progeny differentiate into neurons. Individual DiI-labeled neurons are then repeatedly imaged in vivo over a period of up to 4 days to observe the time course of the elaboration of the axonal and dendritic arbors as well as the migration of the neuron from the ventricular layer into the more mature layers of the tectum.

Cells that extend an axon out of the optic tectum into either the spinal cord or thalamus typically exhibit the following sequence of morphological development: While still close to the ventricular layer, the cell extends a large growth cone that grows toward the pia, where it turns either rostrally or caudally and heads rapidly toward the target region (thalamus or spinal cord). Once the axon growth cone has reached the pia and is heading out of the tectum, the dendrites exhibit a burst of growth and rapidly become more elaborate over the next few days.

These data suggest that dendritic outgrowth may be controlled by contact of the axon with its target. An accurate description of the normal processes of neuronal growth in vivo will now serve as a comparison for future experimental studies on the cellular events controlling axonal outgrowth and the elaboration of dendrites. In particular, we have begun to examine the effect of tCaMKII expression on the development of dendritic structure.

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THE BIOLOGY OF LEARNING

A. Silva R. Bourtchuladze K.P. Giese
B. Frenguelli Z. Marowitz
C.-M. Chen Z. Wang

The key difficulty in the study of learning and memory is the *integration* of behavioral, anatomical, and physiological information into a theory unrestrained by disciplinary boundaries. Gene targeting techniques provide the opportunity to study the impact of the loss of specific genes in the biochemistry, electrophysiology, anatomy, and behavior of mice. Thus, our laboratory is studing the physiology and behavior of mice that are mutant for genes expressed in key forebrain structures, such as the hippocampus and neocortex. The results of these studies suggest that this approach might uncover the cellular processes underlying learning and memory formation in mammals. Summarized here are the key features of the phenotypes of mutants studied in our laboratory.

αCAMKII CAN BOTH PROMOTE AND LIMIT NEUROTRANSMITTER RELEASE

Previously, we found that α-calcium calmodulin kinase II (αCaMKII) is required for long-term potentiation in the CA1 region of the hippocampus. Recently, we have confirmed that this kinase also has a crucial role in presynaptic plasticity. With field and whole-cell studies, we have shown that paired-pulse facilitation is blunted in the CA1 region of mice heterozygous for a targeted mutation of αCaMKII, confirming earlier results with field recordings of αCaMKII homozygotes and indicating that this kinase can promote neurotransmitter release. Unexpectedly, field and whole-cell recordings of post-tetanic potentiation show that the synaptic responses of mutants are larger than those of controls, demonstrating that αCaMKII can also inhibit transmitter release immediately after tetanic stimulation. Thus, αCaMKII has the capacity to either potentiate or depress excitatory synaptic transmission, depending on the pattern of presynaptic activation.

IMPAIRED LEARNING IN MICE WITH ABNORMAL SHORT-TERM PLASTICITY

The loss of the αCaMKII in mice homozygous for a targeted disruption of this kinase leads to a deficit in LTP in the hippocampus (CA1) and to abnormalities in presynaptic plasticity. Heterozygotes show similar presynaptic abnormalities, but no LTP deficit in CA1. To determine the impact of deficits of short-term plasticity in learning and memory, we tested these mutants in water maze tasks. In the visible-platform test (hippocampus-independent), the performance of the heterozygotes was indistinguishable from that of controls. However, in the hidden-platform test (hippocampus-dependent), the heterozygotes were impaired after 3 days of training. In contrast to homozygotes, the heterozygotes learn to find the platform with an additional 2 days of training.

To extend these findings, we tested mice on another task known to require hippocampal function: contextual fear conditioning. After a single trial, the controls, but not the heterozygotes, were able to show contextual fear conditioning (40±5% and 6±4%, respectively). However, the heterozygotes did show conditioning to a discrete tone (43±5%), a behavior that is known to be hippocampus-independent. Similarly to the water maze, the heterozygotes showed significant contextual conditioning (43±4%) with extended training (five trials); homozygotes did not. Our results show that despite impaired short-term plasticity, heterozygotes have normal LTP (CA1) and suggest that their deficits in hippocampal short-term plasticity could underlie the behavioral impairments detected in hippocampus-dependent tasks. To further address this possibility, we looked at another mutant (mice lacking Synapsin II).

MICE WITH A TARGETED MUTATION THAT COMPLETELY ELIMINATES SYNAPSIN II HAVE NORMAL LTP BUT DECREASED PTP AND SLOW LEARNING

The results described above suggest that deficits in short-term plasticity could affect learning. To further address this hypothesis, we tested learning in another mutant known to have normal LTP, but abnormal short-term plasticity. The complete removal of Synapsin II in Sy $II^{-/-}$ mice is known to have a very specific effect in synaptic function: LTP, LTD, and

PPF are normal, even though PTP is decreased. Nevertheless, this mutant appears to groom, eat, drink, walk, and mate normally. Our analysis indicates that the Sy $II^{-/-}$ mutation does not affect sensitivity to shock. Confirmation that the Sy $II^{-/-}$ mutants have normal nociception was obtained with the hot plate test ($p > 0.1$). A study of contextual conditioning shows that similar to αCaMKII heterozygotes, Sy $II^{-/-}$ mutants trained with a single 0.75-mA shock (for 2 sec) show little or no contextual conditioning ($n = 14$; 7±3%), whereas control littermates express clear evidence of conditioning ($n = 13$; 34±7%; $p < 0.002$). Even though the Sy $II^{-/-}$ mutants do not reveal any contextual conditioning, they can be conditioned to a tone (CS) (51±8% vs. 30±4% for controls and mutants, respectively). Just as with αCaMKII heterozygotes, the Sy $II^{-/-}$ mutation seems to affect mostly hippocampus-dependent learning, since contextual, but not cued, conditioning is hippocampus-dependent.

Next, we determined whether additional trials could compensate for the fear conditioning deficits shown by the Sy $II^{-/-}$ mutants. Another group of mice was trained with five trials with 1 minute between trials. Similar to αCaMKII heterozygotes, Sy $II^{-/-}$ mice ($n = 10$) showed nearly normal contextual conditioning with additional training.

The evanescent nature of short-term plasticity excludes its involvement in memory storage. However, electrophysiological and behavioral studies in invertebrates suggest that short-lived changes in synaptic strength could function directly in adaptive responses such as sensitization. Furthermore, electrophysiological studies in the neuromuscular junction of *Drosophila* learning mutants detected alterations in PTP. Recently, modeling work suggested that short-term plasticity could endow networks with the ability to make temporal discriminations, a property essential for complex learning tasks. Our results show that two mutations that affect presynaptic function also compromise learning, even though they do not seem to disrupt LTP, and they suggest that short-term plasticity could have a direct involvement in learning.

THE cAMP RESPONSIVE ELEMENT BINDING PROTEIN (CREB) IS REQUIRED FOR LTP AND FOR LONG-TERM MEMORY

CREB is a factor that mediates transcriptional responses to changes in the intracellular concentration of cAMP and calcium. Interestingly, phosphorylation of CREB at Ser-133 increases dramatically its ability to promote transcription of CREB-containing genes. CaMKII and PKA both phosphorylate CREB at this site. Since studies in our laboratory had involved CaMKII in learning, and in the induction of long-term potentiation (LTP) in the hippocampus, we determined whether CREB is involved in the maintenance of LTP and in memory consolidation. CREB has also been implicated in the activation of protein synthesis required for long-term facilitation in *Aplysia* and long-term memory in *Drosophila*. Our studies with fear conditioning and with the water maze show that mice with a targeted disruption of the α and δ isoforms of CREB are profoundly deficient in long-term memory. In contrast, short-term memory, lasting between 30 and 60 minutes, is normal. Consistent with models claiming a role for LTP in memory, LTP induced with a single tetanus in hippocampal slices from CREB mutants decayed to baseline 90 minutes after tetanic stimulation, even though similar studies in controls revealed stable LTP. However, paired-pulse facilitation and post-tetanic potentiation are normal. These results implicate CREB-dependent transcription in mammalian long-term memory.

MUTATION OF THE NF1 GTPASE ACTIVATING PROTEIN (NF1-GAP) GENE IN MICE AFFECTS BEHAVIOR AND SYNAPTIC FUNCTION

Recent biochemical studies in our laboratory show that αCaMKII can phosphorylate the NF1 GAP (neurofibromin), a protein that when mutated causes the genetic disorder known as neurofibromatosis type I (NF1). NF1 is an autosomal dominant genetic disorder affecting 1/3500 humans. The manifestations of this disorder are complex, but they have a clear impact on tumorigenesis and on the function of the central nervous system: In 30–50% of children affected, the partial loss of the NF1 protein leads to cognitive deficits and learning disabilities. The NF1 protein is the most abundant GAP in the brain, and it is known to be highly enriched in the cytoskeleton and in dendritic endoplasmic reticulum of neurons.

We have studied a mouse with a targeted disruption of the NF1 gene. Similar to humans, the homozygous mutation is lethal in mice. Strikingly, heterozygous mutant mice have subtle but significant "learning" deficits: Performance in the hidden-platform version of the Morris water maze is im-

paired, whereas performance of the mutant mice in the visible-platform version is indistinguishable from that of controls. We also generated mice with heterozygous mutations on NF1, and on the NMDA receptor 1 (NMDAR1). The addition of the NF1 heterozygous mutation exacerbated significantly the "learning" deficits of the NMDAR1 mutant mice. Additionally, electrophysiological analysis showed that the NF1 protein is involved in the modulation of synaptic function. These data demonstrate that the NF1 mutation affects brain function in mice, and they strongly suggest that the mutant mice are an important model for investigating the etiology of the neurological disorders associated with NF1. These studies suggest that this approach might be useful to model neurogenetic disorders.

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REGULATION OF NEUROTRANSMITTER AND RECEPTOR EXPRESSION AND ITS IMPLICATION IN NEURAL PLASTICITY

H. Nawa K. Mizuno
R. Lundsten
M. Waga

Neurons communicate with each other using a large variety of neurotransmitters and neuropeptides. Despite this enormous diversity, each functional neural pathway can be recognized by the particular set of transmitters, neuropeptides, and their receptors employed. Clearly, this "chemical coding" of pathways is critical for determining neural function. The long-term objective of our laboratory is to define the molecular and cellular mechanisms that regulate the synthesis and release of neurotransmitters and neuropeptides as well as expression of their receptors in the nervous system. In particular, we are interested in effects of intercellular protein factors such as cytokines and growth factors. The alteration in expression of neurotransmitters and receptors presumably changes the mode of neurotransmission at each synapse and consequently modulates neural functions such as cognition and memory.

Our previous observations suggest the possibility that many distinct, diffusible factors can influence the development of neurotransmitter/peptide phenotype in the peripheral nervous system. Recently, we found that such factors can also change expression of neurotransmitters and receptors in the brain. For example, brain-derived neurotrophic factor (BDNF), which belongs to the nerve growth factor family (i.e., neurotrophin), increases expression of putative inhibitory neuropeptides and AMPA receptors in forebrain neurons.

Conversely, activin, which is a member of TGF family, suppresses the expression of AMPA receptors. Our findings suggest that expression of neurotransmitters and their receptors is positively and negatively regulated in the brain by various diffusible protein factors.

Our current efforts have been focused on three projects: (1) characterization of neurotransmitter/peptide differentiation activity of neurotrophins in vivo, (2) physiological consequences of regulated neurotransmitter production, and (3) identification of novel factors that influence receptor expression. These studies will elucidate how such diffusible protein factors contribute to neural plasticity such as learning and memory processes.

Induction of Neuropeptide Expression in Neonatal Rat Brain by Neurotrophins

H. Nawa

We have shown that brain-derived neurotrophic factor (BDNF) specifically enhances and maintains the expression of neuropeptide Y and somatostatin in forebrain neurons both in vivo and in vitro. As an extension of this research, neuropeptide differentiation activity was then compared among neurotrophins both in vivo and in vitro. In cultured neocortical neurons, BDNF and neurotrophin-5 (NT-5) remarkably increased levels of neuropeptide Y and somatostatin, and neurotrophin-3 (NT-3) also increased these peptides but required higher concentrations. At elevating substance P, however, NT-3 was as potent as BDNF. In contrast, NGF had negligible or no effect.

Neurotrophins administered into neonatal brain exhibited slightly different potencies for increasing these neuropeptides: The most marked increase in neuropeptide Y levels was obtained in the neocortex by NT-5, whereas that in the striatum and hippocampus was obtained in the neocortex by BDNF, although all three neurotrophins increased somatostatin in similar degrees in all the brain regions examined. Overall spatial patterns of the neuropeptide induction were similar among the neurotrophins: Neurons in deep cortical layers responded to the neurotrophins and initiated expression of neuropeptide Y and somatostatin, whereas substance P was induced most prominently in superficial layers. These observations suggest that all of the neurotrophins can contribute to the complex patterning of neuropeptide expression in the brain.

Regulation of Brain-derived Neurotrophic Factor Protein after Seizures

M. Waga, H. Nawa

Despite numerous previous studies on BDNF mRNA, regulation of the production of BDNF protein has been poorly understood because of a lack of its quantitative measurement. Recently, we have established an ultrasensitive enzyme immunoassay (ELISA) for BDNF protein. The ELISA system enables us to measure trace amounts of BDNF protein in tissues, cells, blood, and cerebrospinal fluid. Using the ELISA system, we will address the question of how BDNF protein production and secretion are regulated by neuronal activity. BDNF content was measured in various brain regions of adult rats. The highest concentration was found in the hippocampus (200 ng/g tissue), in particular, dentate gyrus (>800 ng/g). The BDNF level in the hippocampus is more than a hundred times higher than the NGF levels (2 ng/g tissue) reported. The result suggests that BDNF is one of the most abundant neurotrophic molecules in the brain. After hilus lesion-induced limbic seizures, BDNF protein levels increased rapidly in forebrain regions and reached the maximum after 8–16 hours. In limbic structures, BDNF concentrations remained elevated 4 days after seizure onset, whereas BDNF mRNA is reported to return to basal levels within 46 hours (Isackson et al., *Neuron 6*, 937 [1991]). The temporal and spatial differences of the dynamics of protein and mRNA levels suggest the importance of posttranslational and/or subcellular processes for BDNF production. Persistence of the increases in BDNF content was also reflected in its biological activity, e.g., peptidergic differentiation activity. After limbic seizures, neuropeptide Y content was most markedly and persistently elevated in the entorhinal/amygdaloid region where the most sustained up-regulation of BDNF protein was observed. These results suggest that the sustained increase of BDNF protein in these limbic structures is involved in prolonged post-seizure phenomena including peptidergic alterations.

Influence of Neurotrophins on Transmitter Secretion from Striatal Neurons

K. Mizuno

Recently, we found that neurotrophins can enhance development of GABAergic properties of the striatal neurons both in vivo and in vitro. Among neurotrophins, BDNF and NT-5 specifically elevated cellular GABA content in striatal culture without altering neuronal survival. In the next set of experiments, we addressed the question of whether the increase in GABA content results in elevation of GABA release. GABA secretion was triggered by application of high potassium to striatal cultures. The

amount of GABA released from BDNF-treated neurons was three times higher than that from control neurons. In contrast, levels of glutamate released were not affected by BDNF treatment. The release of GABA from cultured striatal neurons did not require extracellular calcium ion, implying that the GABA release might reflect an activity of GABA transporters. However, blockers for the GABA transporters failed to inhibit the GABA release triggered by potassium. Moreover, the enhancement of GABA release by BDNF was also reproduced by the administration of glutamate instead of potassium. We are currently investigating the molecular mechanism of the GABA secretion.

Activin Inhibits Development of Pyramidal Neurons

R. Lundsten, H. Nawa

Activin is a member of the transforming growth factor (TGF) family, which is now known to be involved in germ-layer differentiation. We have examined the effects of activin on immature neocortical neurons. In cultured neocortical neurons prepared from rat embryo, activin, but not TGF-β, reduced protein levels of a calmodulin-dependent kinase 2, AMPA receptor (types I and II), and glutaminase, which are all expressed predominantly by pyramidal neurons in neocortex. The data were also reproduced at mRNA levels. These results suggest that activin might be a negative regulator for development of cortical pyramidal neurons. Expression of activin itself in the neocortex is also regulated during development. The highest expression of activin mRNA was found in neonatal rats, and its level decreases sharply in a postnatal period. This observation on activin production is quite consistent with phenotypic development of the neocortical neurons. Some of pyramidal neurons are known to be born as early as embryonic day 16, a week before birth of rats, although their phenotypic development initiates only after birth. Thus, activin might have a role in suppressing phenotypic development until the postnatal period, when all types of neocortical neurons are produced. Currently, we are trying to identify the neurons that have activin receptors.

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TRANSMISSION AND PLASTICITY IN MAMMALIAN CENTRAL SYNAPSES

R. Malinow N. Dawkins, B. Frenguelli, H. Hinds, T. Koothan, D. Liao, M. Maletic-Savatic, A. Shirke, A. Weissmann

An understanding of learning and memory will require analysis at several levels of complexity. Synaptic transmission in rat brain slices is complex enough to show glimpses of emergent properties and simple enough to allow hard-nosed biophysical scrutiny. It is our philosophy that there exist key processes in this

system whose understanding is possible and that such an understanding will provide insight into phenomena at higher levels of complexity.

We focus our efforts on understanding how fast signals are propagated from one neuron to another (synaptic transmission) and how the gain of such signals can be modulated (activity-dependent synaptic plasticity). The skeleton of synaptic transmission involves:

1. The invasion of a presynaptic terminal by an action potential;
2. opening of presynaptic calcium channels and rise in presynaptic calcium;
3. interaction of calcium with release machinery producing release of transmitter; and
4. postsynaptic receptor binding of transmitter and channel opening.

At each of these steps, there is the possibility for modulation; thus, we wish to understand each step completely.

Our first year at CSHL has been exhilarating. Two students completed their Ph.D.s: Diana Pettit (now as postdoc with G. Augustine and J. McNamera, Duke University) and Neal Hessler (now postdoc with A. Doupe, Keck Neuroscience Center, UCSF). New arrivals were Bruno Frenguelli and Marjana Maletic-Savatic as postdocs; Heather Hinds as visiting Ph.D. student (from Harvard-MIT M.D./Ph.D. program); and Adriana Weissmann as a predoctoral student. We had two visiting scientists working for short periods in the lab: John Lisman from Brandeis the University and Osvaldo Uchitel from the University of Buenos Aires.

What Is the Basis of the Trial-to-Trial Variability in Transmitter Release?

B. Frenguelli, R. Malinow

It has been known for some time that the release of transmitter is a probabilistic phenomenon; i.e., from trial to trial, there is great fluctuation in transmitter release. We have previously provided evidence that such fluctuation will have an impact on how information is processed by a neuronal circuit (Otmakhov et al., *Neuron 10:* 1101 [1993]). Most investigators are expecting that the critical determinants of this fluctuation are in step 3 above; hence, the intense interest in synaptic vesicle-associated proteins. We have asked if step 2 can be the basis for such fluctuations; i.e., is the rise in presynaptic calcium produced by an action potential always the same or does it vary from trial to trial? Even small fluctuations in calcium could be important because the release of transmitter depends on the fourth power of calcium concentration.

We have addressed this issue by obtaining simultaneous patch-clamp and optical measurements from cortical slice neurons. With such recordings, we assess the role of the variability in calcium rise on transmitter release. Presynaptic boutons on axon collaterals were visualized and rises in intracellular calcium, assessed by Fura-2, were observed in response to single-action potentials. Calcium responses showed trial-to-trial variability and occasional failures despite the faithful conduction of the action potential. These results suggest that a factor contributing to the fluctuation in transmitter release is the variability with which depolarization of a presynaptic terminal produces an increase in intraterminal calcium. Such a fluctuation may be due to the presence of a small number of calcium channels in such structures, and the small probability that any calcium channel will open with an action potential. It will be interesting to determine if this fluctuation in calcium rise can be modulated by activity or pharmacological agents.

Monitoring the Release of Transmitter at Individual Synapses in Cultured Neurons

M. Maletic-Savatic, R. Malinow

We have begun to study the release of transmitter (step 3 above) in cultured neurons with the dye FM-143. This dye is applied in the bath and taken into neurons by endocytosis. Excitation of nerve terminals produces recycling of membrane (to replace the released vesicles), and this dye becomes incorporated into such vesicles. Monitoring (with digital-imaging microscopy) the uptake of the dye indicates location of synaptic sites. Monitoring the loss of dye upon restimulation indicates active release process. With

this technique, we have asked a number of questions: (1) With repeated stimulation, do the synaptic sites remain at the same location? (2) Do the kinetics of release change after repeated stimulation? One interesting aspect of these cultured neurons is that (at the age we study them) they completely lack expression of calcium-calmodulin kinase II (CaMKII), an enzyme we have studied in the context of long-term potentiation (LTP) (see below). We have confirmed this with immunohistochemistry. These neurons are thus effectively CaMKII knock-out tissue. With the transfection methods we have developed (see below), we can ask the question (3) Do these neurons behave differently, with respect to those processes studied with FM-143, if they express CaMKII?

"Silent" Synapses and LTP

D. Liao, R. Malinow

The synapses we study (which are similar to and serve as a model for most excitatory synapses in the vertebrate brain) use glutamate as the neurotransmitter and have two types of postsynaptic glutamate-sensitive receptors: AMPA-type receptors and NMDA-type receptors. These receptors differ in their activation properties as well as their permeation (what they allow to pass into the cell once activated). AMPA-type receptors need only glutamate to open, and NMDA-type receptors require both glutamate and membrane depolarization. The AMPA-type receptor allows Na^+ and K^+ to pass, whereas the NMDA-type receptor also allows Ca^{++} to enter the cell. This year, we found direct evidence that a large fraction of the synapses we study have only NMDA-type receptors. These synapses will thus be silent (even if transmitter is released) when the postsynaptic cell is at resting membrane potential (most of the time), because the postsynaptic receptors will not open if only transmitter is released. We have reached this conclusion by looking at failure rates during transmission elicited when the postsynaptic cell is held at hyperpolarized and depolarized potentials. If there were AMPA-type receptors at all synapses, the failure rates should be the same; we find twice as many failures at hyperpolarized potentials. This difference is not present if NMDA-type receptors are blocked. This and several other pieces of evidence indicate that there are many (in fact, more) synapses with only NMDA receptors.

The classical interpretation of a synaptic failure has been that such an event is due to the failure of transmitter to be released. A change in failure rates after long-term potentiation of synaptic transmission (LTP) has been the strongest evidence indicating that a presynaptic modification has occurred during LTP. This evidence has to be re-interpreted in light of what we have found: Release of transmitter at pure NMDA synapses will be observed as synaptic failures. Specifically, we have tested the hypothesis that pure NMDA synapses add AMPA receptors during LTP.

This process would be seen as a change in synaptic failures (at hyperpolarized potentials, which had been the only conditions studied previously). We have three independent pieces of electrophysiological evidence supporting such a model. We would now like to find biochemical, cell biological, and/or immunocytological evidence for such a process.

These results have led to a specific hypothesis of synapse formation and modulation of gain by LTP: We predict that initial synapses form with only NMDA receptors. These synapses will transmit information only if coactive with other synapses (that have AMPA receptors). LTP induction involves repetitive coactivity producing calcium entry through NMDA receptors and calcium accumulation triggering CaMKII activation. Such activity will promote incorporation of AMPA receptors to the synapse, possibly by a postsynaptic exocytotic process.

Use of Recombinant Viruses to Elucidate Mechanisms in LTP

T. Koothan, H. Hinds, R. Malinow

This year, we published the first successful use of a recombinant virus to modify synaptic transmission. We generated a recombinant vaccinia virus that produces a protein of interest driven by a strong synthetic vaccinia promoter. Micro-application of this virus into brain slices produces efficient expression in cells of a restricted region within several hours. We have used this technique to show that expression of a constitutively active form of CaMKII in postsynaptic neurons can mimic and occlude LTP.

We have begun to look with immunocytochemistry at the cellular localization of recombinant products and to determine conditions that allow expression of recombinant products in cultured neurons. We

are preparing to determine if expression of CaMKII in slices from mice lacking this enzyme can rescue LTP.

We are making viruses that produce an AMPA receptor–GFP fusion protein. This protein shows normal receptor function but can be monitored by the fluorescent GFP protein. We want to see if an LTP-producing protocol will cause such a protein to move from intracellular to plasma membrane sites. This can be monitored in cultured neurons.

Does Injection of Active CaMKII into Postsynaptic Neurons Mimic LTP?

A. Shirke, R. Malinow

We are developing a method to inject with a recording micropipette active CaMKII enzyme into a neuron. With such a protocol, it is possible to compare directly transmission before and after inclusion of this enzyme into the postsynaptic cell. We have preliminary evidence that injection of this enzyme (1) increases transmission and (2) decreases synaptic failures.

It will be interesting to determine if the decrease in failures is due to the activation of AMPA receptors at synapses with only NMDA receptors. If so, will drugs that prevent exocytosis (various well-characterized neurotoxins) prevent this decrease in failures if co-injected with the active CaMKII in the postsynaptic cell? Such a result would indicate that a postsynaptic exocytotic process is necessary for active CaMKII to mimic LTP.

Chimeric Constructs to Study Role of CaMKII in Neuronal Function

A. Weissmann, R. Malinow [in collaboration with G. Enikolopov, Cold Spring Harbor Laboratory]

Chimeric proteins containing steroid-binding domains and protein kinase catalytic domains are being designed. We are making a virus that will generate such a construct. It is expected that the resulting protein will only show catalytic activity in the presence of the hormone. In this manner, we should be able to express this construct in neurons and activate the kinase by exogenous application of the hormone. The activation should occur quickly (within minutes). This will allow localized activation (e.g., localized dendritic or cell body application of the hormone with a pipette) to determine the region of the cell that requires kinase activity to produce effects on synaptic transmission.

Monitoring Activity of Single Synapses in Brain Slices

R. Malinow [in collaboration with J. Lisman, Brandeis University]

This year, we published results in which we resolve activity at single synapses in brain slices using digital fluorescence microscopy. This has allowed us to show that some synapses only transmit when activated in a repetitive manner. Such resolution may be required to unravel the pre- or postsynaptic modification underlying LTP.

In conclusion, we are continuing to elucidate the basic mechanisms involved in central synaptic transmission. Such an understanding is necessary to derive a mechanistic flowchart of plastic processes. We also continue to probe the role of CaMKII in synaptic plasticity.

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SIGNAL TRANSDUCTION IN NEURONS

G. Enikolopov N. Peunova
P. Krasnov
S. John

We study signal transduction in neurons and are interested in how neuronal activity and neuronal development are linked. Recently, we have shown that a gaseous messenger molecule, nitric oxide (NO), may provide such a link. We have found a crucial role for NOS genes in the commitment cascade during neuronal differentiation. We now see a potential for NO to act as a trigger of growth arrest during terminal differentiation in other systems as well. Experiments are now being carried out to investigate the role of NO as an important switch during development of neuronal cells in culture, brain development in *Xenopus* (in collaboration with H. Cline, CSHL), *Drosophila* development (with B. Kuzin, Moscow, Russia), and terminal differentiation in mammals.

NO Triggers a Switch to Growth Arrest during Differentiation of Neuronal Cells and Couples It with Prevention of Apoptosis

N. Peunova, G. Enikolopov

Arrest of cell division is a prerequisite for cells to enter a program of terminal differentiation. Differentiation of neuronal cells induced by nerve growth factor (NGF) involves a proliferative phase that is followed by cytostasis and, ultimately, by manifestation of the fully differentiated phenotype. We have found that NO executes a critical step in differentiation by initiating cytostasis. We have found that:

- NGF treatment induces NOS activity. All three forms of NOS are induced in fully differentiated PC12 cells, but at the first stages, a substantial part of the NO-producing activity comes from an inducible form of the enzyme.
- NO can inhibit proliferation of PC12 cells. Both DNA synthesis and cell proliferation are restored once NO sources are washed away. At low concentration, NO causes the cells to accumulate specifically in G_2 phase while the proportion of cells in S phase decreases. Remarkably, within a range of concentrations of NO donors, the proportion of PC12 cells in G_2 and S phases are similar to the levels reached after prolonged treatment with NGF.
- NOS inhibitor reverses the cytostatic action of NGF and forces the cells to continue to proliferate instead of ceasing to divide after 6–8 days of NGF treatment. Neurite outgrowth, the principal manifestation of the differentiated phenotype, is also prevented by NOS inhibition.
- Mutant cells' capacity to differentiate can be rescued by addition of NO. PC12-U2 cells (described by Burstein and Greene) retain the early steps of the response to NGF but have lost the capacity to execute the later steps. They do not stop dividing after NGF treatment and, as a consequence, do not develop the fully differentiated phenotype; in particular, they do not send out processes. We have shown that this phenotype can be overcome by the addition of NO. Neither NGF nor NO alone had any effect on the phenotype of U2 cells, but in combination, these treatments restored the differentiated neuronal phenotype. Inducible NOS is expressed at much lower levels in U2 cells than in the wild-type PC12 cells. Thus, NGF-treated U2 cells are deficient in inducible NOS and this defect can be rescued by NO.
- The crucial phase of NOS action is cell-cycle-specific, and a critical time window for NOS action exists within the first 2–3 days of NGF treatment.

These experiments indicate a causative role for NO action in NGF-induced growth arrest and differentiation in PC12 cells. We conclude that induction of NOS is an important step in the commitment of neuronal precursors during differentiation and that NOS serves as a growth arrest gene, initiating the switch to cytostasis during differentiation. We propose that NO produced by a group of adjacent neuronal precursors may contribute to the synchronized development of that domain.

What is the target of NO action during differentiation? Guanylate cyclase is among several potential effectors for NO. It can be directly activated by NO and thus NGF/NO-induced growth arrest can be mediated by cGMP- or cAMP-dependent kinases. Our data indicate that NO-mediated changes in the activity of cyclic-nucleotide-dependent protein kinases accompany the NGF action and are critical for the cessation of growth. Another direct target for NO is ribonucleotide reductase (RNR), the rate-limiting enzyme in deoxynucleotide biosynthesis. We have now shown that addition of deoxynucleosides (which permits the bypass of the RNR step by employing a salvage nucleotide biosynthesis pathway) leads to a partial release of the NO-induced block in DNA synthesis in PC12 cells. Inhibition of RNR, beyond preventing DNA synthesis directly by limiting the supply of nucleotides, possibly also serves as a sensor, transmitting the signal further and activating other growth-arrest/checkpoint systems.

An intriguing question is whether antiproliferative NOS action cooperates with other growth arrest systems in the differentiating cell. Growth arrest systems supporting NOS could act in parallel with NOS action (although blockade of NOS activity alone can reverse the constraining action of NGF) or consequent to it, for instance, by inducing other growth arrest genes, such as TGF-β, Rb, and cdk inhibitors, possibly as a direct consequence of NO synthesis. We are currently testing these possibilities.

Finally, we have found that NO can prevent cells from apoptosis. It can both suppress the apoptosis of undifferentiated PC12 cells after withdrawal of serum and prevent the differentiated cells from death after withdrawal of NGF. Establishment of the fully differentiated state of PC12 cells after NGF treatment is accompanied by a switch in NOS gene expression, with expression of the inducible form going down and expression of the neuronal isoform going up. We propose that this switch from the calcium-independent to the calcium-dependent form may underlie the dual role for NOS as a trigger of cytostasis and as an inhibitor of apoptosis during differentiation.

Targeting Proteins to the Nerve Terminals

P. Krasnov, J. Chubb, G. Enikolopov

We want to restrict signal transduction cascades in the nerve cell to the terminals. This way, we may be able to manipulate the signaling in the nerve terminals, including synapses, without affecting the processes in the nucleus and in the cell soma. One approach is to target the molecules that participate in or interfere with signal transduction to the nerve terminals as part of chimeric protein vectors.

We tested several synaptic vesicle proteins for this purpose by fusing them to the HA epitope tag, transfecting them into differentiated PC12 cells, and visualizing the distribution of the chimeric molecules by immunofluorescence. We found one form (rat synaptotagmin II-HA fusion) that permits efficient accumulation of the recombinant molecules in the terminals, with some material being present in the Golgi network.

We prepared an extensive series of deletions covering the entire coding region of the synaptotagmin gene, fused them with the HA epitope, and tested each mutant after transfection of PC12 cells by Western blot analysis and immunofluorescence. We were thus able to visualize the distribution of chimeric molecules in the cell and confirm that different mutant proteins are of predicted lengths and are synthesized at comparable levels. We have shown that the transport to the nerve terminal is dependent on two structural determinants. One signal, located near the transmembrane domain and the dimerization domain, is responsible for the accumulation of the recombinant molecules in the Golgi network. The other signal is located near the carboxyl terminus and is responsible for the actual transport (and/or accumulation) of the molecule to the nerve terminal. This second signal overlaps with the region of neurexin binding, which in turn might determine the location of the synaptotagmin molecules (and, probably the whole synaptic vesicle) near the voltage-gated calcium channels.

We have thus determined which parts of the synaptotagmin molecules are necessary for transport to the terminal. Currently, we are trying to determine if the same short sequences are also sufficient for the targeted transport of the chimeric molecules to the terminal. Our next step is to use these novel targeting vectors to transport protein kinases and recombinant protein kinase inhibitors to the terminals of neuronal cells for the of neuronal plasticity.

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NEURONAL GROWTH AND DIFFERENTIATION

D.R. Marshak Y.S. Bae L. Peña
G.Y. Cha P. Recksiek
N. Chester S. Srinivasan

This laboratory conducts research on the biochemical basis of neuronal growth and differentiation. Overall, the questions that we approach in our research involve the decisions of neuroblasts to cease proliferation and to subsequently elaborate neuritic processes prior to terminal differentiation. These questions thus involve understanding how signal transduction systems that control cell proliferation in the neuroblast are altered upon becoming post-mitotic as well as learning which growth factor molecules control these switches within cells. Specifically, we are interested in the action of a growth-stimulating protein, S100β, that is produced by astrocytes in the brain. The mechanism of action of S100β is compared to that of other growth factors, such as those in the family of neurotrophins or the family of heparin-binding (fibroblast) growth factors. The involvement of such neurotrophic factors in degenerative diseases, such as Alzheimer's disease, has prompted our interest in the role of such factors in neuropathological processes.

Mechanism of Action of S100β

L. Peña, S. Srivasan, D.R. Marshak [in collaboration with N. Arispe, National Institutes of Health]

We have been studying the S100β protein in the context of a typical growth factor that binds to a cell surface receptor, which in turns triggers a Ca^{++}-dependent intracellular second messenger cascade mediated by protein kinases. Although there remains some support for this model, our accumulated data have led us to adopt a different model.

S100β appears to have direct effects on the ion permeability of cell membranes. In our bioassays, (1) a 7ED chick neurite assay and (2) a glial cell proliferation assay, divalent cations such as Ca^{++} are required for S100β action. This is demonstrated by the ability of 1 mM Ni^{++} to block the S100β effect. By itself, this finding could support either the receptor model or the direct membrane permeability model. However, we have data from a cell-free, synthetic lipid bilayer system that supports the latter. In this system, S100β is delivered to a planar lipid bilayer interposed between two chambers fitted with recording electrodes (Fig. 1). The potential across the lipid bilayer was held at +40 mV, with symmetrical baths of 100 mM KCl/1 mM $CaCl_2$ on each side of the lipid bilayer. The tracing shows on/off current flow typical of an S100β ion pore. For comparison with a classical ion channel, the conductance was estimated to be three times that of a typical Na^+ channel, and the pore could remain open for unusually long periods of time. Since S100β shows no sequence similarity to classical ion channels and exhibits different behavior, we provisionally refer to it as an ion "pore" rather than an ion "channel."

We have initiated a mutational analysis of the S100β protein to better understand the structure that permits the formation of the S100β ion pores. In addition to the two bioassays described above, the synthetic lipid bilayer system is now the basis of a new, third assay to study S100β function. In these assays, we can study the effects of strategically placed mutations in S100β protein. The mutations are designed with the aid of a three-dimensional structural model (developed by Jim Pflugrath, formerly of CSHL) patterned after the known structure of ICABP, a member of the superfamily of S100 proteins.

Distribution of S100β in Neurological Disease

D.R. Marshak [in collaboration with W.S.T. Griffin, Arkansas Children's Hospital]

Under the auspices of a major grant from the National Institute on Aging, we are continuing our studies of the brain distribution of S100β, a polypeptide growth factor produced by glial cells in the brain. This is a multisite program grant that brings together

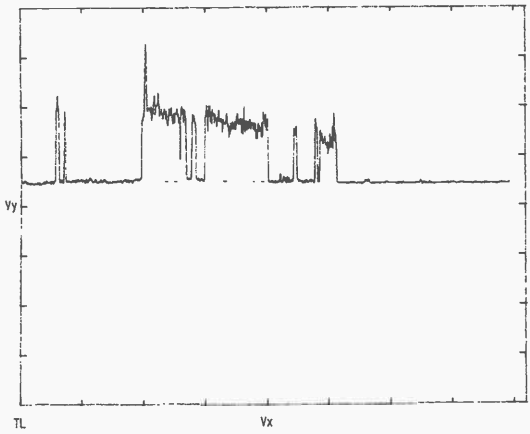

FIGURE 1 Current flow tracing for the S100β ion pore in an artificial planar lipid bilayer.

anatomists, pharmacologists, pathologists, molecular biologists, and biochemists who work on S100β. Several years ago, our lab discovered the function of this molecule and have more recently discovered its abnormal levels in Alzheimer's disease. This work is central to understanding the potential role of this factor in the pathology of neurodegenerative disease. This year, in collaboration with W.S.T. Griffin and colleagues in Arkansas, we found reactive glial cells containing abnormal amounts of the S100β protein in the brains of HIV patients who showed symptoms of dementia in the clinic. This broadens our understanding of the widespread appearance of astrogliosis in neurodegeneration. The observation strengthens the argument that abnormal glia containing S100β are associated with amyloid plaques in the brain.

Regulation of S100β by β-Amyloid

L. Peña, S. Srinivasan, D.R. Marshak

In Alzheimer's disease and Down syndrome, severely afflicted brain regions exhibit up to 20-fold higher protein levels of S100β, and astrocytes surrounding neuritic plaques exhibit higher protein levels of S100β. A major constituent of the plaques is β-amyloid protein, which has been reported to have both neurotrophic and neurotoxic effects in vitro. We examined the responses of central nervous system glia to a β-amyloid peptide. Primary astrocyte cultures, obtained from neonatal rats, and rat C6 glioma cells were synchronized by serum deprivation and treated with βA(1-40), a synthetic fragment of β-amyloid. A weak mitogenic activity was observed, as measured by [^{3}H]thymidine incorporation. Northern blot analysis revealed increases in S100β mRNA within 24 hours in a dose-dependent manner. Nuclear run-off transcription assays showed that βA(1-40) specificially induced new synthesis of S100β mRNA in cells maintained in serum but caused a general elevation of several mRNA species in cells maintained under serum-free conditions. At the protein level, corresponding increases in S100β protein synthesis were observed in response to the βA(1-40) peptide, measured by immunoprecipitation of ^{35}S-labeled cellular proteins. The data indicate that S100β expression can be influenced directly by β-amyloid.

Protein Kinase Modulation during Neuronal Differentiation

Y.S. Bae, L. Peña, D.R. Marshak

We have continued our studies of protein kinase responses to growth factors in neuronal cells. Two model systems are currently in use: chicken embryo neurons stimulated with S100β and rat pheochromocytoma (PC12) cells stimulated with nerve growth factor (NGF) or epidermal growth factor. A complete study of $p34^{cdc2}$ kinase and MAP kinases in PC12 cells that respond to NGF has been continued by Y.S. Bae in conjunction with the work of a former postdoctoral fellow, A. Rossomando. The rat pheochromocytoma cell line, PC12, undergoes morphological and biochemical differentiation into sympathetic neurons in culture under the influence of NGF. The enzyme $p34^{cdc2}$ kinase, which is critical to the induction of mitosis, appears to be down-regulated during NGF-stimulated differentiation. The previously identified p46 protein seems to be a form of MAP kinase in these cells. This protein kinase is known to be stimulated indirectly by growth factor receptor tyrosine kinases and associated molecules. These studies will enable us to work out pathways of signaling from the surface of the neuron to the genome. Our earlier work has demonstrated that the protein kinase CKII is modulated during neuronal development (Diaz-Nido et al. 1994). This reinforces our studies of the regulation of CKII in human cells (I.J. Yu et al., submitted) and that it varies during the cell division cycle as cdc2 activity increased. In a related paper (Suzuki et al. 1994), we demonstrated that the amyloid precursor protein (APP), which gives rise to the pathological form of β-amyloid, is itself phosphorylated by cdc2 in a cell-cycle-dependent manner. These observations point out the interrelationships of the cell-cycle-dependent protein kinases and their alterations in neurological diseases.

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CSH LABORATORY JUNIOR FELLOWS

In 1986, Cold Spring Harbor Laboratory began a Junior Fellow 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 each Fellow to work independently at the Laboratory for a period of up to 3 years on projects of their choice. The Fellow is 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.

Three previous Cold Spring Harbor Fellows, Dr. Adrian Krainer (1987), Dr. Carol Greider (1988), and Dr. Eric Richards (1989), are currently members of the scientific staff at the Laboratory. Dr. David Barford, our Junior Fellow since 1991, left this year to go to Oxford University (Laboratory of Molecular Biophysics) as a university lecturer. Our newest Fellow, Dr. Ueli Grossniklaus, joins us from the Department of Cell Biology, University of Basel, Switzerland.

U. Grossniklaus

Dissection of *Arabidopsis* Ovule Development by Enhancer Detection

U. Grossniklaus, J. Moore, W. Gagliano

The plant life cycle is divided into two generations: the diploid sporophyte and the haploid gametophyte. Whereas in lower plants (e.g., mosses and ferns), the two generations are distinct from each other, tissues of both generations contribute to the ovule in flowering plants. The ovule is the site of megasporogenesis and megagametogenesis, the processes that lead to the formation of the mature embryo sac containing the egg cell. Whereas the mechanisms controlling oocyte development in animals are being studied extensively at the cellular, genetic, and molecular levels, very little is known about the genetic and molecular basis of the corresponding process in flowering plants. Only five mutants affecting ovule development have been reported in *Arabidopsis thaliana:* Two affect the gametophyte (Redei, *Genetics 51:* 857 [1965]; Springer et al., *Science*, in press [1995], see Plant Genetics Section of this Annual Report), and the remaining three affect primarily sporophytic parts of the ovule (for review, see Reiser and Fisher, *Plant Cell 5:* 1291 [1993]).

Within the ovule primordium, a single cell undergoes meiosis, three of the haploid megaspores die and the fourth produces the mature embryo sac by three subsequent mitotic divisions. These eight products differentiate along four alternative developmental pathways to become an oocyte, two synergids, three antipodals, or the binucleate central cell whose two nuclei fuse prior to fertilization (Fig. 1). Following fertilization of both the oocyte and the central cell, the ovule differentiates into a seed. The seven sister cells of the embryo sac are highly specialized. It is likely that the development of each cell type is significantly influenced by its position within the embryo sac and by cell-cell communication. Proper development of the gametophyte appears to depend on interactions between gametophytic and sporophytic tissues of the ovule.

The goal of our research is the identification of genes that are important for the development of the gametophyte and the sporophytic parts of the ovule. We hope to isolate and characterize some of the genes involved in cell specification of the embryo sac cells and of genes that are important for cellular communication processes between sporophytic and gametophytic tissues.

ENHANCER DETECTION/GENE TRAP SCREEN

In *Drosophila*, enhancer detection screens have been very successful in identifying new developmentally regulated genes. Application of a similar approach in

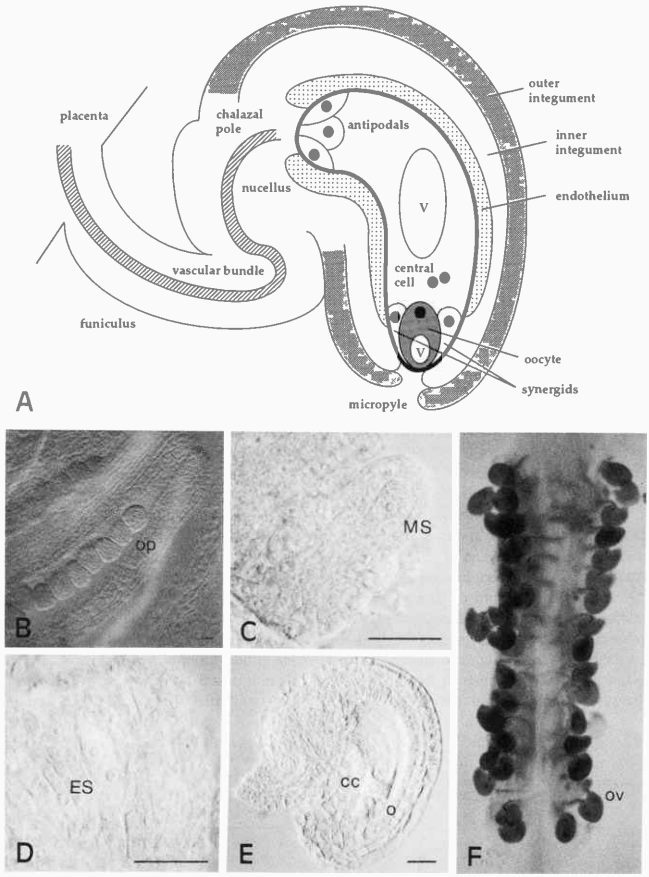

FIGURE 1 Ovule development in *Arabidopsis thaliana*. (*A*) Schematic representation of a mature ovule. The haploid embryo sac or gametophyte is embedded within the diploid sporophyte: The outer and inner integuments consisting of two cell layers each and the innermost endothelial cell layer. The gametophyte is a highly polar structure with three antipodals at the chalazal end and the egg apparatus consisting of the oocyte and two synergids at the micropylar pole. The two nuclei of the large central cell are in proximity of the oocyte. (V) Vacuole. (*B*) Ovule primordia (op) in a carpel of a stage-9 flower. (*C*) Ovule of a stage-11 flower. A single cell in the nucellus has differentiated into the megasporocyte (MS) which will give rise to four megaspores. (*D*) Two-nucleate embryo sac (ES) derived from the surviving megaspore through mitotic division. (*E*) Mature ovule with the gametophyte in the center. The nuclei of the central cell (cc) and oocyte (o) are in the focal plane at the micropylar end of the ovule. (*F*) Carpel of a transposant with specific staining in the ovules (ov). The carpel wall has been removed prior to staining. Bars, 20 mm.

flowering plants should lead to the identification of many genes that control ovule development and gametogenesis. The enhancer detection technique (O'Kane and Gehring, *Proc. Natl. Acad. Sci. 84:* 9123 [1987]) relies on a transposon carrying a reporter gene under the control of a weak constitutive promoter. If this promoter comes under the control of nearby genomic regulatory elements (e.g., enhancers), the reporter gene is expressed in a specific spatial and temporal pattern that reflects the action of these elements. Reporter gene expression thereby indicates the activity of a gene that is usually regulated by these enhancers. A modification of this approach involves the generation of transcriptional fusions to a reporter gene (gene trap). Both approaches have been extremely successful, also allowing the identification of genes that are not easily amenable to classical genetic analyses. They have been especially useful in studying developmental processes occurring late in development, i.e., after the effective lethal phase of a corresponding mutation, and in processes characterized by high complexity and redundancy.

The plant group here at the Laboratory has generated a series of enhancer detector and gene trap constructs. They are based on the *Ac/Ds* transposable element system from maize which also functions in *Arabidopsis*. For detailed description of the system, see Plant Genetics Section of this Annual Report. In brief, a *Ds* element acting as an enhancer detector or gene trap transposon can be mobilized by crossing a *Ds*-containing line to a line bearing an immobile *Ac* element producing transposase. Self-pollination of these F_1 plants will result in some F_2 progeny containing a transposed *Ds* element (transposants). A positive/negative selection system allows the identification of the plants that contain a *Ds* element at a new location by selecting for the transposed *Ds* element but against the donor element and the transposase source. The enhancer detector/gene trap mutagenesis system is a new means of creating random insertions in the *Arabidopsis* genome. An insertion in or close to a gene of interest (as defined by its expression pattern and/or mutant phenotype) provides an entry point to both its molecular and genetic characterization.

A large-scale systematic screen for enhancer detector/gene trap insertions showing specific expression patterns in the *Arabidopsis* ovule and/or mutant phenotypes (female sterility and semisterility) is likely to lead to the identification of genes involved in ovule development. Our goal is to generate 4000 transposants. In collaboration with the other plant groups here at the Laboratory, we should have a collection of approximately 15,000 independent transposants within 1–2 years. This will provide sufficient material for a large-scale screen, although it is far from saturation level. I am interested in insertions that show reporter gene expression in specific cell types or spatially restricted domains of the ovule. At present, virtually nothing is known about the genetic control of cell fate decisions that govern embryo sac cell development. Genes expressed in only one cell type or in a subset of cell types within the embryo sac should provide a basis for studying the process of cell specification. Patterns specific to a subset of sporophytic cells may be involved in cell-cell communication processes, and the corresponding gene products may provide the developing gametophyte with positional cues that direct embryo sac cells to a particular developmental pathway.

During the last year, we have focused our efforts on the large-scale production of transposants. On average, one of four F_2 families yields a transposant (750 seeds per family are screened). Thus, to isolate 4000 transposants, 16,000 F_1 plants must be grown and their F_2 seeds put through the positive/negative selection process. Our progress is summarized in Table 1. I have generated between 20,000 and 25,000 F_1 seeds in more than 1,000 crosses. Three different enhancer detector (*DsE*) and three gene trap (*DsG*) starter lines were crossed to five *Ac* lines (*Ac*). In general, the different combinations yielded similar frequencies of transposition to unlinked sites that were distributed around 25%. There are two exceptions: First, the *DsG1* element gives a higher transposition frequency than the other elements (30%). This may be due to a favorable chromosomal location of this element. Second, the combination of

TABLE 1 Present Status of Enhancer/Gene Trap Screen

F_1 seeds generated	20,000
F_2 families harvested	10,116
F_2 families screened for transposants	6,471
Transposants isolated	1,482
F_3 families screened for steriles (≥10 plants)	574
Male-sterile mutations recovered	9
Female-sterile mutations recovered	1
F_2 plants screened for semisteriles	1,481
Semisterile candidates identified	30
Heritable semisteriles	9
Possibly tagged semisterile mutants	3

DsE2 with *Ac3* gave a very low transposition frequency (13%), suggesting that these two elements are located relatively closely linked on the same chromosome. We have grown 10,000 F_1 plants to maturity and harvested their F_2 seeds. So far, we have screened about 6,500 F_2 families for transposants. The screening of F_2 families is currently proceeding at a steady rate of 300 families per week, and we recover between 70 and 80 transposants every week.

IDENTIFICATION OF MUTANTS AFFECTING OVULE DEVELOPMENT

Two classes of mutations are of primary interest to us. Female sterile mutations are likely to have defects in the diploid sporophytic tissue of the ovule and are typically recessive. The second class of mutations are semisteriles that result from female gametophyte lethality. In such mutants, the defective allele is not transmitted through the haploid female gametophyte and their progeny are therefore heterozygous for the mutation. Half of the gametophytes will receive the mutant allele and abort, leading to only 50% seed production. The transposants we isolate during the selection process (F_2 plants) can be either homozygous or heterozygous for the *Ds* insertion. We can screen these plants for semisteriles but not for steriles. A screen for sterile mutations must be performed in the F_3 generation where one of four plants should show the mutant phenotype.

In collaboration with the other plant groups participating in the enhancer detection/gene trap effort, we have planted 1036 F_3 families whereof we contributed 350 lines. We aimed at growing approximately 12–15 plants per family; 574 of the F_3 families had 10 or more plants (corresponding to a 95% confidence level that a recessive mutation would be detected). Among them, we identified 9 sterile mutations producing no seed and showing an extended flowering period. All of them were reciprocally crossed to wild-type plants to determine whether they were male- or female-sterile. All nine mutations were male-sterile and one line was both male- and female-sterile. Examination of the carpels of this mutant showed that it had no ovules at all. In addition, the mutant had other defects such as missing stamens (→male sterility), altered organ numbers, inflorescence, and leaf abnormalities.

We have screened approximately 1500 F_2 plants for reduced seed production and have identified 30 putative semisterile mutants. Since growth conditions greatly influence seed set, many plants that are not mutant produce a reduced number of seeds. Therefore, the putative mutants are checked for segregation of the kanamycin resistance marker which is linked to the *Ds* element that presumably causes the mutation. A female gametophyte lethal mutation with normal transmission through the male gametophyte should yield a 1:1 segregation of kanamycin-sensitive to -resistant seedlings. We have tested the putative semisterile mutations and three of them behaved as expected, indicating that the mutation may be caused by the *Ds* insertion. They are currently being reciprocally outcrossed to wild type to determine to which degree transmission through the female and male gametophyte is reduced.

SCREEN FOR EXPRESSION PATTERNS

We invested considerable effort into optimizing the staining procedures to detect the β-glucuronidase activity of the reporter gene. We tested numerous fixation methods, different staining conditions, postfixation, and clearing procedures. Unfortunately, β-glucuronidase is very sensitive to these treatments, and we could not find a condition allowing optimal activity and a high degree of product localization at the same time. We decided to use conditions yielding the highest activity in order not to miss any weak expression patterns. Transposants staining in ovules can then be restained under conditions giving better cellular localization. We dissected carpels containing mature ovules of four plants from each of the 1000 F_3 families that were planted. The removal of the thick carpel wall allows the ovules to be directly exposed to the staining solution. We also stained entire inflorescences of these plants, allowing the analysis of the expression pattern at various stages of ovule development. This material is currently being analyzed for ovule-specific expression patterns. The most severe problem we encountered was that the blue product of the enzymatic reaction is forming large crystals in organic clearing solutions. Since the embryo sac is surrounded by five or six cell layers (Fig. 1), it is essential to clear the tissue in order to identify the cells of the gametophyte and the internal cell layers. We tested more than 30 different clearing procedures used in plant and fly labs based on phenol, lactate, chloralhydrate, methylbenzoate, methylsalicilate, clove oil, xylene, and sodium hydroxide. All of them led to the formation of blue crystals, severely obscuring the microscopic analysis of our samples.

The two procedures showing the least crystalization did not clear sufficiently. However, we were able to establish conditions (20% lactate, 20% glycerol in PBS) that lead to the formation of small punctuate crystals rather than the usual large needles. The morphology of the cleared tissue is excellent and the crystalization process takes a few days, leaving us enough time for microscopic inspection.

PUBLICATIONS

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Cadigan, K.M., U. Grossniklaus, and W.J. Gehring. 1994. Functional redundancy: The respective roles of the two sloppy paired genes in *Drosophila* segmentation. *Proc. Natl. Acad. Sci.* **91:** 6324–6328.

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In Press

Andjelkovic, M., P.F. Jones, U. Grossniklaus, P. Cron, A.F. Schier, M. Dick, G. Bilbe, and B.A. Hemmings. 1995. Developmental regulation of expression and activity of multiple forms of the *Drosophila* RAC protein kinase. *J. Biol. Chem.* (in press).

Author Index


COLD SPRING HARBOR
MEETINGS AND COURSES

ACADEMIC AFFAIRS

The academic program continues to bring thousands of scientists to the Laboratory to participate in a series of advanced laboratory and lecture courses in molecular biology, neurobiology and structural biology, a set of conferences including the annual Symposium, and a summer research program for undergraduates. The meetings continue to emphasize presentations of the latest scientific results by junior faculty, postdoctoral fellows, and graduate students as well as their more senior colleagues. The courses, which extend from April through November, succeed in teaching a variety of new techniques and state-of-the-art methodologies by instructors who come from universities, medical schools, and institutes from around the United States and abroad. Thus, the academic program brings together scientists as teachers, students, meetings organizers, and participants to share new scientific knowledge.

In 1994, 15 meetings and 24 courses were held over an 8-month period. The annual Symposium, held this year on the Molecular Genetics of Cancer, provided much discussion and interest as insights into the genetic alterations found in different forms of cancer continue to increase. The Symposium was, in fact, oversubscribed this year, and other meetings that filled the Laboratory to capacity included those on Genome Mapping and Sequencing, Mouse Molecular Genetics, and Retroviruses. Two new meetings were held this year—one on Zebrafish Development and Genetics, which explored research being done on this quite new experimental organism, and one on Epstein-Barr Virus and Associated Diseases. A small special meeting was held in the fall—A Decade of PCR: Celebrating Ten Years of Amplification. This meeting, organized by Stan Rose of Perkin-Elmer and David Stewart, the Meetings Program Director, and sponsored by Perkin-Elmer, brought together scientists from academia and industry. This represents the first in a potential series of biotechnology meetings that David is exploring, to hold in the ever-shrinking off-season courses and conferences.

Although the list of course instructors, assistants, and meetings organizers is listed below, it should be acknowledged here that their great efforts, intelligence, and "grace under pressure" allow the program to run on an intense, almost 24-hour a day schedule. Maintaining and expanding the Academic Program are made possible by support from the National Institutes of Health, National Science Foundation, Department of Energy, and Department of Agriculture which help to support courses and meetings. The Laboratory is also most fortunate to have an education grant from the Howard Hughes Medical Institute, which funds neurobiology courses as well as new courses, thus providing crucial flexibility for the program. Funds from the Esther and Joseph A. Klingenstein Fund and scholarship monies from the Grass Foundation help to support neurobiology courses. The meetings receive support from the Corporate Sponsors Program and the courses receive much support from many companies that donate supplies and lend large amounts of equipment for the courses.

While graduate students, postdoctoral fellows, and faculty participate in the courses and meetings, the Undergraduate Research Program (URP) provides an opportunity for college undergraduates to spend 10 weeks at the Laboratory during the summer. The program, headed by Winship Herr, allows students to do research in the laboratories of staff scientists.

The large numbers of courses and meetings proceed with skill and efficiency, thanks to the collaborative efforts of a large number of people at the Laboratory. The staff of the Meetings Office, ably led by David Stewart and including the Course Registrar Micki McBride, Diane Tighe, Marge Stellabotte, Andrea Stephenson, Nancy Weeks, Drew Mendelsohn, and Jim Koziol, coordinates with both skill and tact the visits of the thousands of scientists who come here to participate in the meetings and courses. Herb Parsons and Ed Campodonico of the audiovisual staff manage with great efficiency and good humor the thousands of slides and projections provided by the scientists at our meetings. The course instructors have come to depend on the skills of Cliff Sutkevich and his staff in setting up and maintaining all of the necessary equipment and supplies, as well as those of the Purchasing Office headed by Phil Lembo and the new Purchasing Agent in charge of the courses, Edie Kappenberg. The many grants that support the academic program are ably coordinated by Mary Horton of the Grants Office.

Terri Grodzicker

59th COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

Molecular Genetics of Cancer

June 1–June 8 421 participants

ARRANGED BY **Bruce Stillman,** Cold Spring Harbor Laboratory

Investigation of the mechanistic aspects of cancer has its roots in the studies on tumor viruses and their effects on cell proliferation, function, and growth. This outstanding progress was well documented in previous Cold Spring Harbor Symposia on Quantitative Biology. The classic 1974 Symposium on Tumor Viruses, followed by the 1979 Symposium on Viral Oncogenes, chronicled the valuable insights that the study of viruses provided to the cancer problem. Studies on the genetics of human cancer arose from cytological observations of the abnormalities that are present in the chromosomes of tumor cells. Quite a number of the genes affected by either a translocation break point, amplification, deletion, or point mutation had been identified by the time that the Laboratory hosted a comprehensive meeting on the Origins of Human Cancer in our centennial year of 1990.

In the early to mid 1980s, progress on the development of chromosome mapping strategies and the accumulation of DNA probes that identified polymorphisms, encouraged by the international Human Genome Project, enabled the identification of other genes that contributed to familial inheritance of high susceptibility to specific cancers. This approach was very successful and led to a degree of optimism that one aspect of cancer, the multistep genetic process from early neoplasia to metastatic tumors, was beginning to be understood. It therefore seemed appropriate that the 59th Symposium on Quantitative Biology focus attention on the Molecular Genetics of Cancer. The concept was to combine the exciting progress on the identification of new genetic alterations in human tumor cells with studies on the function of the cancer gene products and how they go awry in tumor cells.

The meeting was attended by a record 421 participants, with 86 oral presentations in 14 sessions and 185 poster presentations on three separate afternoons. Outstanding first night introductory lectures by Leland Hartwell, Robert Weinberg, Bert Vogelstein, and Suzanne Cory set a high standard that was maintained throughout the next seven days. Harold Varmus, whom we are fortunate to have as the current Director of the National Institutes of Health, presented a masterful Dorcas Cummings Lecture to members of our local community. Ed Harlow, who generously agreed to summarize the meeting, provided the audience with sound advice, good humor, and great insight.

Essential funds to hold such a meeting were provided by the National Cancer Institute (a branch of the National Institutes of Health) and the U.S. Department of Energy. Additional funding came from our Corporate Sponsors who provide increasingly essential funding: Alafi Capital Company, American Cyanamid Company, Amgen Inc., Becton Dickinson and Company, Biogen, Bristol-Myers Squibb Company, Chugai Pharmaceutical Co., Ltd., Ciba-Geigy Corporation, Pfizer Inc., Diagnostic Products Corporation, The Du Pont Merck Pharmaceutical Company, Forest Laboratories, Inc., Genentech, Inc., Glaxo, Hoffmann-La Roche Inc., Johnson & Johnson, Kyowa Hakko Kogyo Co., Ltd., Life Technologies, Inc., Mitsubishi Kasei Institute of Life Sciences, Monsanto Company, New England BioLabs, Inc., Oncogene Science, Inc., Pall Corporation, The Perkin-Elmer Corporation, Research Genetics, Sandoz Research Institute, Schering-Plough Corporation, SmithKline Beecham Pharmaceuticals, Sterling Winthrop Inc., Sumitomo Pharmaceuticals Co., Ltd., Takeda Chemical Industries, Ltd., Toyobo Co., Ltd., Wyeth-Ayerst Research, Zeneca Group PLC.

H. Varmus

M.-C. King

B. Vogelstein

PROGRAM

Welcoming Remarks and Introduction: Bruce Stillman

Control of Cell Cycle and Cell Growth

Chairperson: T. Tlsty, *University of North Carolina School of Medicine*

Genetic Methods for Diagnosis and Cancer Therapy

Chairperson: D.M. Livingston, *Dana-Farber Cancer Institute and Harvard University Medical School*

p53

Chairperson: E. Fearon, *Yale University School of Medicine*

Transcription and Cell Growth Controls

Chairperson: C. Prives, *Columbia University*

Genetic Models

Chairperson: S. Cory, *Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Victoria, Australia*

Signaling by Proto-oncogenes

Chairperson: M.-C. King, *University of California, Berkeley*

Genetic Mechanisms and Neoplasia

Chairperson: M. Skolnick, *University of Utah*

Cell Surface and Metastasis

Chairperson: J.M. Bishop, *University of California, San Francisco*

Dorcas Cummings Lecture: "Why is it important to understand the genetic basis of cancer?"

Speaker: Harold Varmus, *Director, National Institutes of Health*

Cancer Genes

Chairperson: J. Sambrook, *McDermott Center, University of Texas Southwestern Medical School, Dallas*

Cancer Genes and Their Products

Chairperson: D. Hanahan, *University of California, San Francisco*

Apoptosis

Chairperson: J. Rowley, *University of Chicago*

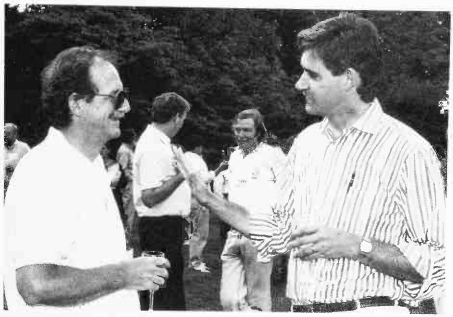
Checkpoints and Genome Stability

Chairperson: E. White, *Rutgers University*

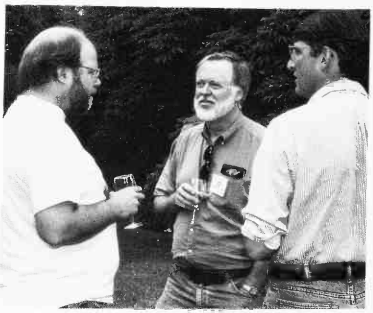
Finale

Chairperson: D. Lane, *University of Dundee, U.K.*

Summary: E. Harlow, *Massachusetts General Hospital Cancer Center, Charlestown*



T. Kelly, B. Stillman



E. Harlow, C. Harris III, D. Beach

MEETINGS

Zebrafish Development and Genetics

April 27–May 1 328 participants

ARRANGED BY **Wolfgang Driever,** Massachusetts General Hospital
Judith Eisen, University of Oregon
David Grunwald, University of Utah Medical School
Charles Kimmel, University of Oregon

This meeting was the first open-invitation meeting to deal specifically with the biology of the zebrafish, an organism that has recently become a major focus for studies of vertebrate biology. Sessions dealing with determinants of cell fate, early morphogenesis and patterning of the embryo, genetics and genomics, nervous system development, growth control, and organogenesis were led by prominent researchers in each field. In addition, one entire session was devoted to developing informational and technical resources that will be made available to the entire zebrafish research community and that would facilitate communication among workers in this field and aid in the development of their research programs.

A major breakthrough for genetic analyses of vertebrate development was announced at the meeting. The laboratories of Nüsslein-Volhard (Tübingen, Germany) and Driever (Boston, USA) reported the recovery of mutations in most of the genes that contribute to embryogenesis in the zebrafish. Many presentations described the initial characterizations of these mutants, including mutants that affected embryonic morphogenesis, how the retina sends topologically relevant signals to the brain, and how certain organ systems such as the heart and hematopoietic system are formed. Thus, it appears that studies with the zebrafish will for the first time offer us a way to study systematically the functions of single genes that are essential for vertebrate development and physiology.

This meeting was funded in part by the National Institute of Child Health and Human Development (a branch of the National Institutes of Health), National Science Foundation, Pet Warehouse, Applied Scientific Instrumentation, Inc., Marine Biotech, Inc., and Society for Developmental Biology.

PROGRAM

Keynote Speaker: Charles Kimmel, *University of Oregon*

Cell Fate
Chairperson: R. Ho, *Princeton University*

Genetics
Chairperson: C. Nüsslein-Volhard, *Max-Planck-Institut für Entwicklungsbiologie, Tübingen, Germany*

Methods
Chairperson: N. Hopkins, *Massachusetts Institute of Technology*

Gastrulation, Patterning, and Morphogenesis I
Chairperson: M. Westerfield, *University of Oregon, Eugene*

Gastrulation, Patterning and Morphogenesis II
Chairperson: N. Holder, *Kings College London, U.K.*

Growth Control
Chairperson: M. Schartl, *Biocenter, University of Würzburg, Germany*

W. Driever, S. Jesuthasan

Nervous System I
Chairperson: J. Campos-Ortega, *Institut für Entwicklungsbiologie, Universität Köln, Germany*

Nervous System II
Chairperson: J. Kuwada, *University of Michigan, Ann Arbor*

Organogenesis I
Chairperson: M.-A. Akimenko, *Loeb Institute for Medical Research, University of Ottawa, Ontario, Canada*

Community Organizing, Gestalt
Chairpersons: W. Driever, *Massachusetts General Hospital*; J. Eisen, *University of Oregon*; D.J. Grunwald, *University of Utah*

Organogenesis II
Chairperson: M. Fishman, *Massachusetts General Hospital*

N. Hopkins

Biology of Heat Shock Proteins and Molecular Chaperones

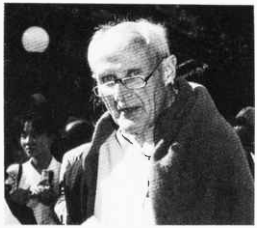
May 4–May 8 408 participants

ARRANGED BY **Costa Georgopoulos,** University of Geneva, Switzerland
Susan Lindquist, University of Chicago
Richard Morimoto, Northwestern University

This 4-day meeting attracted more than 400 participants during which 49 platform presentations and 281 posters were given. The topics covered the areas of (1) regulation of heat shock gene expression in prokaryotic and eukaryotic systems, (2) the GroEL, GroES, HSP60, and TCP-1 molecular chaperones, (3) the HSP70, DnaJ, and grpE family of chaperones, (4) HSP90 and other heat shock proteins and chaperones, (5) protein modification and proteolysis, and (6) the role of heat shock proteins and molecular chaperones in thermotolerance, immunobiology, infectious diseases, and pathophysiology. The 1994 meeting was recognized as a transition to more mechanistic studies on the activities of heat shock transcription factors, biochemical properties of heat shock proteins, and a more exacting understanding of their role in diverse cellular processes. The relationship between protein damage and proteolysis was highlighted by the addition of a new session to stimulate discussion and future research. Finally, the observations on the role of heat shock proteins in human diseases has achieved recognition for understanding how aberrations in the expression or function of heat shock proteins can profoundly influence cellular function.

This meeting was funded in part by the National Institute of General Medical Sciences, National Institute of Diabetes and Digestive and Kidney Diseases (both branches of the National Institutes of Health), National Science Foundation, Affinity BioReagents, Inc., Otsuka Pharmaceutical Factory, Inc., StressGen Biotechnologies Corp., Pharmaceutical Basic Research Laboratories, Japan Tobacco, Inc., and Kureha Chemical Industry Co., Ltd.

C. Georgopoulos, Cori



A. Tissieres

R. Morimoto

PROGRAM

Regulation of Heat Shock Gene Expression
Chairperson: T. Yura, *H.S.P. Research Institute, Kyoto, Japan*

Regulation of Heat Shock Gene Expression
Chairperson: C. Gross, *University of California, San Francisco*

groEL, HSP60, TCP1
Chairperson: E.A. Craig, *University of Wisconsin, Madison*

HSP70 Family, dnaJ, and grpE
Chairperson: F.-U. Hartl, *Memorial Sloan-Kettering Cancer Center*

HSP70 Family and dnaJ
Chairperson: I. Yahara, *Tokyo Metropolitan Institute of Medical Science, Japan*

HSP90
Chairperson: M.-J. Gething, *University of Texas Southwestern Medical Center*

Other HSPs and Chaperones
Chairperson: S. Gottesman, *National Cancer Institute, National Institutes of Health*

Protein Modification and Proteolysis
Chairperson: W. Neupert, *Institut für Physiologische Chemie, München, Germany*

Thermotolerance, Immunobiology, Infectious Diseases, and Pathophysiology
Chairperson: W.J. Welch, *University of California, San Francisco*

Genome Mapping and Sequencing

May 11–May 15 434 participants

ARRANGED BY **Richard Myers,** Stanford University
David Porteous, MRC Human Genetics Unit, Edinburgh, U.K.
Robert Waterston, Washington University School of Medicine

The primary impact of the HGP continues to be in the pursuit of human disease genes. The data and resources of the various mapping efforts have made possible virtually every recent disease gene hunt, and this was fully apparent in the highly competitive search for the breast cancer (BRCA I) gene.

The wealth of data in poster sessions was most impressive, indicating a new depth of field. Reports of the current status of the human mapping projects were done in a new format, with all large groups first presenting posters, followed by a round-table discussion. The poster session gave everyone a chance to review progress in detail. The quality of the recently released CEPH map came under scrutiny, and various problems in the product were raised. Individual chromosome projects, on the other hand, have benefited enormously from the CEPH megaYAC resource, but it is clear that they must be used with caution because of their very high rate of chimerism. The genetic linkage map, spearheaded by J. Weissenbach and the CHLC, continues to be a real success story with the ever-increasing density of informative markers.

Model organism genomes are also a bright spot. Several labs working on the yeast genome have coordinated their sequencing efforts and it seems likely that the entire sequence of the *S.*

D. Porteous, R. Meyers, R. Waterston

S. Brenner

cerevisiae genome will be known by early 1996 at the latest. The *C. elegans* consortium reported more than 5 Mb of completed genomic sequence, another landmark achievement. Groups on several organisms including *Drosophila* and *E. coli* have passed or are rapidly approaching the 1-Mb mark. Among new mapping methods, the single molecule restriction digests presented by D. Schwartz were particularly impressive and point to the possibilities of miniturization in the future.

The EST efforts continue to sample human genes, but the issue of public availability of commercially produced sequences remains a frustration among the wider genome community. Without the ability to compare and analyze the large amount of data held, it is difficult to know how many different genes are represented and what fraction of these may be of the total human complement. The best guesses seemed to indicate that perhaps as many as 30,000–40,000 different genes have been sampled, which might represent about half the full set.

Informatics is increasingly central to progress in genomics, and the communication between informatics and the rest of the community has improved markedly in recent years, as each group begins to appreciate the strengths and weaknesses of the other. The solid progress here was reflected in several excellent talks.

Although several novel approaches to sequencing were presented, it was clear from B. Roe's interminable talk that the current methods of shotgun sequencing followed by directed assembly and using fluorescence-gel-based methods remain powerful and can be satisfactorily applied to human DNA. Automation and optimization of such approaches may indeed prove sufficient to meet the challenge of sequencing the entire human genome.

This meeting was funded in part by the National Center for Human Genome Research (a branch of the National Institutes of Health).

PROGRAM

Human Diseases
Chairpersons: A. Bowcock, *Southwestern Medical Center;* E. Green, *Washington University*

Human Genome Center Poster Symposium
Chairpersons: H. Willard, *Case Western Reserve University;* D. Porteous, *Western General Hospital, Edinburgh*

Model Organisms
Chairpersons: R. Rothstein, *Columbia University College of Physicians & Surgeons;* R. Waterston, *Washington University School of Medicine*

Mapping and Other Methods
Chairpersons: D. Bentley, *The Sanger Centre, U.K.;* J. Weissenbach, *Généthon and Institut Pasteur, France*

cDNA and Gene Cloning
Chairpersons: B. Soares, *Columbia University;* R.M. Myers, *Stanford University*

Informatics
Chairpersons: D. States, *Washington University;* P. Little, *Imperial College, U.K.*

Sequencing and Sequencing Methods
Chairpersons: J.-I. Mao, *Collaborative Research, Inc.;* R. Gibbs, *Baylor College of Medicine*

The Cell Cycle

May 18–May 22 373 participants

ARRANGED BY **David Beach,** Cold Spring Harbor Laboratory
Charles Sherr, St. Jude Children's Research Hospital

After opening remarks by David Beach and Charles Sherr, the sessions were chaired by Fred Cross, Jim Roberts, Lee Johnston, David Morgan, David Beach, Michael Kastan, Mitsuhiro Yanagida, and Helen Piwnica-Worms. The program included scientists from research centers in Europe, the Far East, and the United States. They gathered to discuss their latest research concerning control of the cell cycle and cell proliferation. The program included 75 speakers who discussed such fundamental problems as control of mitosis; entry into the cell cycle; the role of anti-oncogenes and tumor suppressor genes in regulating cell growth; DNA replication; cell differentiation and cessation of proliferation; role of oncogenes and growth factors in control of cell proliferation; and chromosome behavior and division. The participating leading researchers in these areas

D. Glover, L. Bonita

D. Beach

of research use a variety of approaches from genetics to biochemistry to study control of the cell cycle. A variety of model systems from yeast to humans were discussed, and the meeting provided the opportunity to demonstrate how these different approaches complement each other. The meeting was one of great intensity and scientific interchange.

Contributions from Corporate Sponsors provided core support for this meeting.

PROGRAM

Yeast G^1 Control
Chairperson: F. Cross, *Rockefeller University*

Cyclins D and E
Chairperson: J. Roberts, *Fred Hutchinson Cancer Research Center*

Transcription at G_1/S
Chairperson: L. Johnston, *National Institute for Medical Research, London*

Cdk Regulators
Chairperson: D. Morgan, *University of California, San Francisco*

S Phase
Chairperson: D. Beach, *Cold Spring Harbor Laboratory*

Apoptosis/Checkpoints
Chairperson: M. Kastan, *Johns Hopkins University Medical School*

Mitosis
Chairperson: M. Yanagida, *Kyoto University, Japan*

Meiosis and Cdk5
Chairperson: H. Piwnica-Worms, *Harvard Medical School*

Retroviruses

May 24–May 29 432 participants

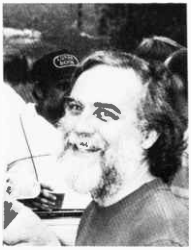
ARRANGED BY **Stephen Goff,** Columbia University
Joseph Sodroski, Dana-Farber Cancer Institute

The annual Retrovirus meeting, originally the RNA tumor virus meeting, stands as one of the longest-running continuously held meetings of Cold Spring Harbor. The 1994 meeting was the largest one to date, both in number of abstracts (372) and attendees; for the first time, applicants had to be turned away from a few large laboratories. The increase in applicants, although in part attributable to the recent expansion in AIDS research, also reflects the strong continued interest in this field. The retrovirus meeting thus brings many long-standing attendees from the early days of the field together with newly recruited HIV investigators.

This year, there were advances in several areas to be considered as highlights of the meeting. Virus receptors were the major focus of one session. Reports described the cloning and characterization of the receptors for several classes of leukemia viruses, including the avian leukosis viruses type A and the amphotropic murine leukemia viruses. The various receptors now known include a


J. Coffen


L. Ratner, P. Jolicouer


S. Goff


M. Eiden

surprising range of types of molecules; retroviruses seem to be able to evolve to the utilization of very diverse surface molecules to mediate their entry. Substantial efforts addressed how the preintegration complex enters the nucleus and suggested that two HIV proteins may facilitate that process in nondividing cells. There was also considerable interest in the viral integrase function, with enhanced understanding of the reactions it catalyzes and the mechanism of action. Finally, the process of virion assembly was advanced by the development of in vitro systems in which Gag proteins assemble to form fibers and particles closely resembling virus cores. All these findings bode well for the future development of antiviral therapies for the control of retroviral disease.

Contributions from Corporate Sponsors provided core support for this meeting.

PROGRAM

Receptors, Envelope and Virus Entry
Chairpersons: M. Eiden, *National Institute of Mental Health,* A.D. Miller, *Fred Hutchinson Cancer Research Center*

Reverse Transcription
Chairpersons: S. Hughes, *Frederick Cancer Research Facility, ABL-Basic Research Program, National Institutes of Health;* M. Roth, *UMDNJ—Robert Wood Johnson Medical School*

Nuclear Transport and Integration
Chairpersons: R. Craigie, NIDDK, *National Institutes of Health;* A. Skalka, *Fox Chase Cancer Center*

Expression I—Transcription
Chairpersons: B. Felber, *Frederick Cancer Research Facility, ABL-Basic Research Program, National Institutes of Health;* J. Lenz, *Albert Einstein College of Medicine*

Expression II—Post Transcriptional Gene Expression
Chairpersons: B. Cullen, *Howard Hughes Medical Institute, Duke University Medical Center;* D. Rekosh, *University of Virginia, Charlottesville*

Accessory Genes
Chairpersons: L. Ratner, *Washington University School of Medicine;* K. Strebel, *NIAID, National Institutes of Health*

Virus Assembly
Chairpersons: E. Hunter, *University of Alabama, Birmingham;* A. Rein, *Frederick Cancer Research Facility, ABL-Basic Research Program, National Institutes of Health*

Virus Assembly and Packaging
Chairpersons: E. Barklis, *Oregon Health Sciences University;* C. Carter, *State University of New York, Stony Brook*

Potpourri
Chairpersons: J. Elder, *Scripps Clinic and Research Foundation;* V. Hirsch, *LID, NIAID, National Institutes of Health*

Pathogenesis
Chairpersons: D. Derse, *NCI, National Institutes of Health;* G. Franchini, *NCI, National Institutes of Health*

Molecular Biology of Papovaviruses and Adenoviruses

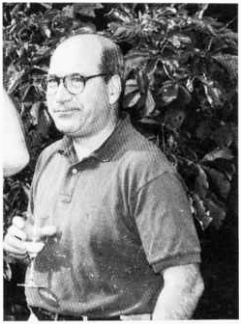
August 17–August 21 265 participants

ARRANGED BY **Terri Grodzicker,** Cold Spring Harbor Laboratory
Peter Howley, Harvard Medical School
David Livingston, Dana-Farber Cancer Institute
Carol Prives, Columbia University

The DNA tumor viruses—SV40, polyoma and adenoviruses—have served as important model systems to study cell growth and transformation as well as cellular transcription, replication, and RNA

P. Howley

T. Grodzicker



D. Livingstone

processing. In recent years, much work using these viruses as well as papillomaviruses have converged, especially with respect to the interaction of viral oncogene proteins with the products of cellular genes, including tumor suppressor genes. Thus, it was most welcome for the meeting to include once more, after a gap of many years, work on the molecular biology of papillomaviruses. A total of 265 scientists gathered at the 1994 meeting to present their latest results.

Much of the emphasis of the meeting concerned the interaction of viral regulatory with cellular proteins. Thus, many talks addressed interactions of viral nuclear proteins such as E1A, SV40 T antigen, and papillomavirus E6 and E7 proteins with Rb and its relatives, p107 and p130; p300 and p53. Studies of interactions of cytoplasmic and membrane-bound proteins such as the PDGF receptor with the BPV-1 E5 oncoprotein and polyoma middle T antigen with proteins involved in signal transduction pathways also received attention. Adenovirus E1A and E1B proteins have come to serve as models for inducers and inhibitors of apoptosis, and several talks and posters addressed this area of research. Much research continues to focus on the analysis of cellular proteins involved in viral DNA replication and their interaction with each other and viral replication proteins. A number of presentations addressed interactions of this type such as PCNA with the cdk inhibitor p21 and RPA with p53 and their likely role in the regulation of replication. The study of transcriptional regulation and interactions of viral E1A proteins, T antigenes, and E2 proteins with components of the transcriptional apparatus using in vitro as well as in vivo systems was extensively discussed. Since several of the cellular transcription factor targets of viral proteins (e.g., p53, E2F, p300) are transcription regulators, these studies addressed issues of growth control as well. The meeting provided a useful and interesting forum for the discussion of new results, and the inclusion of talks and posters on papillomaviruses was most valuable and will be continued in the future.

Contributions from Corporate Sponsors provided core support for this meeting.

PROGRAM

Replication I
Chairperson: L. Chow, *University of Alabama*

Transcription I
Chairperson: W. Herr, *Cold Spring Harbor Laboratory*

Transformation I
Chairperson: T. Roberts, *Dana-Farber Cancer Institute*

Transformation II
Chairperson: D. Galloway, *Fred Hutchinson Cancer Research Center*

Transcription II
Chairperson: J. Alwine, *University of Pennsylvania*

Replication II
Chairperson: J. Hassell, *McMaster University*

Posttranscriptional Regulation
Chairperson: G. Akusjarvi, *Karolinska Institute, Stockholm, Sweden*

Virus-Host Cell Interactions
Chairperson: E. White, *Rutgers University*

Translational Control

August 24–August 28 379 participants

ARRANGED BY **Allan Jacobson,** University of Massachusetts Medical School
Michael Mathews, Cold Spring Harbor Laboratory
Debbie Steege, Duke University Medical Center

The large number of participants at this fourth meeting attests to the vigor of this field and the popularity of the meeting. As has been the case of past meetings, the 1994 meeting encompassed animal, plant, viral and prokaryotic systems, although the continued downward trend in representation from prokaryotic research was disappointing. The meeting continued its focus on controls at each of the steps of translation, the factors, and on translation mechanisms. An increased focus on additional areas was well received by the participants, including developmental regulation mRNA stability, RNA-protein interactions, kinases, and signal transduction.

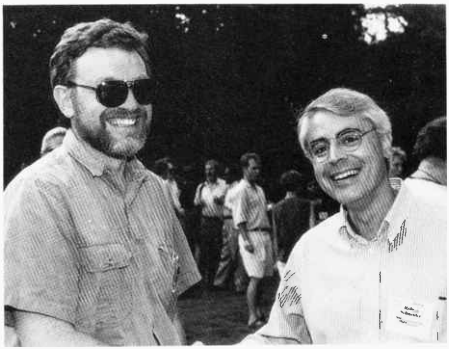
Over the course of the meeting, many excellent talks and posters were presented. One highlight of the developmental regulation session was that RNA localization is emerging as a mechanism for gradient formation in the egg essential for pattern formation. It is also becoming evident that addition and removal of poly(A) tails on mRNA contributes to complex patterns of translational control. A second area of very rapid progress is mRNA turnover in eukaryotic cells, where several possibly related pathways appear to operate. One is initiated by deadenylation and decapping, a second responds to the presence of nonsense codons within the body of the mRNA, and a third responds to *cis*-acting AU-rich elements in the 3′-untranslated region. In the area of initiation, very exciting evidence was presented that eukaryotic ribosomes can translate RNA circles many consecutive rounds, arguing that the free 5′ end of an mRNA is not necessarily the entry point for the small subunit. Evidence is increasing that the 5′ and 3′ ends of eukaryotic mRNAs communicate in some way during translation. Perhaps the newest development in frameshifting is the case in mammalian ornithine decarboxylase antizyme, which appears to be subject to regulation by polyamines. A highlight of the session on translation factors was the discovery of p67, a glycoprotein that functions to protect eIF-2 from inactivation through phosphorylation and is itself subject to regulation at the mRNA level and via O-linked gly-cosylation. A final highlight was the very rapid progress being made in developing second-generation models for the three-dimensional folding of 16S and 23S rRNAs. Much progress is expected during the next 2 years in these and many other areas, a promise that the 1996 Translational Control Meeting will be a great success.

This meeting was funded by RiboGene, Inc. and ICN Biomedicals, Inc.

A. Yoshida, S. Fridman

PROGRAM

Developmental Regulation
Chairperson: M. Wickens, *University of Wisconsin, Madison*
mRNA Stability
Chairperson: R. Parker, *University of Arizona, Tucson*
Initiation
Chairperson: P. Sarnow, *University of Colorado, Denver*
Elongation and Termination
Chairperson: H. Engelberg-Kulka, *Hebrew University-Hadassah Medical School, Jerusalem, Israel*
Kinases
Chairperson: R. Kaufman, *University of Michigan Medical Center, Ann Arbor*
Factors
Chairperson: W. Merrick, *Case Western Reserve University, Cleveland, Ohio*
Ribosomes and RNA-protein Interactions
Chairperson: T. Fox, *Cornell University, Ithaca, New York*
Viral and Cellular Regulatory Mechanisms
Chairperson: E. Ehrenfeld, *University of California, Irvine*



M. Mathews, R. Schneider

Mouse Molecular Genetics

August 31–September 4 429 participants

ARRANGED BY **Anton Berns,** Netherlands Cancer Institute, The Netherlands
Robb Krumlauf, MRC National Institute for Medical Research, U.K.
Andy McMahon, Harvard University
Elizabeth Robertson, Harvard University

There were eight sessions chaired by Bill Dove, Gail Martin, Mario Capecchi, Nadia Rosenthal, Mary Beth Hatton, Luis Parada, Michael Brown, and David Houseman. To complement talks presented by the chairs, each session also included a lecture from invited outside speakers: Rick Woychik, Scott Fraser, Alex Joyner, Marc Tessier-Lavigne, Ashley Dunn, and Neal Copeland.

The participants of the meeting included leading international scientists from North America, Europe, Australia, and the Far East. In addition to the invited chairs/speakers, emphasis was

A. Berns, R. Krumlauf, E. Robertson, A. McMahon

placed on young scientists to provide them with an opportunity to discuss their latest results in diverse areas of mouse molecular genetics, and 67 talks from participants were added to the program. Active areas of discussion included mouse models for human diseases, genetics, lineage analysis, oncogenesis, pattern formation, embryogenesis, organogenesis, cell-cell interactions, receptors and signals, mouse genome projects, mutagenesis, gene regulation, development of the nervous system, and imprinting. To address the growing need to follow the fate of specific cells, a lineage tracing workshop was held to provide information and help on the various types of lineage analysis that might be useful in a number of developmental systems. Although the meeting stressed the value of mouse molecular genetics, there was also an underlying theme of how the results emerging from mouse research relates to other vertebrate systems and how other systems can be used to expand the potential of the mouse as a genetic approach. The meeting provided a stimulating and intense environment for young scientists to exchange information and gain insight into the diverse ways the mouse molecular genetic approach can advance their research goals.

This meeting was funded in part by the Genetics Institute, National Science Foundation, and the following branches of the National Institutes of Health: National Institute of Child Health and Human Development, National Cancer Institute, National Institute of Neurological Disorders and Stroke, and National Institute of General Medical Sciences.

PROGRAM

Genetics
Chairperson: B. Dove, *McArdle Laboratory for Cancer Research, University of Wisconsin, Madison*

Embryogenesis/Lineage
Chairperson: G.R. Martin, *University of California, San Francisco*

Patterning
Chairperson: M. Capecchi, *Howard Hughes Medical Institute, University of Utah, Salt Lake City*

Organogenesis/Muscle
Chairperson: N. Rosenthal, *Massachusetts General Hospital, Charlestown*

Neural Development
Chairperson: M.E. Hatten, *Rockefeller University, New York*

Signals and Receptors
Chairperson: L. Parada, *NCI-Frederick Cancer Research and Development Center, Frederick, Maryland*

Models of Human Diseases
Chairperson: M. Brown, *University of Texas Southwestern Medical Center, Dallas*

Tumorigenesis
Chairperson: D. Housman, *Center for Cancer Research, Massachusetts Institute of Technology, Cambridge*

Epstein-Barr Virus and Associated Diseases

September 7–September 11 307 participants

ARRANGED BY **Joseph Pagano,** University of North Carolina, Chapel Hill
Alan Rickinson, University of Birmingham Medical School, U.K.

The 1994 Cancer Cells meeting was combined with the biannual international meeting of the Epstein-Barr Virus Association to focus on the basic virology and disease associations of the Epstein-Barr virus. The meeting encompassed a broad range of subjects from regulation of viral gene expression to molecular mechanisms of cell immortalization by the Epstein-Barr virus. The bimodal pathways for replication of the virus leading to latent infection, on the one hand, or to virus production, on the other hand, were explored. The pathobiology and immunobiology of EBV infection are complex topics that provide the setting for diseases caused by or associated with EBV and host responses in these diseases. The conference was unusual because it spanned both basic molecular mechanisms and pathobiology of disease. Knowledge about the pathogenesis of EBV lymphomas and nasopharyngeal carcinomas at the molecular pathogenetic level is rapidly emerging and now includes information on the role of EBV in Hodgkin's disease. Finally, newly emerging information was presented on attempts to treat EBV diseases as well as to fashion EBV recom-

H. Zur Hausen, J. Pagano

A. Rickinson, T. Scully

binogenic vaccines. The conference included either as speakers or chairpersons virtually every major researcher in the field plus a large number of new young investigators. Of the invited speakers, more than 50% were invited for the first time to speak at the EBV meeting, and about 80% of the speakers giving short oral presentations were first-timers. Most thought that this was the best International EBV Conference ever held. The meeting was greatly facilitated by the excellent facilities and staff of the Cold Spring Harbor Meetings Office.

This meeting was funded in part by the National Cancer Institute, a branch of the National Institutes of Health; the Burroughs Wellcome Co., Cutter Biomedicals, Miles, Inc., Granbio, Inc., INCSTAR Corp., Viral Antigens, Inc., and The Wellcome Foundation Ltd.

PROGRAM

Welcoming Remarks: Bruce Stillman, *Director, Cold Spring Harbor Laboratory;* Joseph S. Pagano, M.D., *President, International Association for Research on Epstein-Barr Virus and Associated Diseases*

Gertrude and Werner Henle Lecture on Viral Oncology
Presented by: Professor Harald zur Hausen, *German Cancer Research Center, Heidelberg*

Special Lecture: A. Levine, *Dept. of Molecular Biology, Princeton University*

Transformation and Oncogenesis
Chairperson: G. Bornkamm, *Institut für Klinische Molekularbiologie, Hämatologikum der GSF, Munich, Germany*

Replicative Gene Expression
Chairpersons: L. Joab, *CNRS, Institut Gustave Roussy, Villejuif, France;* S. Kenney, *Lineberger Comprehensive Cancer Center, University of North Carolina*

Replication and Glycoproteins
Chairpersons: M. Nonoyama, *Tampa Bay Research Institute, St. Petersburg, Florida;* W. Hammerschmidt, *Institut für Klinische Molekularbiologie und Tumorgenetik, München, Germany*

Transformation and Oncogenesis II
Chairpersons: A. Rickinson, *Institute of Cancer Studies, University of Birmingham Medical School, U.K.;* B. Sugden, *McArdle Laboratory for Cancer Research, University of Wisconsin, Medical School, Madison*

Special Lecture: D. Livingston, *Sidney Farber Cancer Research Institute, Dept. of Medical Oncology, Harvard University*

Immunobiology and Pathobiology
Chairpersons: D. Moss, *Queensland Institute of Medical Research, Brisbane, Australia;* G. Pearson, *Georgetown University Medical Center, Washington, District of Columbia*

Immunobiology and Pathology
Chairpersons: E. Klein, *Microbiology and Tumorbiology Center, Karolinska Institutet, Stockholm, Sweden;* T. Tursz, *Institut Gustave Roussy and Institut de Recherche sur le Cancer, Villejuif, France*

Lymphomas
Chairpersons: D. Crawford, *London School of Hygiene and Tropical Medicine, U.K.;* I. Magrath, *National Cancer Institute, National Institutes of Health, Bethesda, Maryland*

Latent Gene Expression
Chairpersons: I. Ernberg, *Microbiology and Tumor Biology Center, Karolinska Institutet, Stockholm, Sweden;* P. Farrell, *Ludwig Institute for Cancer Research, St. Mary's Hospital Medical School, London, U.K.*

Nasopharyngeal Carcinoma and Epithelial Infection
Chairpersons: M. Ng, *University of Hong Kong, Republic of China;* U. Prasad, *University of Malaya, Kuala Lumpur, Malaysia*

Intervention and Diagnosis
Chairpersons: E. Littler, *Wellcome Foundation, Kent, U.K.;* H. Wolf, *Institute for Medical Microbiology, University of Regensberg, Germany*

Gene Therapy

September 21–September 25 382 participants

ARRANGED BY **Theodore Friedmann,** University of California, San Diego
Y.W. Kan, University of California, San Francisco
Richard Mulligan, Massachusetts Institute of Technology and Whitehead Institute

Gene therapy has become an established driving concept in modern medicine and is rapidly progressing toward practical clinical application. Because of the importance of this new field of medicine, Cold Spring Harbor Laboratory sponsored its second meeting on Human Gene Therapy. The meeting, organized by Theodore Friedmann, Y.W. Kan, and Richard Mulligan, included discussions of basic molecular genetic and cell biological studies for gene transfer into human and other mammalian cells and their therapeutic implications. Of special importance were descriptions of new and improved gene transfer techniques, both viral and nonviral, and very impressive advances toward modification of viral and nonviral gene delivery tools to allow targeted tissue-specific delivery of therapeutic genes in vivo. Applications toward cancer, AIDS, hematological disease and neurological disorders demonstrated clearly that the techniques and principles of human gene therapy will have increasingly important roles in the development of more effective therapies for a wide variety of genetic and acquired human diseases. Because of the importance of this area of biomedical research, a third meeting on Human Gene Therapy has been scheduled in 1996.

The following branches of the National Institutes of Health provided support for this conference: National Institute of Child Health & Human Development, National Cancer Institute, National Institute of Neurological Disorders & Stroke, National Institute of Allergy and Infectious Diseases, and the National Institute of Diabetes and Digestive and Kidney Diseases.

PROGRAM

Welcome and Orientation: Theodore Friedmann, *University of California, San Diego*

The Hematopoietic System
Chairperson: R. Mulligan, *Whitehead Institute for Biomedical Research and Massachusetts Institute of Technology*

Hematopoietic System: Other Vectors and Models
Chairperson: T. Friedmann, *University of California, San Diego*

Adenoviral Vectors
Chairperson: J. Wilson, *Institute for Human Gene Therapy, University of Pennsylvania, Philadelphia*

Gene Delivery
Chairperson: Y.W. Kan, *University of California, San Francisco*

Cancer
Chairperson: P. Greenberg, *University of Washington and Fred Hutchinson Cancer Research Center, Seattle*

Muscle Gene Therapy
Chairperson: H.M. Blau, *Stanford University School of Medicine*

Cell Grafting Approaches
Chairperson: H.M. Blau, *Stanford University School of Medicine*

New Developments and Models
Chairperson: R.M. Blaese, *National Institutes of Health*

Cancer, AIDS
Chairperson: G. Nabel, *Howard Hughes Medical Institute, University of Michigan, Ann Arbor*

T. Friedman

Y.-W. Kan

S. Leff, H. Blau

Ribosome Synthesis and Nucleolar Function

September 28–October 2 167 participants

ARRANGED BY **Richard Gourse,** University of Wisconsin, Madison
Robert Perry, Fox Chase Cancer Center
Barbara Sollner-Webb, Johns Hopkins University
Michael Wormington, University of Virginia

Ribosome synthesis involves the regulation of numerous processes, encompassing transcription by all three RNA polymerases, RNA processing, translation, RNA-protein interaction, and nucleocytoplasmic transport. In addition, the synthesis of ribosomal components is coordinated and balanced with the production of many other cell constituents. The regulation of ribosome synthesis also impacts on virtually every other cellular component, responding to regulatory networks that control cell growth, proliferation, and development.

Modern genetic and biochemical methodologies have greatly increased our knowledge about many aspects of ribosome biogenesis. These include the eukaryotic rRNA gene transcription machinery, rRNA maturation, and an emerging family of nucleolar snRNAs that likely are involved in rRNA processing and/or ribosome assembly. Recent studies are also revealing unexpected alternative roles for ribosomal proteins outside of the ribosome and the unexpected nucleolar localization of other known proteins. Thus, molecular mechanisms that govern ribosome biogenesis are being elucidated, but many key elements of how this regulation occurs and how these processes are integrated remain largely uncharacterized.

The Ribosome Synthesis and Nucleolar Function meeting was successful in bringing together researchers working on these various aspects of ribosome synthesis and nucleolar function in both prokaryotes and eukaryotes. Presentations were largely made by graduate students, postdocs, and young independent investigators.

This meeting was funded in part by the National Institute of General Medical Sciences, a branch of the National Institutes of Health.

PROGRAM

rRNA Biosynthesis
Chairperson: M. Nomura, *University of California, Irvine*

Regulation of rRNA Biosynthesis
Chairperson: R. Gourse, *University of Wisconsin, Madison*

rRNA Processing
Chairperson: B. Sollner-Webb, *Johns Hopkins School of Medicine*

Small Nucleolar RNAs: Synthesis and Function
Chairperson: J. Steitz, *Howard Hughes Medical Institute, Yale University*

Synthesis and Regulation of Ribosomal Proteins
Chairperson: B. Perry, *Fox Chase Cancer Center*

Function and Organization of Ribosomal Components
Chairperson: M. Wormington, *University of Virginia, Charlottesville*

Localization, Assembly, and Activity of Nucleolar Proteins
Chairperson: G. Blobel, *Howard Hughes Medical Institute, Rockefeller University*

Nucleolar Organization
Chairperson: J. Warner, *Albert Einstein College of Medicine*

J. Steitz, L. Lindahl

M. Nomura

J. Warner

Molecular Approaches to the Control of Infectious Diseases

October 5–October 9 201 participants

ARRANGED BY **Fred Brown,** USDA, Plum Island Animal Disease Center
Robert Chanock, NIAID, National Institutes of Health
Harold S. Ginsberg, Columbia University College of Physicians & Surgeons
Erling Norrby, Karolinska Institute, Sweden

The 12th Annual Meeting on Modern Approaches to New Vaccines (now renamed Molecular Approaches to the Control of Infectious Diseases) was held in October. As in the previous year, keynote speakers introduced a diverse program which included sessions on Antibody Design and Therapy, DNA Immunization, Vectors for Vaccine Delivery, and Vaccine Design in addition to those dealing with individual bacteria, parasites, and viruses.

The renewed interest in vaccination worldwide has led to several innovative approaches to the design of vaccines. None has attracted more attention than the potential of using directly DNA coding for immunogenic proteins. Some of the experiments that were described have been spectacularly successful, and information on the way this type of approach provides protection is eagerly awaited. At the practical level, the view of control authorities such as the Food and Drug Administration on the use of DNA is awaited with some trepidation.

The sophisticated technology that is being applied to the design and production of antibodies in the test tube was also a hot topic at the meeting. In addition, there were extremely interesting and innovative presentations on the basic sciences underlying vaccines. It is somewhat invidious to pinpoint individual presentations in a meeting of such high quality. Nevertheless, it was refreshing to hear about such diverse topics as the expression of a variety of immunogens in plants and the protection of dogs against lethal parvovirus infection by a synthetic peptide, the first description of a successful peptide vaccine in the natural host. Maybe there is still hope for chemists in the field of vaccination. Pasteur would have been pleased.

The meeting also included the inaugural Albert B. Sabin Memorial Lecture. This was delivered by Dr. D.A. Henderson, Deputy Assistant Secretary for Health, who spoke on prospects for the global eradication of poliomyelitis. Dr. Henderson was an appropriate choice because he was a leading figure in the World Health Organization's successful campaign to eradicate smallpox—so far the only infectious disease that has been eradicated. These annual lectures will be sponsored by the Albert B. Sabin Vaccine Foundation, which is the creation of Mr. H.R. Shepherd.

This meeting was funded in part by American Cyanamid Company, Lederle-Praxis Biologicals Division; and Pharmacia LKB Biotechnology.

E. Norrby

H. Ginsberg, R. Chanock

Fred Brown

PROGRAM

Antibody Design and Therapy
Chairperson: D. Burton, *Scripps Research Institute*
DNA Immunization
Chairperson: H. Robinson, *University of Massachusetts*
Bacteriology and Mycology
Chairperson: J. Collier, *Harvard Medical School*
HIV–SIV
Chairperson: J. Berzofsky, *National Cancer Institute*
Vectors for Vaccine Delivery
Chairperson: R. Curtiss III, *Washington University*
Parasitology
Chairperson: A. Mahmoud, *Case Western Reserve University*
Vaccine Design
Chairperson: I. Ramshaw, *John Curtin School of Medical Research, Canberra, Australia*
Immunology
Chairperson: R. Zinkernagel, *Institute of Experimental Immunology, Zürich, Switzerland*
Virology
Chairperson: B. Murphy, *LID, NIAID, National Institutes of Health*
Albert B. Sabin Vaccine Foundation Inaugural Award Ceremony and Memorial Lecture
Introduction: Mr. David Luke III, *Cold Spring Harbor Laboratory*
Award Ceremony
Mr. H.R. Shepherd, *Albert B. Sabin Vaccine Foundation;* Dr. P.K. Russell, *Albert B. Sabin Vaccine Foundation;* Mrs. Heloisa Sabin
1994 Albert B. Sabin Memorial Lecture
Dr. Donald A. Henderson, *Deputy Assistant Secretary for Health, Department of Health and Human Services*
Closing Remarks: Mr. David Luke III, *Cold Spring Harbor Laboratory*

Learning and Memory

October 12—October 16 124 participants

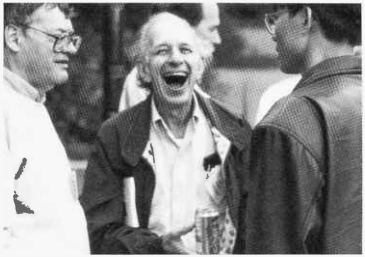
ARRANGED BY **Jack Byrne,** University of Texas Medical School
Richard Morris, University of Edinburgh Medical School, U.K.
Charles Stevens, The Salk Institute
Richard Thompson, University of Southern California, Los Angeles

The second biennial meeting on Learning and Memory at Cold Spring Harbor consisted of platform and poster presentations and discussions of current research and concepts in the biological basis of memory. Discussion focused on mechanisms underlying memory, including molecular genetic substrates, biophysical and biochemical cellular/synaptic processes, developmental processes, neural systems, and behavioral phenomena. One key aspect of the meeting was sessions seeking common ground between vertebrate and invertebrate systems, from which several principles that may have general relevance to adult plasticity emerged: short-term forms of learning and memory require changes in existing neural circuits, which may involve multiple cellular mechanisms within single neurons; second messenger systems appear to have a key role in mediating cellular changes; changes in the properties of membrane channels are commonly correlated with learning and memory; and long-term, but not short-term, memory requires new protein synthesis. Another key feature of the meeting was the new and rapidly developing field concerned with molecular genetic mechanisms of memory.

Yet another aspect of the meeting related neural mechanisms and systems to the behavioral phenomena of learning and memory. Widespread interest in associative synaptic plasticity (such as LTP) as a neural substrate of learning also prompted a reassessment of the enormous scope of associative learning principles with respect to such diverse phenomena as recognition memory, spatial learning, and the role of context in occasion-setting and retrieval and in instrumental habit-learning.

This meeting was funded in part by the National Science Foundation.

R. Morris



C. Stevens, E. Kandel, C. Chen

J. Byrne

PROGRAM

Introductory Comments: Jack Byrne, *University of Texas Medical School*

Higher Brain Systems and Memory
Chairperson: M. Mishkin, *National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland*

Basic Processes of Learning and Memory I
Chairperson: J.L. McGaugh, *University of California, Irvine*

Basic Processes of Learning and Memory II
Chairperson: R. Morris, *University of Edinburgh, U.K.*

Basic Processes of Learning and Memory III
Chairperson: R. Thompson, *University of Southern California, Los Angeles*

Brain Plasticity and Development
Chairperson: C. Shatz, *University of California, Berkeley*

Biophysics and Molecular Biology of Synaptic Transmission and Memory I
Chairperson: J. Byrne, *University of Texas, Houston*

Biophysics and Molecular Biology of Synaptic Transmission and Memory II
Chairperson: S. Tonegawa, *Massachusetts Institute of Technology*

Biophysics and Molecular Biology of Synaptic Transmission and Memory III
Chairperson: C. Stevens, *Salk Institute, La Jolla, California*

Biophysics and Molecular Biology of Synaptic Transmission and Memory IV
Chairperson: E. Kandel, *Columbia University, New York*

SPECIAL MEETING

A Decade of PCR: Celebrating Ten Years of Amplification

September 12–14

ARRANGED BY **Stan Rose,** Perkin-Elmer Corporation
David Stewart, Cold Spring Harbor Laboratory

This special meeting was held to commemorate the first decade since the discovery of the polymerase chain reaction by Nobel laureate Kary Mullis, who gave the opening perspective for the meeting. The polymerase chain reaction, which permits the faithful amplification of tiny quantities of DNA into manageable quantities, has transformed molecular biology, greatly expanding horizons in gene mapping and discovery, HIV research and diagnosis, human identity testing, and genetic analysis. This meeting brought together 221 practitioners in the field of DNA amplification ranging from basic studies of the thermostable enzymes and oligonucleotide primers that fuel the reaction, recent advances in automation, quantitation, and miniaturization, to applications ranging from criminal forensics to the study of human evolution and migration. Summarized by Maynard Olson, the meeting provided an exciting and informative review of PCR's first decade of application, while providing new insights into the future directions of this valuable technique. Videotapes of the meeting were made available through the Cold Spring Harbor Laboratory Press.

The meeting was wholly sponsored by The Perkin-Elmer Corporation. This represents a departure from the traditional Cold Spring Harbor meeting in that both the scheduling and choice of program and invited speakers were a joint decision of the Laboratory and the sponsor organization. The Perkin-Elmer Corporation has supported the Laboratory's program of meetings through the corporate sponsor program since 1989. This special meeting arose since the Laboratory has decided to offer corporations that have already demonstrated their support for academic meetings through the corporate sponsor program, the opportunity to sponsor individual technical meetings in Grace Auditorium.

S. Rose, J. Chamberlain

T. Caskey, J. Grace, K. Mullis, G. Kelly

PROGRAM

Introduction: J.D. Watson, *Cold Spring Harbor Laboratory*
Perspective: K. Mullis

An In-Depth Look at PCR
Chairperson: J. Chamberlain, *University of Michigan*

Primers, Oligos, and Hybridization, W. Rychlik, National Biosciences, Inc.
Biology of DNA Polymerases, T. Kunkel, National Institutes of Health
PCR Automation, Genotyping, S. Rose, Perkin-Elmer Corporation

Applications of PCR I
Chairperson: J. Chamberlain, *University of Michigan*

Human Genome Project, G. Evans, University of Texas
Human Genetics, H. Erlich, Roche Molecular Systems
Molecular Diagnostics, T. Caskey, Baylor College of Medicine

Applications of PCR II
Chairperson: S. Rose, *Perkin-Elmer Corporation*
Forensic Analysis, B. Budowle, FBI Academy
Ancient DNA/Gene Evolution, S. Paabo, University of Munich
Agriculture and the Third World, R. Jefferson, CAMBIA
Gene Expression in Single Cells, J. Eberwine, University of Pennsylvania
In Situ PCR, A. Haase, University of Minnesota
Combinatorial Libraries and Rapid Evolution, A. Ellington, Indiana University
PCR and Education, D. Micklos, Cold Spring Harbor Laboratory

The Future of PCR
Chairperson: R. Gibbs, *Baylor College of Medicine*
Transposon PCR, E. Rose, Perkin-Elmer Corporation
PCR Quantitation, F. Ferre, The Immune Response Corporation
Analysis of PCR Products in Microchips, S. Fodor, Affymetrix
RNA Differential Display, P. Liang, Dana Farber Cancer Institute
Representational Difference Analysis, N. Lisitsyn, Cold Spring Harbor Laboratory
Summary, M. Olson, *University of Washington*

POSTGRADUATE COURSES

The summer program of Postgraduate Courses at Cold Spring Harbor Laboratory is aimed at meeting the special need for training in interdisciplinary subjects that are either so new or so specialized that they are not adequately treated by universities. Our aim is to provide intensive study in the most recent developments and techniques in these subjects and to prepare students to enter directly into research in a particular area. To ensure up-to-date coverage of current research work, we bring together a workshop staff from many laboratories around the world and supplement this staff with a series of seminar speakers.

Early Development of *Xenopus laevis*

April 6–13

INSTRUCTORS
Grainger, Robert, Ph.D., University of Virginia
Sive, Hazel, Ph.D, Whitehead Institute

ASSISTANTS
Amaya, Enrique, University of California, Berkeley
Papalopulu, Nancy, Salk Institute for Biological Studies

This course provided extensive laboratory exposure to the biology and manipulation of embryos from the frog, *Xenopus laevis*. The course was particularly suited for investigators who had current training in molecular biology and knowledge of developmental biology, but had no experience with *Xenopus*. Intensive laboratory sessions were supplemented by daily lectures from experts in both experimental and molecular embryology. Six main areas were covered: (1) care and handling of adults and embryos, (2) stages of embryonic development and anatomy, (3) whole mount in situ hybridization and immunocytochemistry, (4) microinjection of eggs and oocytes, (5) micromanipulation of embryos, and (6) induction assays. Lecturers and instructors included E. Amaya, R. Elinson, J. Gurdon, R. Harland, R. Keller, N. Papalopulu, and C. Wylie.

PARTICIPANTS

Alonso, D., M.S., Conicet, Argentina
Bellefroid, E., Ph.D., George August Universitat, Germany
Beltman, J., Ph.D., Stanford University School of Medicine
Delvecchio, R., Ph.D., Cold Spring Harbor Laboratory
Guadagno, S., Ph.D., University of California, San Diego
Holland, L., Ph.D, University of Missouri School of Medicine
Huard, V., B.S.c., Hotel-Dieu de Quebec, Canada
Jones, T., Ph.D., National Cancer Institute
Kabler, B., M.D., M.S.c., University of Pisa, Italy
Lagna, G., Ph.D., Rockefeller University
Moos, M., M.D., Ph.D., Food & Drug Administration
Neilson, K., M.S., American Red Cross
Puccini, D., University of Genova, Italy
Shain, D., M.S., Colorado State University
Strobel, S., Ph.D., University of Colorado
Zhang, J.J., M.S., Mt. Sinai School of Medicine

SEMINARS

Amaya, E., University of California, Berkeley. Pseudogenetic analysis of *Xenopus* development.
Elinson, R., University of Toronto. Cytoplasmic organization of the egg and dorsoventral polarity of the embryo.
Grainger, R., University of Virginia. Completing the body plan: Inductive mechanisms leading to formation of particular tissues and organs.
Gurdon, J., Wellcome/Cancer Research Campaign Institute. From egg to muscle: Localized determinants or cell interactions?
Harland, R., University of California, Berkeley. Molecular nature of Spemann's organizer: Role of peptide growth factors.
Keller, R., University of California, Berkeley. Morphogenesis of the early *Xenopus* development.
Papalopulu, N., Salk Institute. Neuroanatomy and neural development in *Xenopus*.
Sive, H., Massachusetts Institute of Technology. Anteroposterior patterning in *Xenopus*.
Wylie, C., University of Minnesota. Early development and maternal control of development in *Xenopus*.

Protein Purification and Characterization

April 6–19

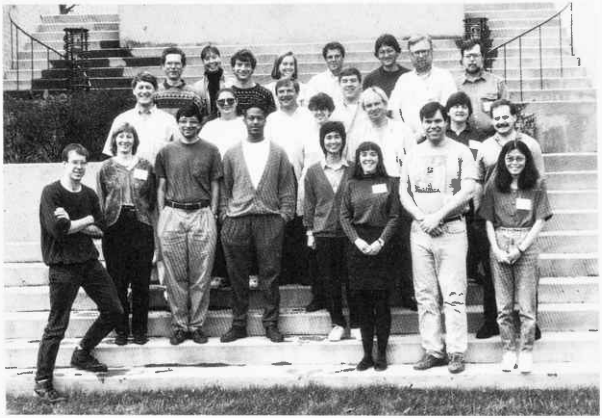
INSTRUCTORS

Burgess, Richard, Ph.D., University of Wisconsin, Madison
Kadonaga, James, Ph.D., University of California, San Diego
Lin, Sue-Hwa, Ph.D., M.D., Anderson Cancer Center, University of Texas
Marshak, Daniel, Ph.D., Cold Spring Harbor Laboratory

ASSISTANTS

Burke, Thomas, University of California, San Diego
Carpino, Nicholas, Cold Spring Harbor Laboratory
Earley, Karen, University of Texas
George, Catherine, University of California, San Diego
Grabski, Anthony, University of Wisconsin, Madison

This course was intended for scientists who are not familiar with techniques of protein isolation and characterization. It was a rigorous program that included laboratory work all day and a lecture with discussion session every evening. Each student became familiar with each of the major techniques in protein purification by actually performing four separate isolations including: (1) a regulatory protein from muscle tissue; (2) a sequence-specific, DNA-binding protein; (3) a recombinant protein overexpressed in *Escherichia coli*; and (4) a membrane-bound receptor. A variety of bulk fractionation, electrophoretic, and chromatographic techniques were employed including precipitation by salts, pH, and ionic polymers; ion exchange, gel filtration, hydrophobic interaction, and reverse-phase chromatography; lectin affinity, oligonucleotide affinity, and immunoaffinity chromatography; polyacrylamide gel electrophoresis, and electroblotting; and high-performance liquid chromatography. Procedures were presented for solubilizing proteins from inclusion bodies and refolding them into active monomeric forms. Methods of protein characterization were utilized, in-



cluding immunological and biochemical assays, 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, modifications of proteins, methodologies for protein purification and characterization, and applications of protein biochemistry to cell and molecular biology. Guest lecturers included K. Collins, N. Pace, Y. Paterson, G. Rose, J. Rothman, B. Stillman, and N. Tonks.

PARTICIPANTS

Chung, B.-C., Ph.D., Duke University
Corda, D., Ph.D., Consorzio Mario Negri Sud., Italy
Futcher, B., Ph.D., Cold Spring Harbor Laboratory
Genetta, T., Ph.D., University of Pennsylvania Medical School
Grether, M., Ph.D., Massachusetts Institute of Technology
Hans, S., University of California, San Francisco
MacDonald, M.E., Ph.D., Massachusetts General Hospital
McCarthy, D., Ph.D., University of Oklahoma
Mohnen, D., Ph.D., University of Georgia
Parent, S., Ph.D., Merck Research Laboratories
Paty, P., M.D., Memorial Sloan-Kettering Cancer Center
Rawson, R., Ph.D., University of Texas Southwestern Medical Center
Rupp, B., Ph.D., Lawrence Livermore National Laboratory
Vasil, M., Ph.D., University of Colorado Medical School
Warrior, R., Ph.D., University of Southern California
Williams, K., B.S., Meharry Medical College

SEMINARS

Burgess, R., University of Wisconsin, Madison. (1) Protein purification: Basic methods. (2) Protein purification: Advanced methods.
Collins, K., Cold Spring Harbor Laboratory. Purification of telomerase.
Kadonaga, J., University of California, San Diego. Purification of transcription factors.
Lin, S.-H., University of Texas. Cell surface proteins.
Marshak, D., Cold Spring Harbor Laboratory. Applications to microsequencing.
Pace, N., Texas A&M University. Thermodynamics of protein folding.
Paterson, Y., University of Pennsylvania. Designing synthetic antigens.
Rose, G., Washington University. Fundamentals of protein structure.
Rothman, J., Memorial Sloan-Kettering Institute. Protein trafficking.
Stillman, B., Cold Spring Harbor Laboratory. Eukaryotic DNA replication proteins.
Tonks, N., Cold Spring Harbor Laboratory. Protein tyrosine phosphatases.

Cloning and Analysis of Large DNA Molecules

April 6–19

INSTRUCTORS

Abderrahim, Hadi, Ph.D., Cell Genesys, Inc.
Birren, Bruce, Ph.D., California Institute of Technology
Vollrath, Doug, Ph.D., Stanford University

ASSISTANTS

Dewer, Ken, University of Laval, Canada
Gschwend, Michele, Stanford University School of Medicine
Mendez, Michael, Cell Genesys, Inc.

This course covered the theory and practice of manipulating and cloning high-molecular-weight DNA. The course focused on the use of yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), and bacteriophage P1 cloning systems for library construction and techniques of pulsed-field gel electrophoresis (PFGE). Lectures and laboratory work included an introduction to yeast genetics, the isolation and manipulation of high-molecular-weight DNA from a variety of sources, and preparative and analytical PFGE. YAC, BAC, and P1 clones were produced and characterized by a number of approaches, including library screening, contig assembly, long-range restriction mapping, and recovery of YAC ends. A variety of size standards for PFGE were prepared and their separations on many different types of PFGE gel boxes were compared. Lectures by outside speakers on topics of current interest supplemented the laboratory work.

PARTICIPANTS

Bentley, E., B.S., University College London, United Kingdom
Berx, G., University of Ghent, Belgium
Carriero, F., B.S., Metapontum Agrobious, Italy
Du, L., Ph.D., Shriner's Hospital, Canada
Fulmek, S., M.D., Mt. Sinai Medical Center
Galli, J., Ph.D., Karolinska Institute, Sweden
Leung, M., B.S., Tulane University Medical Center
Lindpainter, K., M.D., Children's Hospital
Liu, Q., Ph.D., Myriad Genetics, Inc.
Millar, A., Ph.D., University of British Columbia, Canada
Peichel, C., M.A., Princeton University
Quackenbush, J., Ph.D., Salk Institute
Schutte, M., Ph.D., Johns Hopkins University
Smith, E., Ph.D., Tuskegee University
Swanson, K., B.S., Research Genetics
Wight, D., Ph.D., Ohio University

SEMINARS

Lai, E., University of North Carolina, Chapel Hill. Physical mapping using PFGE and BAC cloning.
Lisitsyn, N., Cold Spring Harbor Laboratory. Representational differential analysis.
Lovett, M., University of Texas Southwestern Medical College. Identification of genes from large genomic regions by cDNA selection.
Myers, R., Stanford University. Integrated mapping approaches for the Human Genome Project.
Shepherd, N., DuPont Merck Pharmaceutical Company. P1 cloning: Principles and applications.
Shizuya, H., California Institute of Technology. The BAC system: Library construction, clone analysis, and mapping.
Strauss, W., Whitehead Institute/MIT Center for Genome Research. Construction of transgenic mice using YACs.
Strobel, S., University of Colorado. Chromosome dissection: Chemical and enzymatic techniques for cleaving complex DNA at unique sites.

Advanced Bacterial Genetics

June 10–30

INSTRUCTORS

Maloy, Stanley, Ph.D., University of Illinois, Urbana
Stewart, Valley, Ph.D., Cornell University
Taylor, Ronald, Ph.D., Dartmouth Medical School

ASSISTANTS

Lin, Janine, Cornell University
Pfau, James, Dartmouth Medical School
Muro-Pastor, Alicia, University of Illinois, Urbana

This laboratory course demonstrated genetic approaches that could be used to analyze biological processes and their regulation, as well as detailed structure/function relationships of genes. Techniques covered included isolation, characterization, and mapping of mutations; use of transposable genetic elements as mutagens, linked selectable markers and portable regions of homology; construction and analysis of operon and gene fusions; use of bacteriophage in genetic analysis; molecular cloning and restriction endonuclease mapping; allele exchange; Southern blot analysis; polymerase chain reaction; and site-specific mutagenesis. The course consisted of a series of experiments that employed these techniques in the genetic analysis of diverse bacterial and bacteriophage species. Lectures and discussions concentrated on the application of genetic analysis to contemporary questions in bacterial physiology, diversity, and pathogenesis. Guest lecturers were J. Beckwith, V. Miller, I. Herskowitz, A. Grossman, and N. Trun.

PARTICIPANTS

Barsom, L., B.S., University of Pittsburgh
Boot, H., M.S., University of Amsterdam, The Netherlands
Calia, K., M.D., Massachusetts General Hospital
Dominguez, A., M.D., Hospital Bellvitge, Spain
Feng, X., M.S., Washington State University
Gold, H., M.D., New England Deaconess Hospital
Gupte, G., M.S., Arizona State University
Hendrick, J., Ph.D., Memorial Sloan-Kettering Cancer Center
Hentschel, U., B.S., University of California, San Diego
Holmes, A., M.D., Boston University
Hoover, T., Ph.D., University of Georgia
Krajewska-Gynkiewicz, K., Ph.D., Polish Academy of Sciences, Poland
Macfadyen, L., B.S., University of British Columbia, Canada
Madison, L., B.S., Harvard Medical School
Troup, B., M.S., Philipps Universitat Marburg, Germany
Van Dissel, J., Ph.D., University Hospital Leiden, The Netherlands

SEMINARS

Bassler, B., The Agouron Institute. Intercellular communication in luminous bacteria: The glow of social interaction.
Highlander, S., Baylor College of Medicine. Using gene fusion in *E. coli* to identify and characterize regulators of *Pasteurella haemolytica* leukotoxin expression.
Silhavy, T., Princeton University. Genetic analysis of protein secretion in *E. coli*.
Shuman, H., Columbia University. Genetic analysis of *Legionella:* Identification of genes required for intracellular manipulation and host cell killing.
Winkler, M., University of Texas Medical School. Mediation of spontaneous mutagenesis and global control by genes required for intracellular multiplication and host killing.
Westpheling, J., University of Georgia. Transcriptional regulation of genes for carbon utilization and morphogenesis and streptomyces.

Molecular Approaches to Ion Channel Structure, Expression, and Function

June 10–30

INSTRUCTORS

Margulies, Jody, Ph.D., University of California, Los Angeles
Robertson, Gail, Ph.D., University of Wisconsin
Ruben, Peter, Ph.D., University of Hawaii

ASSISTANT

Henteleff, Mark, University of Hawaii

This intensive laboratory/lecture course was designed to introduce students to the combined use of molecular biological techniques and electrophysiological analysis for the study of ligand-gated and voltage-gated ion channels. The course covered expression of cloned channels and receptors in

Xenopus oocytes and cultured cells, including in vitro preparation of RNA transcripts, microinjection into oocytes, site-directed mutagenesis, and characterization of channels and receptors using two-electrode voltage clamp and patch clamp. Lectures covered molecular biology and electrophysiology techniques as well as the theory and analysis of ionic currents. Students were encouraged to bring their own samples to study using the techniques taught in the course. Guest lecturers included J. Adelman, O. Andersen, F. Bezanilla, R. MacKinnon, G. Mandel, F. Sigworth, J. Trimmer, and M. White.

PARTICIPANTS

Berthelier, V., B.S., CNRS, France
Cheffings, C., B.A., University of Oxford, United Kingdom
Fraser, D., B.S., University of Calgary, Canada
Huang, C.-C., M.S., University of Oxford, United Kingdom
Linne, M.-L., M.S., University of Tampere, Finland
Lundbaek, J., M.D., Cornell University
Moroni, A., Ph.D., University of Milan, Italy
Piedras-Renteria, E., M.S., University of Illinois, Urbana
Rosenthal, J., B.S., Brown University
Wolff, M., M.S., Rhone Poulec AG Company
Yun, L., M.D., Karolinska Institute, Sweden
ZhuGe, R., D.V.M., Iowa State University

SEMINARS

Bennett, M., Albert Einstein College of Medicine. NMDA receptors.
Bezanilla, F., University of California, Los Angeles. Voltage-dependent gating.
Czajkowski, C., Columbia University. Mapping the agonist binding site and channel of the nicotinic Ach receptor.
Goldin, A., University of California, Irvine. Sodium channel inactivation.
MacKinnon, R., Harvard Medical School. Structure/function studies in cation selective channels.
Mandel, G., SUNY, Stony Brook. Molecular studies of neural-specific sodium channels.
Margulies, J., University of California, Los Angeles. Functional analysis of novel signaling proteins (RC3 and Gamma 7) in oocytes.
Nerbonne, J., Washington University School of Medicine. Molecular basis of functional K^+ channel diversity.
Papazian, D., University of California, Los Angeles. Complementation of folding defects identifies electrostatic interactions of S4 voltage sensor in Shaker K channel.
Robertson, G., University of Wisconsin, Madison. Gating and modulation in the eag family of potassium channels.
Ruben, P., University of Hawaii. (1) Introduction to voltage clamping. (2) Gating modes in rat brain sodium channels.
Sigworth, F., Yale University School of Medicine. How do voltage-gated channels work?
White, M., Medical College of Pennsylvania. Probing the pocket and pore of the Ach receptor.

Molecular Embryology of the Mouse

June 10–30

INSTRUCTORS

Mann, Jeff, Ph.D., Beckman Research Institute of the City of Hope
Soriano, Philippe, Ph.D., Fred Hutchinson Cancer Research Center

CO-INSTRUCTORS

Behringer, Richard, Ph.D., University of Texas
Papaioannou, Virginia, Ph.D., Columbia University

ASSISTANTS

Nichols, Jenny, University of Edinburgh, United Kingdom
Pease, Shirley, California Institute of Technology

This course was designed for biologists interested in applying their expertise to the study of mouse embryonic development. Laboratory components provided an intensive introduction into the technical aspects of working with and analyzing mouse embryos, and lecture components provided the conceptual basis for current research. Procedures that were described included isolation and culture of pre- and postimplantation embryos, oviduct and uterus transfer, formation of aggregation chimeras, isolation of germ layers in gastrulation-stage embryos, establishment, culture, and genetic manipulation of embryonic stem cell lines, in situ hybridization to whole mounts and sections of embryos, immunohistochemistry, microinjection of DNA into pronuclei, and microinjection of embryonic stem cells into blastocysts. Speakers were R. Behringer, A. Bradley, M. Bronner-Fraser, V. Chapman, P. Donovan, T. Gridley, B. Hogan, R. Jaenisch, N. Jenkins, T. Jessell, R. Lovell-Badge, G. Papaioannou, L. Parada, G. Rinchik, J. Rossant, R. Schultz, S. Strickland, P. Wassarman and D. Wilkinson.

PARTICIPANTS

Bates, G., Ph.D., UMDS, Guy's Hospital, United Kingdom
Battaglino, M.S., Mount Sinai School of Medicine
Bowles, J., Ph.D., University of Queensland, Australia
Brophy, P., Ph.D., University of Stirling, United Kingdom
Brusa, R., B.S., University of Heidelberg Germany
Hamre, K., Ph.D., University of Tennessee
Hanlon, D., Ph.D., University of California, San Diego
Heyer, B., B.S., Max-Planck Institute, Germany
Levi, G., Ph.D., INSERM U368, France
Lutz, B., Ph.D., Baylor College of Medicine
Mo, F.-E., M.S., University of Illinois
Panicker, M., Ph.D., Tata Institute, India
Peralta, D., Ph.D., University of Texas
Wang, Y., Ph.D., Johns Hopkins University

SEMINARS

Behringer, R., University of Texas. Research talk.
Bradley, A., Baylor College of Medicine. (1) Introduction to ES cells. (2) Homologous recombination in ES cells.
Bronner-Fraser, M., University of California, Irvine. Cell lineage in the neural crest.
Jenkins, N., Frederick Cancer Research & Development Center. History of mouse genetics and research talk.
Jessell, T., Columbia University. Establishing symmetry and pattern in the vertebrate nervous system.
Lovell-Badge, R., National Institute for Medical Research, United Kingdom. (1) Introduction to sex determination. (2) Sex and Sox genes in development.
Mann, J., Beckman Research City of Hope Medical Center. Genomic imprinting in ES cells and embryos.
McLaren, A., The Wellcome/CRC Institute, United Kingdom. Primordial germ cells.
McMahon, A., Harvard University. Research talk.
Papaioannou, G., Columbia University. Chimeras in experimental embryology and research talk.
Parada, L., Frederick Cancer Research & Development Center. Research talk.
Rinchik, G., Sarah Lawrence College. Genetic resources.
Robertson, L., Harvard University. Research talk.
Rossant, J., Mount Sinai Hospital, Canada. Preimplantation development and research talk.
Solter, D., Max-Planck Institute of Immunology, Germany. Genomic imprinting and cellular and nuclear totipotency in mammals.
Soriano, P., Fred Hutchinson Cancer Research Center. Research talk.
Strickland, S., SUNY at Stony Brook. Maternal control and the initiation of development.
Tam, P., Children's Medical Research Institute, Australia. Postimplantation development and research talk.
Wassarman, P., Roche Institute of Molecular Biology. Mechanisms of mammalian fertilization and research talk.
Wilkinson, D., National Institute for Medical Research, United Kingdom. Segmentation in the vertebrate embryo and research talk.

Genetic-Epidemiologic Studies of Complex Diseases

June 15–21

INSTRUCTORS

Risch, Neil, Ph.D., Yale University
Squires-Wheeler, Elizabeth, Ph.D., Columbia University

This course considered the difficulties in studying the genetic basis of complex disorders, such as diabetes, cardiovascular disease, cancer, Alzheimer's disease, migraine, affective disorder, alcoholism, and epilepsy, with a particular emphasis on neuropsychiatric conditions. Discussions were held on genetic-epidemiologic study designs, including family, twin, and adoption studies, as well as mode of inheritance analyses, and their role in setting the framework for understanding the genetic and nongenetic components of a disease.

A major focus was on the identification of specific gene effects using both linkage and association analyses. Discussions took place on the efficiency and robustness of different designs for such analysis, as well as how evidence from genetic epidemiologic studies inform both the design and interpretation of molecular genetic studies. Recent discoveries of genes for both Mendelian and non-Mendelian diseases guided the discussion of the various methodologic issues.

PARTICIPANTS

Borglum, A., M.D., Aahus University, Denmark
Buu, N., B.S., Montreal General Hospital, Canada
Buxbaum, J., Ph.D., Rockefeller University
Ely, J., Ph.D., Trinity University
Gavras, H., M.D., Boston University School of Medicine
Grice, D., M.D., Yale University
Kaufmann, C., M.D., Columbia University
Kelner, K., Ph.D., Science Magazine
Kockum, I., B.A., Karolinska Institute, Sweden
Kowallis, G., M.D., St. Vincent's Hospital
Marder, K., M.D., Columbia University College of Physicians/Surgeons
McNamara, J., M.D., Duke University Medical Center
Murray, J., B.S., University Hospital, Canada
O'Connor, D., M.D., University of California, San Diego
Petronis, A., Ph.D., Clark Institute of Psychiatry, Canada
Santangelo, S., Sc.D., New England Medical Center
Tracey, M., Ph.D., Florida International University
Ulrich, M., Ph.D., Elon College
Wadelius, C., Ph.D., University Hospital, Sweden

SEMINARS

Eaves, L., Medical College of Virginia. Twin and adoption designs.
Easton, D., Institute of Cancer Research, United Kingdom. Breast cancer and related disorders.
Keats, B., Louisiana State University Medical Center. Linkage analysis: Introduction and standard approaches.
Kidd, K., Yale University. Population genetic principles.
Merikangas, K., Yale University. (1) Trait assessment, prevalence, and family studies. (2) Neurologic and psychiatric disorders.
Ott, J., Columbia University. Complex disorders: Two locus models and intervening variables.
Ottman, R., Columbia University. Epidemiologic principles.
Pericak-Vance, M., Duke University Medical Center. Alzheimer's disease.
Peterson, G., Johns Hopkins University. Colon cancer.
Sing, C., University of Michigan Medical School. Coronary artery disease: Intervening variables.
Spielman, R., University of Pennsylvania. Association studies.
Suarez, B., Washington University. Linkage analysis: Nonparametric approaches.
Todd, J., University of Oxford, United Kingdom. Diabetes.

Neurobiology of Human Neurological Diseases: Mechanisms of Neurodegeneration

June 23–29

INSTRUCTORS:

Choi, Dennis W., Washington University
Mobley, William C., University of California, San Francisco

Why do neurons and glia die in specific acute or chronic human neurological disorders? Do different pathological deaths share common mechanisms? What practical treatments can be contemplated? This lecture course explored possible answers to these important questions. Recent advances in 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, Huntington's disease, and stroke. Taking advantage of small class size and extensive discussion, invited faculty lecturers examined critical issues in their areas of expertise. Overview was provided and course participants did not need to be familiar with neurological diseases, but the course did focus principally on the specific hypotheses and approaches driving current research. Emphasis was placed on the highly dynamic interface between basic and clinical investigations, including the interdependence of clinical research and disease model development, and the value of disease research for understanding the nature of the normal nervous system. An interview was conducted with a patient with Huntington's disease. Last year's faculty were: M. Chalfie, R. Ciaranello, I. Diamond, K. Fischbeck, A. Goate, D. Landis, J. McNamara, J. Mazziotta, H. Monyer, L. Ptacek, S. Prusiner, and D. Selkoe.

PARTICIPANTS

Bernstein, H.-G., Ph.D., University of Magdeburg, Germany
Darius, S., M.D., Ph.D., University of Magdeburg, Germany
Dopazo, A., Ph.D., Instituto Cajal, Spain
Doster, S.K., M.D., Ph.D., Washington University Medical School
Gottron, F., B.S., Washington University
Harrigan, M., B.S., University of Washington School of Medicine
Hoban, C., D.Sc., Cambridge NeuroScience, Inc.
Jung, I., B.S., University of Arizona
Kurada, P.K., M.S., University of Notre Dame
Laake, J., M.D., University of Oslo, Norway
Lee, A., B.A., Cyto Therapeutics, Inc.
Chun, W.-L., Ph.D., Abbott Laboratories
Malchiodi-Albedi, F., M.D., Ph.D., Instituto Superiore Di Sanita, Italy
Markopoulou, K., Ph.D., University of Nebraska Medical Center
McKee, M.A., M.D., National Institutes of Health
McLaughlin, B.A., B.A., University of Pennsylvania
Montemayor, M., B.A., Ph.D., Columbia University College of Physicians & Surgeons
Neff, N., Ph.D., Cephalon, Inc.
Oron, L., Ph.D., Tel Aviv University
Sheffield, L., M.A., University of Kansas Medical Center
Talbot, C., B.S., Washington University School of Medicine
Tishler, T., B.A., University of California, Los Angeles

SEMINARS

Chalfie, M., Columbia University. Genetically determined neurodegeneration.
Choi, D., Washington University School of Medicine. (1) Patient presentation. (2) Excitotoxicity.
Dawson, V., Johns Hopkins University School of Medicine. Nitric oxide and neurodegeneration.
Edwards, R., UCLA School of Medicine. Dopamine uptake and neurodegeneration.
Fischbeck, K., University of Pennsylvania School of Medicine. Molecular genetics of neurological diseases.
Goate, A., Washington University School of Medicine. Genetics of familial Alzheimer's disease.

Hefti, F., Genetech Inc. Therapeutic approaches to neurodegeneration.
Hsiao, K., University Hospital, Minneapolis. Animal models of neurodegeneration diseases.
Kriegstein, A., Columbia University. Epilepsy and neurodegeneration.
Landis, D., Case Western Reserve School of Medicine. Role of glia in CNS injury.
Mobley, W., University of California School of Medicine. (1) Neuronal growth factors. (2) Taped patient presentation.
Monyer, H., University of Heidelberg, Germany. Glutamate receptors in health and disease.
Price, D., Johns Hopkins University. Motor neuron disease and Alzheimer's disease.
Selkoe, D., Brigham & Women's Hospital. Molecular pathogenesis of Alzheimer's disease.

Computational Neuroscience: Vision

July 1–14

INSTRUCTORS
Heeger, David, Ph.D., Stanford University
Movshon, J. Anthony, Ph.D., New York University
Simoncelli, Eero, Ph.D., University of Pennsylvania

ASSISTANTS
Carandini, Matteo, New York University
Darrell, Trevor, Massachusetts Institute of Technology

Computational approaches to neuroscience have produced important advances in our understanding of neural processing. Prominent successes have come in areas where strong inputs from neurobiological, behavioral, and computational approaches can interact. Through a combination of lectures and hands-on experience with a computer laboratory, this intensive course examined several areas, including feature extraction, motion analysis, binocular stereopsis, color vision, higher-level visual processing, visual neural networks, and oculomotor function. The theme was that an understanding of the computational problems, the constraints on solutions to these problems, and the range of possible solutions can help guide research in neuroscience. Students had experience in neurobiological or computational approaches to visual processing. A strong background in mathematics was beneficial.

Past lecturers have included E. Adelson, R. Andersen, H. Bulthoff, R. Desimone, R. von der Heydt, E. Hildreth, P. Lennie, S. Lisberger, J. Malik, J. Maunsell, S. McKee, M. Morgan, K. Nakayama, W. Newsome, T. Poggio, T. Sejnowski, D. Sparks, S. Ullman, and B. Wandell.

PARTICIPANTS

Backus, B., M.A., University of California, Berkeley
Beutter, B., Ph.D., NASA Ames Research Center
Buracas, G., M.S., Salk Institute
Chen, C.-C., B.S., University of California, Santa Barbara
Cohen, G., M.S., Stanford University Medical Center
Dykstra, T., M.D., University of Nijmegen, The Netherlands
Duvdevani-Bar, S., B.S., M.S., Weizmann Institute of Science, Israel
Groh, J., M.S., Ph.D., Stanford University School of Medicine
Lin, L.-M., M.S., University of Chicago
Niyogi, S., B.S., M.S., Massachusetts Institute of Technology
Parnas, B., Ph.D., NASA Ames Research Center
Ringach, D., M.S., New York University
Ruksenas, O., Ph.D., Vilnius University, Lithuania
Skoczenski, A., Ph.D., New York University
Speigle, J., B.S., University of California, Santa Barbara
Spivey-Knowlton, M., B.A., University of Rochester
Sumnall, J., B.S., M.S., St. John's College, United Kingdom
Tang, C., M.S., New York University
Teo, P., M.S., Stanford University
Thomas, J.I., Ph.D., University of Pennsylvania
Verghese, P., Ph.D., NASA Ames Research Center
Weiss, Y., M.S., Massachusetts Institute of Technology
Zhou, Y.-X., Ph.D., New York University

SEMINARS

Adelson, E., Massachusetts Institute of Technology. (1) The elements of early vision. (2) Lightness, transparency, and shading.
Glimcher, P., New York University. Neurophysiology of saccadic eye movements.
Graham, N., Columbia University. Visual segregation among textured regions, with an emphasis on low-level mechanisms.
Heeger, D., Stanford University. (1) Psychophysical methods, signal detection theory, and color matching. (2) Normalization of neural responses in primary visual cortex. (3) Methods for recovering three-dimensional motion and shape from image sequences.
Kersten, D., University of Minnesota. Visual perception of the shapes and depth relationships of surfaces.
Lennie, P., University of Rochester. Color physiology.
Lisberger, S., University of California, San Francisco. (1) Visual control of eye movements I. Smooth pursuit. (2) Visual control of eye movements II. Learning in the vestibulo-ocular reflex.
McKee, S., Smith-Kettlewell Institute. The psychophysics of stereopsis.
Movshon, J.A., New York University. (1) Anatomy and physiology of the retina, lateral geniculate nucleus, and primary visual cortex. (2) Analysis of pattern motion by area MT neurons.
Ohzawa, I., University of California, Berkeley. Physiology of binocular vision and stereopsis.
Shadlen, M., Stanford University. (1) Visual motion perception I: Psychophysics and physiology. (2) Visual motion perception III: Decisions.
Simoncelli, E., University of Pennsylvania. (1) Introduction to signal and image processing. (2) Visual motion perception II: Algorithms and models.
Tanaka, K., Riken Institute, Japan. Processing of visual images of objects in the inferotemporal cortex.
Wandell, B., Stanford University. (1) Functional imaging of the human brain. (2) Color appearance: Theory and measurements.

Arabidopsis Molecular Genetics

July 4–24

INSTRUCTORS

Chory, Joanne, Ph.D., Salk Institute
Ecker, Joseph, Ph.D., University of Pennsylvania
Theologis, Athanasios, Ph.D., University of California, Berkeley

ASSISTANTS

Abel, Steffer, University of California, Berkeley
Dunn, Patrick, University of Pennsylvania
Soowal, Lara, Salk Institute
Telfer, Abby, University of Pennsylvania

This course provided an intensive overview of current topics and techniques in *Arabidopsis* biology, with an emphasis on molecular genetics. It also introduced approaches used in yeast that have the potential to be utilized for the advancement of *Arabidopsis* molecular genetics. It was designed for scientists with experience in molecular techniques who are working or wish to work with *Arabidopsis*. The course consisted of a rigorous lecture series, a hands-on laboratory, and informal discussions. Speakers provided both an in-depth discussion of their work and an overview of their specialty. Speakers included Frederick Ausubel, Development of Model Pathogenesis Systems in *Arabidopsis*; Tony Cashmore, The Blue Light Photoreceptor in *Arabidopsis*; Joanne Chory, Molecular and Genetic Analysis of Light-regulated Development; Gloria Coruzzi, Molecular Genetics of Nitrogen Assimilation into Amino Acids in *Arabidopsis*; Nigel Crawford, The Nitrate Assimilation Pathway: The First Steps in the Acquisition of N2 from the Environment; Gary Drews, Applications of In Situ Hybridization; Joe Ecker, Physical Mapping of the *Arabidopsis* Genome; Pamela Green, Control of mRNA Stability in Plants; Gerry Fink, Molecular Genetics of Growth Regulation; David Marks, Trichome Development in *Arabidopsis* as a Model System to Study Cell Fate and Differentiation; David Meinke, Genetic Dissection of Embryogenesis in *Arabidopsis*; June Nasrallah, Pollination Responses in Crucifers; Neil Olzewski, Molecular Genetic Approaches to Understanding Gibberellin Signal Transduction; Daphne Preuss, Cell-Cell Communication during *Arabidopsis* Fertilization; Ian Sussex, Organization and Function of Apical Meristems; Abby Telfer, Genetic Regulation of Plant Morphogenesis; Athanasios Theologis, Early Events in Plant Hormone Action; Richard Vierstra, Organization, Function, and Expression of the Ubiquitin Proteolytic Pathway in *Arabidopsis*; Robert Waterston, The *Caenorhabditis elegans* Genome Sequencing Project; Detlef Weigel, Genetic Hierarchy Controlling Flower Development in *Arabidopsis*; and Patricia Zambryski, Biology of *Agrobacterium*.

The laboratory sessions covered *Arabidopsis* genetics and development; transient gene expression assays in protoplasts; complementation of yeast mutants for cloning *Arabidopsis* genes; two-hybrid system in yeast; transformation by *Agrobacterium*; in situ detection of RNA; biochemical analysis of transcription factors; pulsed-field gel electrophoresis; analysis of yeast artificial chromosomes containing the *Arabidopsis* genome, and techniques for PCR-based mapping of mutations.

PARTICIPANTS

Della Penna, D., Ph.D., University of Arizona
Bergelson, J., Ph.D., Washington University
Chen, L., B.S., University of Illinois, Urbana
Jeddeloh, J., B.A., Washington University
Jones, A., B.S., Washington University
Kauschmann, A., B.S., GmbH, Germany
McKhann, H., Ph.D., C.N.R.S., France
Mundy, J., Ph.D., Copenhagen University, Denmark
Murali, D., Ph.D., Center for Cellular & Molecular Biology, India
Neuenschwander, U., Ph.D., CIBA, Agricultural Biotechnology Research
Osteryoung, K., Ph.D., University of Arizona
Overvoode, P., B.S., Washington State University
Pascal, E., Ph.D., University of Illinois, Urbana
Schrick, K., Ph.D., University of Washington, Seattle
Ward, S., B.S., University of Warwick, United Kingdom
Werner, J., Ph.D., University of California, Santa Barbara

SEMINARS

Ausubel, F., Harvard Medical School. Development of model pathogenesis systems in *Arabidopsis*.
Bender, J., Whitehead Institute. (1) Yeast complementation. (2) Gene isolation by complementation in yeast.
Cashmore, T., University of Pennsylvania. The blue light photoreceptor in *Arabidopsis*.
Chory, J., Salk Institute. Molecular and genetic analyses of light-regulated development.
Corruzzi, G., New York University. Molecular genetics of nitrogen assimilation into amino acids in *Arabidopsis*.
Drews, G., University of Utah. Applications of in situ hybridization.
Ecker, J., University of Pennsylvania. Physical mapping of the *Arabidopsis* genome.
Fink, G., White Institute. Molecular genetics of growth regulation.
Green, P., Michigan State University. Control of mRNA stability in plants.
Marks, D., University of Minnesota. Trichome development in *Arabidopsis* as a model system to study cell fate and differentiation.
Meinke, D., Oklahoma State University. Genetic dissection of embryogenesis in *Arabidopsis*.
Nasrallah, J., Cornell University. Pollination responses in crucifers.
Olszewski, N., University of Minnesota. Molecular genetics approaches to understanding gibberellin signal transduction.
Preuss, D., Stanford University. Cell-cell communication during *Arabidopsis* fertilization.
Sussex, I., University of California, Berkeley. Organization and function of apical meristems.
Telfer, A., University of Pennsylvania. Genetic regulation of plant morphogenesis.
Theologis, A., University of California, Berkeley. Early events in plant hormone action.
Vierstra, R., University of Wisconsin, Madison. Organization, functions, and expression of the ubiquitin proteolytic pathway in *Arabidopsis*.
Waterston, R., Washington University, St. Louis. The *C. elegans* genome sequencing project.
Wiegel, D., Salk Institute. Genetic hierarchy controlling flower development in *Arabidopsis*.
Yang, M., SUNY, Stony Brook. The two-hybrid system in yeast.
Zambryski, P., University of California, Berkeley. Biology of *Agrobacterium*.

Molecular Cloning of Neural Genes

July 4–24

INSTRUCTORS

Blakely, Randy, Ph.D., Emory University, Atlanta, Georgia
Boulter, James, Ph.D., Salk Institute, La Jolla, California
Chao, Moses, Ph.D., Cornell University, New York, New York
Julius, David, Ph.D., University of California, San Francisco
Lai, Cary, Ph.D., Scripps Research Institute

ASSISTANTS

Bauman, Andrea, Emory University School of Medicine
Elgohyen, Belen, Salk Institute
Kong, Haeyoung, University of Pennsylvania
Sun, Linda, University of California, San Francisco

This intensive laboratory/lecture course was intended to provide scientists with a basic knowledge of the use of the techniques of molecular biology in examining questions of neurobiological significance. Particular emphasis was placed on using and discussing methods for circumventing the special problems posed in the study of the nervous system; for example, examination of low abundance mRNAs in extremely heterogeneous cell populations.

The laboratory work included mRNA quantitation methods (nuclease protection, etc.), preparation of hybridization probes, library construction (λ ZAP and IST procedure), plaque-screening techniques (probe hybridization, antibody interaction), DNA sequencing, PCR amplification, and RNA amplification. A major emphasis of the laboratory work was devoted to characterization and analysis of nucleic acids. A portion of the laboratory concentrated on gene transfer techniques, including the use of *Xenopus* oocytes and transfection in cultured cells. Other methodologies that were emphasized were in situ hybridization and DNA sequence analysis. The lecture series, presented by invited speakers, focused on emerging techniques and how they may be applied to the study of the nervous system. Guest lecturers were R. Edwards, B. Ganetzky, D. Julius, G. Lemke, C. Lai, G. Mandel, J. Morgan, R. Neve, M.-M. Poo, R. Reed, and A. Silva.

PARTICIPANTS

Arribas, M., Ph.D., Yale University School of Medicine
Balice-Gordon, R., Ph.D., University of Pennsylvania
Blumenstein, S., M.S., Weizmann Institute of Science, Israel
Buser, C., Ph.D., SUNY, Stony Brook
Carnell, L., B.S., University of California, Berkeley
Giffard, R. M.D., Ph.D., Stanford University School of Medicine
Jenner, A., B.A., Harvard Medical School
Kelley, D., Ph.D., Columbia University
Lindenboim, L., M.S., Tel Aviv University, Israel
Lois, C., M.D., Rockefeller University
McBain, C., Ph.D., National Institutes of Health
Nitsch, R., M.D., Ph.D., Massachusetts Institute of Technology
Osterhout, D., Ph.D., University of Michigan
Rubin, W., B.A., Duke University Medical Center
Storey, K., Ph.D., University of Oxford, United Kingdom
Wigston, D., Ph.D., Emory University School of Medicine

SEMINARS

Barres, B., Stanford University School of Medicine. Cell death and control of cell survival in the oligodendrocyte lineage.
Cline, H., Cold Spring Harbor Laboratory. Retinotectal plasticity: Moving toward a molecular genetic analysis.
Eberwine, J., University of Pennsylvania. RNA-binding proteins and neurological diseases.
Ingraham, H., University of California, San Francisco. Transcriptional regulators in the hypothalamic-pituitary axis.
Lemke, G., The Salk Institute. Targeted disruption of mammalian peripheral nervous system development.
Morgan, J., Roche Institute. Genetic control of neural function: From yeast to transgenic mice.
Niswander, L., Sloan-Kettering Institute. In situ hybridization—Methods and applications.
O'Malley, K., Washington University School of Medicine. In vitro and in vivo analysis and neural-specific gene expression.
Poo, M.-M., Columbia University. Nerve growth, transmitter secretion, and synaptogenesis: Random walks in culture dish.
Quinn, A.M., Yale University School of Medicine. Navigating the World Wide Web: An introduction to Internet services for the molecular biologist.
Weinmaster, G., University of California, Los Angeles. Functional analysis of rat *notch* genes
Zinn, K., California Institute of Technology. Cell-fate decisions and axon guidance in the insect CNS.

Neurobiology of *Drosophila*

July 4–24

INSTRUCTORS

Bieber, Allan, Ph.D., Purdue University
Hardie, Roger, Ph.D., University of Cambridge, United Kingdom
Taghert, Paul, Ph.D., Washington University School of Medicine

ASSISTANTS

Renn, Susan, Washington University School of Medicine
Rutherford, George, Purdue University

This laboratory/lecture course was intended for researchers at all levels who want to use *Drosophila* as an experimental system for studying neurobiology. Daily seminars introduced the history behind special topics and further developed those topics by including recent contributions and outstanding questions. Guest lecturers brought original preparations for viewing and discussion and/or direct lab exercises and experiments in their areas of special interest.

The course introduced students to various preparations useful for studying *Drosophila* neurobiology: the larval and adult nervous systems for studying physiology and behavior and the embryonic and metamorphosing nervous systems for studying development. Students learned a broad range of methods including electrophysiological, anatomical, and behavioral techniques that are critical for the study of *Drosophila* neurobiology. Topics and techniques of special value in *Drosophila* (e.g., an introduction to genetics, early embryogenesis, spreading of chromosomes, and embryo injections) were also included.

Physiological emphasis was given to genetics and molecular biology of excitability, developmental emphasis to neurogenesis, axonal pathfinding and synaptogenesis, and behavioral emphasis to courtship, learning and memory, and biological rhythms. Guest lecturers included C.F. Wu, R. ffrench-Constant, E. Isacoff, B. Ganetzky, V. Hartenstein, G. Technau, S. Crews, N. Patel, C. Doe, A. Tomlinson, H. Keshishian, B. Taylor, K. White, R. Davis, R. Jackson, and T. Tully.

PARTICIPANTS

Atashi, J., B.S., University of North Carolina
Baldwin, D., B.A., University of Washington, Seattle
Forjanic, J.P., B.S., Max-Planck Institute, Germany
Lenz, B., B.A., Max-Planck Institute, Germany
Pereira, H., B.S., York University , Canada
Petersen, C., B.S., Vanderbilt University
Pimentel, A., M.A., City College, New York
Sawin, E., B.S., M.S., McMaster University, Canada
Sayeed, O., B.A., M.A., Indiana University
Sun, Y., M.S., Oregon State University
Therianos, S., B.A., University of Basel, Switzerland
Van de Goor, J., Ph.D., University of California, San Francisco

SEMINARS

Bieber, A., Purdue University. Axon guidance.
Campos-Ortega, J., University of Koln, Germany. Early neurogenesis
Carlson, J., Yale University. Sensory transduction.
Dickinson, M., University of Chicago. Wing structure and function.
ffrench-Constant, R., University of Wisconsin, Madison. Neurotransmitter receptors.
Hardie, R., Cambridge University, United Kingdom. Physiology of the visual system.
Hartenstein, V., University of California, Los Angeles. Embryonic development and early neurogenesis.
Iverson, L., Beckman Research Institute City of Hope. Ion channels 2.
Jackson, R., Worcester Foundation. Circadian rhythms.
Keshishian, H., Yale University. Muscle development and synaptogenesis.
Patel, N., Carnegie Institute of Washington. Neuronal determination.
Restifo, l ., University of Arizona. Development of the larval nervous system.
Sokolowski, M., York University, Canada. Behavioral genetics.
Strausfeld, N., University of Arizona. Brain structure.
Taghert, P., Washington University. Peptide neurotransmitters.
Taylor, B., Oregon State University. Sex determination and behavior.
Tully, T., Cold Spring Harbor Laboratory. Learning and memory.
Wu, C.-F., University of Iowa. Ion channels 1.

Molecular Neurobiology: Brain Development and Function

July 17–30

INSTRUCTORS

McKay, Ronald, Ph.D., National Institutes of Health
Reichardt, Louis, Ph.D., University of California, San Francisco
Schwarz, Thomas, Ph.D., Stanford University

This lecture course presented both basic concepts and currently exciting research problems in molecular neurobiology. It focused on approaches and methods now used to study the development and function of the nervous system. Topics covered included gene expression, receptor structure and function, ion channel cloning, second messenger systems, learning, sensory transduction, behavioral genetics, neural induction, cell lineage, immortal cell lines, cell adhesion, oncogenes, and neurite outgrowth. The course provided the opportunity to discuss this rapidly expanding research area with invited lecturers. Individuals from a wide variety of backgrounds (graduate students to faculty) were encouraged to apply. Lectures were given by T. Albright, C. Bargmann, J. Byrne, K. Campbell, H. Cine, S. Crews, K. Harris, M. Hatten, R. Jahn, D. Jay, A. Joyner, D. Julius, L. Katz, S. McConnell, R. McKay, J. Patrick, R. Reed, U. Rutishauser, E. Schuman, T. Schwarz, M. Simon, and G. Yancopoulos.

PARTICIPANTS

Condie, B., Ph.D., University of Utah
Francesconi, A., Ph.D., Cornell University Medical College
Gadient, R., Ph.D., University of Basel, Switzerland
Goldner, F., M.D., Baylor College of Medicine
Holz, A., B.S., University of Zurich, Switzerland
Hong, A.-S.T., B.S., National University of Singapore
Hu, B., B.S., University of California, Los Angeles
Laferriere, N., B.S., University of Ottawa, Canada

Lopez-Rubalcava, C., B.S., CINVESTAV, Mexico
Mackarehtschian, K., M.A., Princeton University
Nakagawa, Y., M.D., Tokyo University, Japan
Okada, A., B.A., Columbia University
Rokhsar, D., Ph.D., University of California, Berkeley
Roth, M., Ph.D., Northwestern University
Scott, K.E., B.S., University of California, San Diego
Serpente, N., Ph.D., National Institute of Medical Research, United Kingdom
Sommer, W., M.D., Karolinska Institute, Sweden
Sutula, T., M.D., Ph.D., University of Wisconsin
Astride V., M.D., Clark Institute of Psychiatry, Canada
Williams, R., Ph.D., Karolinska Institute, Sweden
Yeo, S.-L., B.S., University of Singapore

SEMINARS

Bialek, W., NEC Research Institute, Princeton, New Jersey. Modeling vision.

Bredesen, D., University of California, Los Angeles. Genetic control of neural cell death.

Cleveland, D., Johns Hopkins University. Transgenic models of motoneuron disease.

Doupe, A., University of California, San Francisco. The neural basis of vocal learning in songbirds.

Hudspeth, J., University of Texas, Dallas. How hearing happens: Mechanoelectrical transduction, frequency tuning, and synaptic transmission by hair cells of the inner ear.

Jahn, R., Yale University. Exocytosis of synaptic vesicles: A neurobiologist's view.

Kaplan, D., National Cancer Institute. Neurotrophins, *trk* receptors, and neuronal signal transduction.

Katz, L., Duke University Medical Center. Linking layers and connecting columns: The formation of cortical circuits.

Malinow, R., Cold Spring Harbor Laboratory. Mechanisms of synaptic transmission and plasticity in the central nervous system.

Matthews, G., SUNY, Stony Brook. Neuronal exocytosis and endocytosis: Physiological perspective.

Mayford, M., Columbia University. Genetic approaches to synaptic plasticity, learning, and memory.

McKay, R., National Institutes of Health. Neuronal stem cells in mammals.

Meister, M., Harvard University. Multineuronal signals in brain development and function.

Movshon, J.A., New York University. Neural basis of visual motion perception.

Reichardt, L., University of California, San Francisco. Studies of neuronal survival, differentiation, and axon outgrowth.

Rothman, J., Memorial Sloan-Kettering Cancer Center. Membrane transport in cells and synapses.

Rubenstein, J., University of California, San Francisco. Patterning of the vertebrate forebrain.

Schuman, E., California Technical Institute. Diffusible signals and long-term potentiation.

Sejnowski, T., Salk Institute. Predictive Heggian learning.

Schwarz, T., Stanford University. Synaptotagmin mutations and synaptic function.

Ts'O, D., Baylor College of Medicine. Functional organization and connectivity in visual cortex.

Wilson, M., Massachusetts Institute of Technology. Sleep, memory, and behaviorally induced changes in hippocampal ensemble activity.

Zuker, C., University of California, San Diego. Molecular genetics of sensory transduction in *Drosophila*.

Yeast Genetics

July 26–August 15

INSTRUCTORS

Kaiser, Chris, Ph.D., Massachusetts Institute of Technology
Michaelis, Susan, Ph.D., Johns Hopkins University
Mitchell, Aaron, Ph.D., Columbia University

CO-INSTRUCTOR

Adams, Alison, University of Arizona

ASSISTANTS

Frand, Alison, Massachusetts Institute of Technology
Li, Weishi, Columbia University
Romano, Julia, Johns Hopkins University
Nijbroek, Gaby, Johns Hopkins University

The major laboratory techniques used in the genetic analysis of yeast were studied, including the isolation and characterization of mutants, tetrad analysis, complementation, and mitotic recombination. Micromanipulation used in tetrad analysis was carried out by all students. Molecular genetic techniques, including yeast transformation, gene replacement, analysis of gene fusions, and electrophoretic separation of chromosomes, were applied to the analysis of yeast DNA. 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

Batiza, A., M.A., University of Wisconsin, Madison
Biosca, J., Ph.D., Universitat Autonoma, Spain
Dunstan, H., Ph.D., University of Oregon
Grant, A., B.S., Emory University
LaMarco, K., Ph.D., Tularik, Inc.
Loidl, J., Ph.D., University of Vienna, Austria
Mitchell, T., Ph.D., Duke University Medical Center
Moczko, M., B.S., Universitat Freiburg, Germany
Mulhaupt, Y., B.S., Ciba Geigy Ltd., Switzerland
Shani, N., Ph.D., Johns Hopkins University School of Medicine
Srinivasan, S., B.S., M.S., National Institutes of Health
Stochaj, U., Ph.D., McGill University, Canada
Tesseur, I., M.S., Katholieke Universiteit Leuvan, Belgium
Thelin, A., Ph.D., Dartmouth College
Toikkanen, J., M.S., VTT Biotechnology & Food Research, Finland
Young, K., Ph.D., American Cyanamid Company

SEMINARS

Adams, A., University of Arizona. Genetic analysis of yeast cytoskeleton.
Errede, B., University of North Carolina, Chapel Hill. Organization and dynamics of map-kinase activation pathways in yeast.
Carlson, M., Columbia University. Transcriptional control in response to glucose availability.
Fields, S., SUNY, Stony Brook. The two-hybrid system to detect protein-protein interaction.
Fink, G., Whitehead Institute. Single transduction in haploids and diploids.
Fox, T., Cornell University. Mitochondrial genetics: Shoot first, ask questions later.
Futcher, B., Cold Spring Harbor Laboratory. The yeast cell cycle engine.
Gottschling, D., University of Chicago. Unstable and near the end: The dynamics of telomeric position effects.
Herskowitz, I., University of California, San Francisco. Specialized cell types in yeast.
Hieter, P., Johns Hopkins Medical School. Chromosome transmission, yeast genetics, and human disease.
Kaiser, C., Massachusetts Institute of Technology. Genes and protein of the secretory pathway.
Michaelis, S., Johns Hopkins Medical School. Maturation and export of the mating pheromone α-factor.
Mitchell, A., Columbia University. Control of meiotic gene expression.
Petes, T., University of North Carolina, Chapel Hill. Macro- and microrearrangements of yeast genome.
Pringle, J., University of North Carolina, Chapel Hill. Bud-site selection and polarity establishment in yeast.
Rose, M., Princeton University. Nuclear fusion in yeast: A window onto the functions of microtubules, the SPB, and the nucleus.
Wigler, M., Cold Spring Harbor Laboratory. RAS and signal transduction.
Winston, F., Harvard Medical School. Analysis of histones, the TATA-binding protein, and other transcription factors of yeast.

Advanced Molecular Cloning and Expression of Eukaryotic Genes

July 26–August 15

INSTRUCTORS

Burtis, Ken, Ph.D., University of California, Davis
Freedman, Len, Ph.D., Sloan-Kettering Institute
Learned, Marc, Ph.D., University of California, Davis

ASSISTANTS

Towers, Terri, Sloan-Kettering Institute
Case, Ryan, University of California, Davis

This course focused on both the cloning and the characterization of eukaryotic genes to probe their structure, function, and expression. As a model, they examined *cis*- and *trans*-acting components involved in the regulation of eukaryotic gene expression. Eukaryotic transcription factors were expressed in *E. coli* and purified by affinity chromatography. Mutations were generated in the DNA-binding domain of these factors by oligonucleotide directed and random mutagenesis procedures and characterized by DNA sequencing and DNA-binding assays. Using both the wild-type and mutant proteins, students learned the techniques and theory for detecting and characterizing the interaction between regulatory DNA sequences and *trans*-acting protein factors. In addition, transfection was used to introduce cloned DNA molecules that had been manipulated in vitro into mammalian tissue culture cells. The regulated expression of these transfected DNAs was analyzed by nuclease protection and enzymatic assays. Finally, expression libraries from various organisms were prepared and screened with recognition site probes for specific DNA-binding proteins as a means of learning the theoretical and practical aspects of constructing cDNA libraries. Guest lecturers discussed current problems in eukaryotic transcriptional regulation as well as technical approaches to their solutions. Experience with basic recombinant DNA techniques was a prerequisite for admission to the course.

PARTICIPANTS

Au, K., Ph.D., Glaxo Research Institute
Barasch, J., M.D., Ph.D., Columbia University College of Physicians & Surgeons
Brown, M., Ph.D., Emory University
Ciani, E., B.S., University of Bologna, Italy
Cosby, N.C., Ph.D., University of Missouri, Columbia
Fiddler, T., B.S., Oregon Health Sciences University
Huang, S., M.S., University of Georgia
Krisinski, S., B.S., University of New Mexico
Melendez, P., B.S., University of Puerto Rico
Nel, A., M.D., Ph.D., University of California, Los Angeles
Robles, J., M.S., Promega Corporation
Roxstrom, K.A., B.S., Stockholm University, Sweden
Sastry, G., Ph.D., University of Leeds, United Kingdom
Slezynger, T., Ph.D., Empresas Polar, Venezuela
Smith, E., B.A., M.S., University of Chicago
Turetsky, D., B.S., Washington University Medical School

SEMINARS

Burley, S., Rockefeller University. X-ray crystallographic studies of eukaryotic transcription factors.
Burtis, K., University of California, Davis. Structure and function of Doublesex proteins of *Drosophila*.
Carey, M., University of California Medical School, Los Angeles. Mechanism of DNA binding and transcriptional activation by GAL4-VP16.
Curran, T., Roche Institute of Molecular Biology. The complex world of Fos and Jun.
Desplan, C., Rockefeller University. Transcriptional control during early *Drosophila* development.
Freedman, L., Sloan-Kettering Institute. Modulation of nuclear receptor target gene selectivity by receptor dimerization.
Fields, S., SUNY, Stony Brook. The two-hybrid system to detect protein-protein interaction.
Kornberg, R., Stanford Medical School. Yeast RNA polymerase II transcription: Structure, mechanism, and regulation.
Learned, M., University of California, Davis. Molecular genetics of isoprenoid biosynthesis in *Arabidopsis*.
Smale, S., University of California, Los Angeles. Transcriptional regulation during lymphocyte development: Analysis of initiator elements and of a composite regulatory region.
Stillman, B., Cold Spring Harbor Laboratory. Mechanism and regulation of eukaryotic DNA replication.
Struhl, K., Harvard Medical School. Molecular mechanisms of transcriptional regulation in yeast.
Winston, F., Harvard Medical School. Analysis of histones, TATA-binding protein, and other transcription factors of yeast.
Young, R., Whitehead Institute for Biomedical Research. A pre-assembled complex of general transcription factors and RNA polymerase II in yeast.
Zaret, K., Brown University. Mechanism of gene activation in hepatocyte development.

Imaging Structure and Function in the Nervous System

July 26–August 15

INSTRUCTORS

Augustine, George, Ph.D., Duke University
Lichtman, Jeff, Ph.D., Washington University
Smith, Stephen, Ph.D., Stanford University

ASSISTANTS

Balice-Gordon, Rita, University of Pennsylvania
Dailey, Michael, Stanford University School of Medicine
Eilers, Jens, University of Saarlandes, Germany
Guild, Jeff, Cornell University
Tang, Nina, Duke University
Zipfel, Warren, Cornell University

Advances in optical microscopy and digital image processing, coupled with the development of a variety of powerful fluorescent probes, present expanding opportunities for visualizing the structure and function of neurons, synapses, and networks in the brain. This intensive laboratory/lecture course provided participants with the theoretical and practical tools to utilize these emerging technologies. The primary emphasis of the course was on optical microscopy—including fluorescence, differential interference (Normarski), phase, and confocal microscopy—as well as the application of video cameras and digital image processors to enhance microscopic images. Students used calcium-sensitive probes (e.g., fura-2), "caged" compounds, exocytosis tracers, and other methods to explore neuronal function in a variety of neural systems, including living animals, brain slices, peripheral synapse preparations, acutely dissociated neurons, and cultured cells. A new feature of this year's course was to have lectures (A. Konnerth) and laboratory exercises on the use of whole-cell patch-clamp methods to microinject fluorescent indicators into single cells. Applicants had a strong background in the neurosciences or in cell biology. Lecturers included W. Betz, J. Connor, S. Fraser, A. Grinvald, T. Inoue, L. Katz, R. Lewis, J. Nerbonne, W. Ross, R.Y. Tsien, W. Webb, and R. Wick.

PARTICIPANTS

Albuquerque, C., B.S., Columbia University
Avery, R., B.S., Baylor College of Medicine
Beierlein, M., M.S., Brown University
Carey, M., B.S., M.S., Oregon Health Sciences University
Galuske, R., M.D., Max-Planck Institute, Germany
Jordan, C., Ph.D., University of California, Berkeley
Korschina, S., Ph.D., Max-Planck Institute, Germany
Pettit, D., B.A., Duke University Medical Center
Ross, L., Ph.D., Ohio University
Shear, J., B.S., Stanford University
Stoeckli, E., Ph.D., Case Western Reserve University
Tombaugh, G., Ph.D., Duke University Medical Center

SEMINARS

Augustine, G., Duke University Medical Center. (1) Calcium indicators. (2) Calibration of fluorescent Ca indicator dyes.
Betz, W., University of Colorado School of Medicine. Fluorescence measurements of synaptic vesicle cycling.
Cohen, L., Yale University School of Medicine. Voltage-sensitive dyes.
Conner, J., Roche Institute of Molecular Biology. Calcium imaging in brain slices.
Fay, F., University of Massachusetts Medical School. (1) Digital deconvolution of blurred images. (2) Volume rendering.
Fraser, S., Beckman Institute. MRI imaging.
Heuser, J., Washington University School of Medicine. (1) Reflectance microscopy. (2) Demonstration of reflectance microscopy.
Inoue T., Universal Imaging Corporation. Image processing.

Katz, L., Duke University Medical Center. Flash photolysis in brain slices.
Konnerth, A., University of Saalandes, Germany. Patch-clamp measurements from CNS neurons in slices.
Lewis, R., Stanford University School of Medicine. Calcium signaling pathways.
Lichtman, J., Washington University. (1) Structure and function of the light microscope. (2) Fluorescence microscopy. (3) Introduction to confocal microscopy.
Nerbonne, J., Washington University School of Medicine. (1) Design of caged compounds. (2) Acute dissociation of central neurons.
Smith, S., Stanford University School of Medicine. (1) Interference microscopy. (2) Detectors for imaging, Part I. (3) Detectors for imaging, Part II. (4) Properties of video signals. (5) Laser confocal microscopy.
Tsien, R., University of California, La Jolla. Design and function of indicator dyes.
Webb, W., Cornell University. 2-Photon microscopy.

Advanced *Drosophila* Genetics

August 1–14

INSTRUCTORS

Ashburner, Michael, Ph.D., University of Cambridge, United Kingdom
Hawley, Scott, Ph.D., University of California, Davis

This intensive seminar course provided an introduction to the theory and practice of methods used to manipulate the *Drosophila* genome. It was suitable for graduate students and researchers with some experience with *Drosophila* who are interested in expanding their knowledge of the wide range of genetic techniques now available for use with this organism. Topics covered included chromosome mechanics, the design and execution of genetic screens, and the use of transposable elements as genetic tools.

PARTICIPANTS

Afshar, K., B.S., University of California, Davis
Bhattacharya, A., M.S., Indian Institute of Science, India
Birman, S., Ph.D., University of Virginia
Broadie, K., Ph.D., University of Cambridge, United Kingdom
Brummel, T., B.A., University of California, Irvine
Burgess, R., B.S., Stanford University Medical School
Diamond, M., M.D., Ph.D., University of California, Berkeley
Grammont, M., INSERM, France
Grieder, N., B.S., University of Basel, Switzerland
Haenlin, M., Ph.D., LGME, France
Kelly, D., B.S., University of Glasgow, United Kingdom
Marques, G., Ph.D., University of California, Irvine

Myers, M., Ph.D., Rockefeller University
Mythreyi, D.S., B.S., Iowa State University
Pinter, M., Ph.D., Hungarian Academy of Sciences, Hungary
Swanson, B., M.D., CNRS, France
Scholz, H., B.S., University of Koln, Germany
Wisotzkey, R., Ph.D., Massachusetts General Hospital
Zars, T., M.A., University of Notre Dame
Zhang, N., Ph.D., Roche Institute of Molecular Biology

SEMINARS

Ashburner, M., Cambridge University, United Kingdom. Evolution of the genus *Drosophila*.
Bingham, P., SUNY, Stony Brook. Nuclear structure, gene structure, and gene isolation in *Drosophila*.
Cherbas, P., University of Wisconsin, Madison. Somatic cell genetics of *Drosophila*.
Cline, T., University of California, Berkeley. Genetic dissection of the sex determination gene hierarchy in D.
Engels, W., University of Wisconsin, Madison. P element biology and gene replacement techniques.
Ganetsky, B., University of Wisconsin, Madison. Neurogenetics of ion channels in *Drosophila*: From mutants to molecules.
Hall, J., Brandeis University. What can behavioral analysis tell us about *Drosophila* genetics and its genome?
Hawley, S., University of California, Davis. (1) An introduction of *Drosophila* genetics. (2) Chromosome rearrangements. (3) Meiosis in *Drosophila* males and females.
Lehmann, R., Whitehead Institute. Making and analyzing developmental mutants.
Perrimon, N., Harvard Medical School. Mosaic analysis.
Spradling, A., Carnegie Institution of Washington. Single P element insertional mutagenesis.
Theukauf, W., SUNY, Stony Brook. Looking at the cytoskeleton during oogenesis and embryogenesis.

Macromolecular Crystallography

October 13–26

INSTRUCTORS

Furey, William, Ph.D., V.A. Medical Center
Gilliland, Gary, Ph.D., Center for Advanced Research in Biotechnology
McPherson, Alexander, Ph.D., University of California, Riverside
Pflugrath, James, Ph.D., Molecular Structure Corporation

ASSISTANT

Ji, Xinhau, Center for Advanced Research in Biotechnology

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 are new to macromolecular crystallography. Topics covered included protein crystallization, crystal characterization, data collection, cryo-crystallography, data reduction, anomalous dispersion, phase determination, molecular replacement and averaging, electron density interpretation, structure refinement, molecular graphics, molecular dynamics, and multidimensional nuclear magnetic resonance. Participants learned through extensive hands-on experiments, informal discussions, and lectures on current applications of these and related procedures given by outside speakers.

PARTICIPANTS

Adams, A., Ph.D., University of Dublin, United Kingdom
Braig, K., Ph.D., Yale University
Darimont, B., Ph.D., University of California, San Francisco
de la Calle, A., M.S., Harvard Medical School
Donlan, M., Ph.D., Molecular Simulations Inc.
Groppe, J., Ph.D., Biozentrum, Switzerland
Ladias, J., M.D., Harvard Medical School
Larsen, T., B.S., University of Copenhagen, Denmark
Mohammadi, M., Ph.D., New York University Medical Center
Montoya, G., Ph.D., EMBL, Germany
Reichert, P., M.D., Shering-Plough Research Institute
Ribeiro, S., B.S., Max-Planck Institute, Germany
Schad, E., B.S., M.S., University of Lund, Sweden
Smith, C., B.S., University of Michigan
Tran, A., M.A., Brookhaven National Laboratory
West, A., Ph.D., University of Medicine & Dentistry of New Jersey

SEMINARS

Brunger, A., Yale University. Simulated annealing refinement and the free R-factor.
Cheng, X., Cold Spring Harbor Laboratory. DNA modification by methyltransferase.
Clore, G.M., National Institutes of Health. Determination of high-resolution structures of proteins by 3D and 4D NMR.
Fitzgerald, P., Merck Research Laboratories. (1) Molecular replacement: An overview. (2) HIV protease: A target for structure-based drug design.
Furey, W., V.A. Medical Center. (1) Noncrystallographic symmetry and molecular averaging. (2) Phasing statistics: What do those numbers mean? Do they lie? (3) Isomorphous replacement.
Gilliland, G., Center for Advanced Research in Biotechnology. (1) Development of crystallization strategies. (2) Glutathione S-transferase: Crystallographic analysis of catalysis and substrate specificity.
Ginell, S., Brookhaven National Laboratory. Cryocrystallography and the immortal crystal.
Harrison, S., Harvard University. Applications of molecular averaging in virus crystallography and elsewhere.
Heinemann, U., Max-Delbruck Centrum, Germany. (1) Crystallization and structure analysis of DNA and DNA/protein complexes. (2) The universal nucleic-acid-binding cold-shock domain.
Kjeldgaard, M., Aarhus University, Denmark. Electron density map interpretation for A to O.
McPherson, A., University of California, Riverside. (1) Crystallization of macromolecules. (2) What is a crystal? Miller indices, Bragg's Law. (3) Fourier transforms and the electron density equations.
Newcomer, M., Vanderbilt University. Crystallographic studies on retinoid-binding proteins.
Otwinowski, Z., University of Texas Southwestern Medical Center. (1) Scaling and merging of data. (2) Sources of errors in measurements.
Pflugrath, J., Molecular Structure Corporation, Texas. Data Collection: Design and setup.
Smith, J., Purdue University. (1) MAD phasing: From theory to experiment. (2) Crystallographic problems and solutions in the structure determination of GMP synthetase.
Sprang, S., University of Texas Southwestern Medical Center. Mechanism of G-protein α subunits.
Sweet, R., Brookhaven National Laboratory. X-ray sources and optics.
Tronrud, D., University of Oregon. Macromolecular refinement.

Analysis and Genetic Manipulation of Yeast Artificial Chromosomes (YACs)

October 13–26

INSTRUCTORS

Carle, Georges, Ph.D., Universite de Nice, France
Green, Eric, Ph.D., National Institutes of Health
Huxley, Clare, Ph.D., St. Mary's Hospital Medical School, United Kingdom

ASSISTANTS

Courseaux, Anouk, Universite de Nice, France
Borbye, Lisbeth, National Institutes of Health
Manson, Ania, St. Mary's Hospital Medical School, United Kingdom

Cloning in yeast artificial chromosomes (YACs) is rapidly being applied to a wide variety of molecular genetic problems. This course provided basic scientific expertise in current techniques for the analysis and manipulation of YACs. In general, a blend of theoretical and practical information was provided, with the goal of establishing a strong foundation for applying YAC cloning to a diversity of scientific problems. Topics included standard yeast genetic techniques (such as the propagation and storage of cells, tetrad dissection, colony hybridization, and DNA transformation), YAC library screening, characterizing YAC inserts by pulsed-field gel electrophoresis and PCR, manipulating YAC clones by recombination-mediated disruption, targeted integration, and YAC-YAC recombination, identifying genes in YACs, and techniques for introducing YACs into mammalian cells. Participants learned through hands-on experience, informal discussions, and lectures given by prominent experts designed to complement the experimental activities.

PARTICIPANTS

Alderborn, A., Ph.D., University Hospital, Sweden
Banga, S., Ph.D., New Jersey Medical School
Bilbe, G., M.D., Ciba-Geigy, Switzerland
Byrne, K., B.S., University of Queensland, Australia
Compton, S., M.A., National Institutes of Health
Hiemisch, H., German Cancer Research Center, Germany
Kaname, T., M.D., Ph.D., Kumamoto University School of Medicine, Japan
Kimbro, K., Ph.D., The Children's Hospital
Kulkarni, G., Ph.D., University of North Texas
Matza, Y., B.S., Tel Aviv University, Israel
McCormick, S., Ph.D., Gladstone Institute of Cardiovascular Disease
Ngan, B., M.D., Ph.D., FRCP. University of Toronto, Canada
Perez-Jimenez, J., B.S., University of Puerto Rico
Szalay, A., B.S., M.S., Ph.D., University of Alberta, Canada
Xu, J., Ph.D., Purdue University

SEMINARS

Carle, G., Universite de Nice, France. Meiotic and mitotic recombination between overlapping YACs.
Cook, G., MRC Laboratory of Molecular Biology, United Kingdom. Analyzing complex gene families.
Gnirke, A., Mercator Genetics, Inc. RARE cleavage analysis of YACs.
Green, E., National Institutes of Health. Application of YAC cloning to the mapping of human chromosomes.
Huxley, C., St. Mary's Hospital Medical School, United Kingdom. Transfer of YACs into mammalian cells.
Krizman, D., National Institutes of Health. Identifying genes in genomic clones by exon trapping.
Lovett, M., University of Texas. Identifying genes in genomic clones by direct selection.
Rodstein, R., Columbia University. Introduction to yeast.
Schwartz, D., New York University. New physical approaches to genome analysis.
Spencer, F., Johns Hopkins University School of Medicine. Methods for YAC analysis: The Johns Hopkins tool kit for YAC manipulation.

Advanced In Situ Hybridization and Immunocytochemistry

October 18–31

INSTRUCTORS

Hough, Paul, Ph.D., Brookhaven National Laboratory
Jacobson, Ken, Ph.D., University of North Carolina, Chapel Hill
Mastrangelo, Iris, Ph.D., Brookhaven National Laboratory
Spector, David, Ph.D., Cold Spring Harbor Laboratory
Ried, Thomas, Ph.D., National Institutes of Health

ASSISTANTS

Derby, Robert, Cold Spring Harbor Laboratory
Oliver, Tim, University of North Carolina, Chapel Hill
Sheets, Erin University of North Carolina, Chapel Hill

This course focused on specialized techniques in microscopy related to localizing DNA sequences and proteins in cells and preparing DNA and DNA-protein spreads for microscopic examination. The course emphasized the use of the latest equipment and techniques in epifluorescence microscopy, confocal laser scanning microscopy, electron microscopy, and digital image processing. The aims of the course were designed to provide state-of-the-art technology and scientific expertise in the use of microscopic applications to address basic questions in genome organization and cellular and molecular biology. The course was designed for the molecular biologist who was in need of microscopic approaches and for the cell biologist who was not familiar with the practical application of advanced techniques presented in the course. Among the methods presented were the preparation of tagged nucleic acids probes, fixation methods, detection of multiple DNA sequences in single nuclei or chromosome spreads, use of a variety of reporter molecules and non-antibody fluorescent tags, indirect antibody labeling, and detection of multiple proteins in a single cell. In addition, molecular electron microscopy was used to examine DNA-protein interactions. In

each method, several experimental protocols were presented allowing the students to assess the relative merits of each and to relate them to their own research. Students were encouraged to bring nucleic acid or protein probes to the course, which were used in addition to those provided by the instructors. The laboratory portion of the course was supplemented by invited lecturers who gave up-to-the-minute reports on current research using the techniques being presented in the course.

PARTICIPANTS

Bear, D., Ph.D., University of New Mexico Cancer Center
Bonner, W., Ph.D., National Institutes of Health
Heby, O., Ph.D., University of Umea, Sweden
Hendricks-Munoz, K., M.D., Ph.D., New York University Medical Center
Huie, P., M.A., Stanford University Medical Center
Lewis, A., D.V.M., Children's Hospital Research Foundation
Lund, L., Ph.D., University of California, San Francisco
Moseley, C., B.S., Meharry Medical College
Richardson, J., D.V.M., Ph.D., University of Texas Southwestern Medical Center
Rothenpieler, U., M.D., University of Munich, Germany
Sandbrink, R., Ph.D., University of Heidelberg, Germany
Soderberg, C., M.S., B.S., University of Uppsala, Sweden
Stein, E., B.S., Vanderbilt University
Thouless, M., Ph.D., University of Washington, Seattle
Tsou, H., M.D., Beth Israel Hospital
Yamanaka, S., M.D., Ph.D., Gladstone Institute of Cardiovascular Disease, California

SEMINARS

Bagasra, O., Thomas Jefferson University. In situ PCR and its applications in research and diagnosis.
Brinkley, W., Baylor College of Medicine. Ultrastructural organization of the kinetochore.
Hough, P., Brookhaven National Laboratory. Image production in the electron microscope and brief overview of different applications.
Jacobson, K., University of North Carolina, Chapel Hill. Basic introduction to light microscopy and fluorescence.
Lanni, F., Carnegie-Mellon University. Near field and standing wave microscopy.
Murray, J., University of Pennsylvania. Use of confocal microscopy and deconvolution techniques.
Oshira, M., Hamamatsu Photonic Systems, Inc. Video microscopy.
Ried, T., National Institutes of Health. Comparative genomic hybridization.
Singer, R., University of Massachusetts Medical School. Cytoplasmic organization of mRNA.
Spector, D., Cold Spring Harbor Laboratory. (1) Immunocytochemistry. (2) An integrated microscopic approach to examining nuclear organization.
Waggoner, A., Carnegie-Mellon University. Development of fluorochromes and filters for fluorescence microscopy.

Molecular Genetics, Cell Biology, and Cell Cycle of Fission Yeast

November 1–14

INSTRUCTORS

Chappell, Tom, Ph.D., University College London, United Kingdom
Young, Paul, Ph.D., Queens University, Canada

ASSISTANTS

Balasubramanian, Mohan, Vanderbilt University School of Medicine
Den Haese, Greg, Vanderbilt University School of Medicine
Panaretou, Barry, University College London, United Kingdom

Studies with the fission yeast *Schizosaccharomyces pombe* have contributed greatly to the field of cell cycle research and have prompted a broad interest in the biology of this organism. *S. pombe* is increasingly being chosen as a model organism for investigations into other aspects of cell biology and genetics using the powerful molecular and genetical techniques available. The content of the course reflected all these areas of interest and provided participants with the skills necessary to pursue their own investigations. Topics covered included mutagenesis and analysis of mutants, transformation and gene transplacement techniques, extraction of proteins, preparation of nuclear DNA, plasmid recovery from yeast into bacteria, cell cycle methods, cytology, and immunological techniques. In addition to hands-on experience, participants had the opportunity to learn through informal group discussions and formal lectures given by prominent *S. pombe* researchers drawn from the expanding international community.

PARTICIPANTS

Brisken, C., M.D., Whitehead Institute
Champlin, E., M.S., Colby College
Cooper, J., Ph.D., University of Colorado, Boulder
Ghazvini, M., B.S., Institut Pasteur, France
Higgins, R., Ph.D., Memorial Sloan-Kettering Cancer Center
Kelman, B.S., M.S., Cornell University Medical College
Martin, B.S., Ph.D., Memorial Sloan-Kettering Cancer Center
May, K., B.S., University College London, United Kingdom
Ritchie, S., M.S., Simon Fraser University, Canada
Rupes, I., Ph.D., St. John's University

SEMINARS

Balasubramanian, M., Vanderbilt University School of Medicine. Cytoskeletal elements associated with septum formation in fission yeast.
Chappell, T., University College London, United Kingdom. Regulation of Golgi development in fission yeast.
Connelly, T., Cold Spring Harbor Laboratory. Cell cycle control.
Den Haese, G., Vanderbilt University School of Medicine. Threonine-14 phosphorylation of *cdc2* is mediated by *wee1* in fission yeast.
Enoch, T., Harvard Medical School. Checkpoint control in fission yeast.
Hoffman, C., Boston College. G proteins and tyrosine phosphatase activators of *S. pombe* adenylate cyclase.
Klar, A., ABL-Basic Research Program. Developmental decisions in yeast and mice.
McLeod, M., SUNY, Brooklyn. Initiation of meiosis.
Russell, P., Scripps Research Institute. Cell cycle control in fission yeast.
Wigler, M., Cold Spring Harbor Laboratory. RAS signal transduction.
Young, P., Queens University, Canada. Ion transport and pH regulation in yeast.

Computational Genomics

November 2–7

INSTRUCTORS

Marr, Thomas, Ph.D., Cold Spring Harbor Laboratory
Pearson, William, Ph.D., University of Virginia
Smith, Randall, Ph.D., Baylor College of Medicine

This course was intended to be a comprehensive overview of the theory and practice of some of the major computational methods and tools for genomic analysis. Topics included tools for accessing computational resources over the Internet, the analysis of complex genetic traits, theory, and practice of DNA sequence assembly, protein evolution and analysis (including tools for viewing and analyzing 2-D and 3-D structures of proteins from the perspective of primary sequence analysis), theory and practice of DNA and protein sequence analysis and statistical scoring systems, and interpretation of matches, multiple sequence alignment, and sequence clustering techniques. State-of-the-art UNIX workstations and computer programs were used and made available to students. The course was intended for people with a solid fundamental knowledge of using UNIX workstations who wanted to acquire significant skills in the area of computational genome analysis. This course was ideal for computer core directors and staff for molecular biology and genetics resources, for biologists wishing to acquire advanced skills in genome analysis, and for scientists from other disciplines, such as computer science and physics, who wished to gain an overview of the state-of-the-art work in this area. Funding for this course was provided by the National Center for Human Genome Research at the National Institutes of Health. Instructors included S. Altschul, W. Chang, D. Davison, J. Kececioglu, G. Myers, and G. Schuler.

PARTICIPANTS

Bouffard, G., B.S., National Institutes of Health
Bryer, J., B.S., Simon Fraser University, Canada
Cowles, S., Ph.D., Stanford University
Feldmann, K., M.S., Max-Planck Institute, Germany
Grimaila, R., B.S., M.S., Burroughs Wellcome Company
Hastwell, C., M.S., SmithKline Beecham, United Kingdom
Learn, G., Ph.D., Stanford University
Lewitter, F., Ph.D., Whitehead Institute
Maidak, B., Ph.D., University of Illinois, Urbana
Miller, M., Ph.D., National Institutes of Health
Nakai, K., Ph.D., National Institute for Basic Biology, Japan
Omori, K., B.S., DuPont Central Research & Development
Price, C., B.A., M.S., University of Nebraska Medical Center
Reid, R., B.S., SmithKline Beecham Pharmaceuticals
Roter, A., Ph.D., Sequana Therapeutics
Rousley, S., B.S., University of California, San Diego
Sartiel, A., M.S., Tel Aviv University, Israel
Servenius, B., Ph.D., Lund University, Sweden

SEMINARS

Altschul, S., National Institutes of Health. Sequence matching—Theory and statistical evaluation.

Chang, W., Cold Spring Harbor Laboratory. Sequence matching—More theory and practice.

Davison, D., University of Houston. Molecular biology resources on Internet.

Kececioglu, J., University of California, Davis. Multiple sequence alignment—Theory and a new algorithm.

Marr, T., Cold Spring Harbor Laboratory. (1) Introduction and overview. (2) Wrap-up, summary, course evaluation.

Myers, G., University of Arizona. DNA sequence assembly.

Pearson, W., University of Virginia. (1) Protein evolution. (2) Sequence matching—Practice.

Schuler, G., National Institutes of Health. MACAW and NCBI applications.

Smith, R., Baylor College of Medicine. (1) Pattern-induced multiple sequence alignment. (2) Multiple sequence alignment laboratory.

Molecular Markers for Plant Breeding and Plant Genetics

November 8–21

INSTRUCTORS

Burr, Ben, Ph.D., Brookhaven National Laboratory
Doerge, Rebecca, Ph.D., Cornell University, Ithaca
Tingey, Scott, Ph.D., DuPont Experimental Station

ASSISTANT

del Tufo, Joseph, DuPont Experimental Station

The course was designed to explore both theoretical and practical concepts for the use of molecular markers in plant genetics and plant breeding. This was accomplished through invited lecturers, lab work, interactive instruction, and computational analysis. Participants learned approaches to problems such as single gene introgression, analysis of genetic diversity, gene mapping, and quantitative trait analysis. The techniques employed were DNA-amplification-based and included simple-sequence repeat polymorphisms, RAPDs, RFLPs, and bulk segregant analysis. Computational work included utilization of databases, gene mapping, quantitative trait mapping, and germplasm analysis. A variety of mapping techniques from both plant and animal systems were examined with respect to their strengths for specific purposes. Experimental design and potential future strategies were emphasized. The 15 students were outstanding and represented a broad variety of backgrounds. The lecturers were W. Beavis, M. Clegg, D. Duvick, M. Gale, M. Mazur, R. Michelmore, A. Rafalski, J. Romero-Severson, R. Sederoff, S. Tanksley, and J. Wendel.

PARTICIPANTS

Cervera, M., Ph.D., University of Gent, Belgium
Chaparro, J., Ph.D., Forbia Pty, Australia
Devaux, P., M.S., Ph.D., Florimond Desprez Seed Company, France
DeVerno, L., M.S., Petawawa National Forestry Institute, Canada
Dookun, A., B.S., M.S., Ph.D., Mauritius Sugar Industry Research Institute, Mauritius
Garcia, G., M.S., North Carolina State University
Ghislain, M., Ph.D., International Potato Center, Peru
Lio, P., M.S., University of Florence, Italy
Prosen, D., Ph.D., Harris Moran Seed Company
Satagopan, J., M.S., University of Wisconsin, Madison
Sebastian, L., Ph.D., PhilRice Research Institute, Philippines
Shoemaker, J., M.S., North Carolina State University
Sterling, F., B.S., Chiquita Brands International, Costa Rico
Taramino, G., B.S., Ph.D., E.I. DuPont de Nemours
Vogel, J., B.S., Ph.D., E.I. DuPont de Nemours

SEMINARS

Beavis, W., Pioneer Hi-Bred International. Lessons from comparative linkage mapping and QTL analysis.
Clegg, M., University of California, Riverside. Molecular diversity in plant species.
Duvick, D., Iowa State University. Molecular markers in plant breeding—A view from the field.
Gale, M., John Innes Centre, United Kingdom. Wheat genome research, maps, markers, and comparative genetics.
Mazur, B., DuPont Company. Development of improved quality grains through biotechnology.
Michelmore, R., University of California, Davis. The future of molecular markers and the manipulation of disease resistance.
Rafalski, A., DuPont Company. Choosing technologies for marker-assisted breeding and genetic mapping.
Romero-Severson, J., Mycogen Company. Statistical treatment of molecular markers and quantitative trait loci.
Sederoff, R., North Carolina State University. Application of molecular markers to novel genetic systems.
Tanksley, S., Cornell University. Map-based cloning of a disease resistance gene in tomato.
Wendel, J., Iowa State University. The evolution of cotton and its genome.

Monoclonal Antibodies from Combinatorial Libraries

November 8–21

INSTRUCTORS

Barbas, Carlos, Ph.D., Scripps Research Institute
Burton, Dennis, Ph.D., Scripps Research Institute

CO-INSTRUCTOR

Silverman, Gregg, Ph.D., University of California, San Diego

ASSISTANTS

Bastidas, Raiza, Scripps Research Institute
Briones, Amelia, Scripps Research Institute
Pilkington, Glenn, American Bio-Technologies

Recent advances in the generation and selection of antibodies from combinatorial libraries allow for the rapid production of antibodies from immune and nonimmune sources. This laboratory/lecture course focused on the construction of combinatorial antibody libraries expressed on the surface of phage and selection of desired antibodies from libraries by panning. Students learned the theoretical and practical aspects of constructing combinatorial libraries from immune and nonimmune sources as well as the construction of synthetic antibody libraries. Production, purification, and characterization of Fab fragments expressed in *E. coli* were also covered. The lecture series, presented by a number of invited speakers focused on 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, antibody diversity, catalytic antibodies, and recent results on the use of antibodies in therapy.

PARTICIPANTS

Almeida, F., B.S., University of Lisbon, Portugal
Berry, J., B.S., University of Manitoba, Canada
Chen, L., Ph.D., National Institutes of Health
Haahr-Hansen, M., M.S., DAKO A/S, Denmark
Klasse, P.J., M.D., Ph.D., Institute of Cancer Research, United Kingdom
Lee, M.-C., Ph.D., Beckman Instruments, Inc.
Lesseur, M.C., B.S., Empresas Polar, Venezuela
Machado, D., M.S., University of Sheffield, United Kingdom
Masataka, T., Ph.D., Tokai University, Japan
Minenkova, O., Ph.D., University of Rome, Italy
Ojala, P., M.S., Medix Biochemica, Finland
Penneck, D., M.S., USAMRIID
Rimarachin, J., M.D., Ph.D., Cornell University Medical College
Shearing, L., B.S., La Trobe University, Australia
Sinclair, C., B.S., Ph.D., University of Glasgow, United Kingdom
van den Bos, C., M.S., Ph.D., Cold Spring Harbor Laboratory

SEMINARS

Barbas, C., The Scripps Research Institute. Synthetic human antibodies.
Burton, D., The Scripps Research Institute. Human antibodies from phage display libraries.
Carter, P., Genetech, Inc. Expression of antibodies and antibody fragments.
Janda, K., The Scripps Research Institute. Catalytic antibodies.
Larrick, J., Palo Alto Institute for Molecular Medicine. An overview of antibodies in biotechnology.
Model, P., Rockefeller University. Phage biology.
Sanz, I., University of Texas Health Science Center. Generation and features of antibody diversity.
Scott, J., Simon Fraser University, Canada. Phage display.
Silverman, G., University of California, San Diego. Evaluation of diversity in combinatorial antibody libraries.
Wilson, I., The Scripps Research Institute. Structural basis of antigen recognition by antibody.

The Laboratory would like to acknowledge the generosity of the following companies who loaned equipment and reagents to the various courses:

Adams & List Assoc. Ltd.
Alpha Innotech Corporation
Ambion, Inc.
AMRESCO, Inc.
Axon Instruments Inc.
Baxter Scientific
Becton-Dickinson
BIO 101, Inc.
Bio Control
Bio-Rad Laboratories
Biometra Inc.
Boehringer Mannheim Corp.
Brinkmann Instruments, Inc.
C.B.S. Scientific Co Inc.
Cellex Biosciences Inc.
Chroma Technology Corp.
Dage-MTI, Inc.
Denton Vacuum
Digital Instruments
Drummond Scientific Co.
DYNAL, Inc.
Edge Scientific Instrument Corporation
Electron Microscopy Sciences
Epicentre Technologies
Eppendorf
FMC Corporation
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Gatan Inc.
General Valve Corporation
Grass Instrument Company
Hamamatsu Photonic Systems
Hewlett-Packard Co.
Hitachi
Hoefer Scientific Instruments
Instrutech
Intermountain Scientific
IntelliGenetics, Inc.
Invitrogen Corporation
Kodak/Scientific Imaging Systems
Kramer Scientific
Leica
Life Technologies, Inc.
Medical Systems Corp.
Micro Video Instruments
Millipore Corp.
MJ Research Inc.
Molecular Probes Inc.
Nalgene
Narishige USA Inc.
New England Biolabs, Inc
Newport Corp.
Nikon, Inc.
NORAN Instruments Inc.
Novagen, Inc.
Olympus Corporation
Omega Optical Inc.
Oncor Inc.
Operon Technologies Inc.
Optronics
Organon Teknika Corp.
Paultek Imaging
Perkin-Elmer Cetus
Perkin-Elmer, Applied Biosystems Division
Pharmacia LKB
Photometrics
Promega Corporation
Qiagen, Inc.
Sarstedt Inc.
Savant Instruments, Inc.
Scanalytics
Sigma Chemical Co.
Spectra Physics
Stoelting Autogenics
Stratagene Cloning Systems
SUN BIOscience, Inc.
Sutter Instrument Co.
Technical Products International Inc.
USB/Amersham
Vector Laboratories, Inc.
Wallac, Inc.
Warner Instrument Corp
Carl Zeiss, Inc.

SEMINARS

Cold Spring Harbor 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 joined the Laboratory during the summer. The seminars also afford a necessary opportunity for the graduate students and postgraduate staff to develop their skills in defending, organizing, and presenting their research.

1994

January

Angus Wilson, Cold Spring Harbor Laboratory. The VP16-induced complex: A protein jigsaw.

Jorge Galan, State University of New York, Stony Brook. Molecular mechanisms of *Salmonella* entry into mammalian cells. (Host: Winship Herr)

Adrian Krainer, Cold Spring Harbor Laboratory. Mechanisms of splice site selection.

George Reeke, Rockefeller University. Neural Darwinism: Connecting brain and behavior. (Host: John Anderson)

Sridar Natesan, Cold Spring Harbor Laboratory. Structural organization of the c-fos promoter by transcription factor YY1.

February

Ward Odenwald, National Institutes of Health, NINDS. Functional analysis of the *Drosophila castor/pollux* gene cluster in CNS development and the role of ectopical mini-*white* gene expression in sexual behavior. (Host: Yi Zhong)

George Tokiwa, Cold Spring Harbor Laboratory. Inhibition of G_1 cyclin activity by the RAS/cAMP pathway in yeast.

Don Rio, University of California, Berkeley. Mechanism of *Drosophila* P element transposition and control by tissue-specific RNA splicing. (Host: Adrian Krainer)

Mary Lou Pardue, Massachusetts Institute of Technology. *Drosophila* telomeres: Transposable elements earning an honest living? (Host: David Spector)

Ray O'Keefe, Cold Spring Harbor Laboratory. The nuclear organizaton of pre-mRNA splicing.

Peter Gergen, State University of New York, Stony Brook. Functions of the *Drosophila* Runt protein, a member of a new family of heteromeric DNA-binding transcriptional regulators. (Host: Michael Gilman)

Stefan Stamm, Cold Spring Harbor Laboratory. Is the neuron-specific splicing mechanism of clathrin light chain B exon EN used by other neuron-specific exons?

Thomas Meier, Albert Einstein College of Medicine. Nuclear-cytoplasmic transport on tracks. (Host: David Spector)

Ann Sutton, Cold Spring Harbor Laboratory. The role of the Sit4 phosphatase in the yeast cell cycle.

March

Michel Charbonneau, Scripps Research Institute. Phosphorylation of Cdc25 in the fission yeast. (Host: Kim Arndt)

Jerry Yin, Cold Spring Harbor Laboratory. CREB and long-term memory in *Drosophila*.

Toshiya Yamada, Columbia University. Factors controlling cell patterning in the vertebrate nervous system. (Host: Hiroyuki Nawa)

Johannes Hofmann, Cold Spring Harbor Laboratory. G_1/S transition in fission yeast: Target genes of the cell-cycle-regulated transcription factor $cdc10^+$.

David Allis, Syracuse University. Linker histone (H1) phosphorylation: Opening or closing functional domains in chromatin? (Host: Carol Greider)

David Barford, Cold Spring Harbor Laboratory. The structure of protein tyrosine phosphatase 1B.

Joe Gray, University of California, San Francisco. Genetic progression in breast and ovarian cancer. (Host: David Spector)

Jacek Skowronski, Cold Spring Harbor Laboratory. HIV-1 Nef interaction with CD4 and $p56^{lck}$ protein tyrosine kinase.

Lester Lau, University of Illinois, Chicago. Endocrine and neurogenic regulation of the orphan nuclear receptor Nur-77. (Host: Nicholas Tonks)

April

Rob Martienssen, Cold Spring Harbor Laboratory. Enhancer-trap and gene-trap transposons in *Arabidopsis*.

Gary Ruvkun, Harvard Medical School. Pattern formation in *C. elegans* by an antisense RNA and by POU proteins. (Host: Winship Herr)

Arne Stenlund, Cold Spring Harbor Laboratory. DNA replication of papillomaviruses.

Raymond White, University of Utah, Salt Lake City. Molecular genetics of the *APC* gene. (Host: Alcino Silva)

Catherine Weiss, Cold Spring Harbor Laboratory. Molecular analysis of heterotrimeric G protein(s) in plants.

Paul Kaufman, Cold Spring Harbor Laboratory. Chromatin assembly during DNA replication.

October

Stephen Sprang, HHMI/University of Texas Southwestern Medical Center. Mechanism of G-protein alpha subunits. (Host: James Pflugrath)

Linda Van Aelst, Cold Spring Harbor Laboratory. Ras function and map kinase cascade.

Jim Bliska, State University of New York, Stony Brook. Signal transduction during yersinia-mammalian cell interactions. (Host: Nicholas Tonks)

Xiaodong Cheng, Cold Spring Harbor Laboratory. How does DNA become methylated? The crystal structure of *Hha*I methylase in complex with DNA,

Brad Olwin, Purdue University. FGF receptor complexes and signaling pathways in skeletal muscle cells. (Host: Nicholas Tonks)

Cindy Sadowski, Cold Spring Harbor Laboratory. Targeting TBP to the PSE, a non-TATA box *cis*-regulatory element present in snRNA promoters.

November

William Theurkauf, State University of New York, Stony Brook. Cytoskeletal functions during early *Drosophila* development. (Host: Dave Helfman)

Carol Greider, Cold Spring Harbor Laboratory. Telomerase mechanism, reconstitution, and potential role in cellular immortalization.

Tom Maniatis, Harvard University. Mechanisms of regulated alternative splicing and pre-mRNA editing. (Host: Adrian Krainer)

Anthony Rossomando, Cold Spring Harbor Laboratory. Negative regulation of map kinase kinase (MKK).

Joanne Chory, Salk Institute. Signal transduction pathways controlling light-regulated development in plants. (Host: Venkatesan Sundaresan)

December

Roger Traub, IBM. Network oscillations in the hippocampal slice. (Host: Ancino Silva)

Hong Sun, Cold Spring Harbor Laboratory. MKP-1, a phosphatase that dephosphorylates and inactivates MAP kinases in vivo.

Claude Desplan, Rockefeller University. Functional specificity of homeoproteins during early *Drosophila* development. (Host: Ueli Grossniklaus)

Yi Zhong, Cold Spring Harbor Laboratory. Genetic analysis of a neuropeptide action in *Drosophila*.

Mike Rosbash, Brandeis University. Analysis of pre-mRNA structure and HIV REV function in yeast. (Host: Adrian Krainer)

Dafna Bar-Sagi, Cold Spring Harbor Laboratory. The Ras signaling complex.

UNDERGRADUATE RESEARCH

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, 418 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 major problem areas under investigation; (3) a better understanding of the physical and intellectual tools for modern research and the pertinence of this information to future training; and (4) a personal acquaintance with research, research workers, and centers for study.

The following students, selected from more than 180 applicants, took part in the program, which was supported by Bio-Rad Laboratories, Burroughs-Wellcome Fund, C. Bliss Memorial Fund, Gardner, The Garfield Internship, Hanson Industries, Libby Internship, National Science Foundation, Olney Fund, Phillips Petroleum Foundation, Inc., Powers Foundation, William Shakespeare Internship, and Frederica von Stade Internship.

Omar Antar, Harvard University
Advisor: **Xiaodong Cheng**
Sponsor: Burroughs-Wellcome Fund
Toward solving the three-dimensional structure of p16: Crystallization trials.

Nadine Bewry, Tennessee State University
Advisor: **Holly Cline**
Sponsor: National Science Foundation
BDNF in the development of retinal axon arbors in *Xenopus.*

Timothy Chan, Harvard University
Advisor: **David Beach**
Sponsor: Phillips/Bio-Rad
The p21 cyclin-dependent kinase inhibitor modulates DNA repair by association with a cyclin-like uracil-DNA glycosylase.

Jonathan Chubb, Cambridge University
Advisor: **G. Enikolopov**
Sponsor: Hanson Industries
Synaptotagmin II targeting to synapses.

Hannah Cross, Cambridge University
Advisor: **Robert Martienssen**
Sponsor: Wm. Shakespeare
Isolating a derivative allele in *Arabidopsis.*

Michelle DaCosta, Yale University
Advisor: **Arne Stenlund**
Sponsor: National Science Foundation
Characterizing the bovine papillomavirus protein E2.

Daniel Debowy, Yale University
Advisor: **Michael Hengartner**
Sponsor: Burroughs-Wellcome Fund
Construction and modification of episomes for a two-hybrid system assay on the *C. elegans* programmed cell death suppressor protein CED-9.

Romy Hoque, Columbia University
Advisor: **Thomas Marr**
Sponsor: Burroughs-Wellcome Fund
Hydrophobic character of transcriptional activation domains.

Jerry Hsu, Harvard University
Advisor: **Hiroyuki Nawa**
Sponsor: National Science Foundation
Identification of a possible agrin isoform in the rat brain.

Frank Lee, Duke University
Advisor: **Alcino J. Silva**
Sponsor: Olney Fund
Mutation of the NF1 GTPase activating protein (NF1-GAP) and aCaMKII genes in transgenic mice affects synaptic plasticity and performance on learning tests.

Ulo Maivali, Tartu University
Advisor: **Michael Mathews**
Sponsor: Gardner
Studies on unusual translation in mammalian cells.

Steve Miller, Pomona College
Advisor: **Ann Sutton**
Sponsor: National Science Foundation
Molecular and genetic analyses of the yeast *PDL3* gene.

Jill Nemacheck, Purdue University
Advisor: **V. Sundaresan**
Sponsor: Burroughs-Wellcome Fund
Cloning and analysis of *indeterminate.*

Elizabeth O'Connor, C.W. Post College/L.I.U.
Advisor: **Michael Wigler**
Sponsor: Burroughs-Wellcome Fund
Cloning homologs of *Schizosaccharomyces pombe* morphogenic and mating genes from *Drosophila melanogaster* and humans.

Loren del Mar Peña, Duke University
Advisor: **Winship Herr**
Sponsor: National Science Foundation
Exploring the cellular function of host cell factor (HCF).

Samanthi Perera, Mount Holyoke College
Advisor: **Adrian Krainer**
Sponsor: Libby
Selection for high-affinity binding sequences in RNA for hnRNP A2 and B1.

Caroline Roberts, Cedar Crest College
Advisor: **Erich Grotewold**
Sponsor: National Science Foundation
Myb homologs in *Arabidopsis* flowers.

Elaine Round, Washington University
Advisor: **Kim Arndt**
Sponsor: National Science Foundation
Characterization of CTR9.

Ibis Sánchez-Serrano, Iowa State University
Advisor: **Tim Tully**
Sponsor: Frederica von Stade Fund
Comparisons between *cs, linotte,* and *nalyot* brains.

Thomas Su, University of California, Los Angeles
Advisor: **Hong Ma**
Sponsor: National Science Foundation
Investigation of AGL2 DNA binding.

Yong Yu, University of Utah
Advisor: **Bruce Stillman**
Sponsor: Bliss/Powers
Characterization of the CAF-I large subunit p150.

NATURE STUDY PROGRAM

The Nature Study Program gives elementary and secondary school students the opportunity to acquire a greater knowledge and understanding of their environment. Through a series of specialized field courses, younger students can engage in introductory programs such as Nature Bugs, Nature Detectives, and Nature Discovery, and older students can enroll in more advanced programs such as Marine Biology and Nature Photography.

During the summer of 1994, a total of 406 students participated in 28 courses within the program. The classes were held outdoors, weather permitting, at the West Side School. The Laboratory has equipped and maintains classroom and laboratory facilities as well as a darkroom at West Side School. This facility is used as a base for the students' exploration of the local environment. Field classes are held on Laboratory grounds, St. John's Preserve, Fire Island National Seashore, Caumsett State Park, the Cold Spring Harbor Fish Hatchery and Aquarium, as well as other local preserves and sanctuaries.

In addition to the three 2-week sessions, the Adventure Education course meets on two Fridays for trips. The students go on a 10-mile bicycle hike to Sagamore Hill and a 12-mile canoe trip on the Nissequogue River.

PROGRAM DIRECTOR

William M. Payoski, M.A., Adjunct Professor, Nassau Community College

REGISTRAR

Amy Anderson, Cold Spring Harbor Laboratory

INSTRUCTORS

Jennifer Gensel, Elementary Teacher, Hicksville School District
Linda Payoski, M.S., Science Teacher, Uniondale High School
Marjorie Pizza, B.A., Science Teacher, Locust Valley School District
Donna Stokes, Teaching Candidate
Camille Walker, B.S., Biological Oceanography
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
Bird Study
Freshwater Life
Seashore Life
Marine Biology
Nature Photography
Adventure Education


BANBURY
CENTER

BANBURY CENTER DIRECTOR'S REPORT

Banbury Center maintained the numbers and quality of its meetings during 1994. There were again 16 meetings at the Center, attended by 510 participants. In addition, five neurobiology courses were held over the summer months and the Center was made available to ten other groups. All told, the Center was used on 31 different occasions in 1994!

Participants

For the second year, we can give some demographic information about our participants, although these figures are approximate because some individuals come to more than one meeting each year. Of the 510 participants, 418 came from the United States. This year, New York (85) supplanted California (64) as the leading source of participants. Massachusetts (42) and Maryland (41) were third and fourth. These four states accounted for 45% of visitors to Banbury Center in 1994, the remaining U.S. participants coming from 26 other states. Ninety-two participants (18%) came from 17 foreign countries, figures very similar to those in 1993. Again most came from the United Kingdom, with equal numbers (10) from Germany and Japan. It was especially pleasing that we were able to maintain attendance by foreign participants despite increased travel costs.

Scientific Meetings

It becomes ever more difficult to categorize the diverse topics of our meetings, so this year I shall describe them in chronological order.

The year opened with an outstanding meeting, **Secretory Pathways: The Molecular Basis for Their Specificity**. This is a very exciting area of research, one that endeavors to understand the mechanisms by which proteins move from the sites of their synthesis in the cytoplasm to the places where they are needed—elsewhere in the cytoplasm or to the outside of the cell. This presents many problems to the cell, not the least of which is that it involves complex interactions between different sorts of molecules. We were honored to have Nobel laureate, George Palade, one of the founders of the field, attend and deliver the closing remarks.

Banbury Center has established a tradition for meetings dealing with both scientific and policy aspects of genome projects. In this case, a group of scientists working on the *Arabidopsis* genome came to Banbury for **The *Arabidopsis* Genome** meeting to review the scientific progress being made and to make plans for future efforts. The cross-disciplinary nature of genomic research was evident in the first session that included contributions from scientists working on yeast, nematode, fruit fly, and mouse genomes.

The third meeting of the year, **Melatonin: Mechanisms and Actions**, was unusual in that it covered a physiological subject, the role of the chemical melatonin in regulating circadian rhythms. Quite apart from the fascinating biology involved in the ways in which organisms set and maintain internal clocks, understanding this for human beings could have significant implications for the millions of people who work hours that do not coincide with the light-dark cycle of the day and for those of us who find it difficult to adapt as we cross time zones.

The final scientific meeting of the spring, **Genetics of Learning and Memory,** could not have been held even a few years ago. Here, the tools of recombinant DNA are being used to dissect the processes underlying the ways in which organisms learn from their experiences and retain those memories while discarding memories of no functional significance. Once again, it was impressive

Robertson House provides dining and housing accommodations at Banbury Center

how studies on very different organisms—fruit fly, nematode, and mouse—are being brought together through the use of genetics, and in so doing, findings on each organism are reinforced by research on the others.

Scientific meetings began again in September with a meeting, **Targets for Specific Therapies in Leukemia**. These cancers have been studied intensively for many years, and there have been significant advances in understanding their genetics and molecular pathology, and basing therapies on that knowledge. This meeting brought together the leaders in these topics to review the current standing and future directions. It was a special pleasure to have Baynard Clarkson as one of the organizers. Barney has been a devoted supporter of the Laboratory as a trustee and as chairman of the trustees; it was good to put his scientific skills to use as well!

Some topics justify having follow-up meetings because of their intrinsic interest and because of the benefits that would accrue if the topics could be advanced. **Protein Design/Folding** is an excellent example. The problem of predicting how a peptide chain will fold is a great intellectual challenge and one, if solved, would help with designing proteins with new or modified activities.

A paradox of chromosome replication is that chromosomes will become progressively shorter because of the way that DNA is made. To prevent loss of DNA from their ends, chromosomes terminate in structures called telomeres, and an enzyme complex called telomerase made of RNA and protein maintains the telomeres. It appears that telomerase may have an important role in cancer and in aging. The October meeting entitled, appropriately, **Telomeres**, was the first intensive meeting devoted to telomeres and telomerase, a meeting in which the advances of the previous years were reviewed in detail.

Genomes played a large part in the Center's activities this year. Following the example set by the ***Arabidopsis*** meeting in the Spring, **Grass Genomes** was also intended to assess the current status and potential benefits of having the complete DNA sequence of grasses available for genetic manipulation.

Banbury Center has a long tradition of promoting studies of genetic disorders. There have been several very notable examples, and the meeting **Candidate Gene Approaches to ALS** was an excellent example of what such meetings can achieve. Advances in this field have been rapid and now the Amyotrophic Lateral Sclerosis Association wanted to look forward to the next step in their research program. We brought scientists working on ALS together with scientists from other areas who have expertise and approaches that are likely to be important in the next phase of ALS research.

The next meeting also dealt with genomes but with genes and genomes in general. The genome sequencing projects are beginning to produce very large amounts of sequence data, data that should make it possible to evaluate the various ideas proposed to account for the present arrangement of genes within species and for the differences between species. **Evolution of Genes and Genomes** was designed to carry out such an evaluation. It was clear that we can expect major evolutionary insights to come from comparisons of the genome sequences of the "model" organisms being sequenced.

The major challenge facing human genetics is the analysis of so-called complex disorders where mutations in more than one gene are necessary for development of the disorder or where mutations in different genes may lead to the same disorder. The **Molecular Genetics of Diabetes** meeting provided a snapshot of the tools being developed to tackle the genetic analyses of these types of disorders.

The most successful practical applications of biomedical research have come in the area of public health, where the health of many millions of people has been improved. Vaccines exemplify this, but for reasons both technical and social, the development of new vaccines is fraught with the difficulties. **Planning for the Next Generation of Vaccines** was sweeping in its coverage of these issues, ranging from new technical advances through analyses of those factors that influence the decisions of scientists, companies, and governments to undertake and support the development of new vaccines.

Robert Wood Johnson Foundation Meeting

The Robert Wood Johnson Foundation is funding two meetings at Banbury Center to examine the role that human genetics should play in primary health care and how genetic services can be provided. The first meeting, held in August, was a **Workshop on Human Genetics and Health Care**. It was notable for including individuals involved in different areas of health care provision who are concerned that genetics is properly introduced in primary health care. As well as clinical geneticists and genetic counsellors, there were nurses, primary care physicians, social workers, nurse practitioners, and representatives of professional organizations.

JP Morgan–Cold Spring Harbor Laboratory Meeting for Executives

This meeting, sponsored by J.P. Morgan, for the senior executives of pharmaceutical, biotechnology, and venture capital companies tackled **The Biology of Human Behavior** for the ninth meeting in this series. The origins of recent interest in this subject can be traced to the work of E.O. Wilson, who established the field called sociobiology in the mid 1970s. More recently, human molecular genetics has found some evidence for the genetic basis of traits such as male homosexuality and anti-social behavior. The controversy over the claims made in the book *The Bell Curve* demonstrates how interesting and contentious such findings are, especially when it is urged that public policy be based on these findings. This was an extraordinarily interesting and exciting meeting, made especially memorable by the participation of E.O. Wilson.

Charles A. Dana Foundation Project on Manic-Depressive Illness

As part of the Cold Spring Harbor Laboratory contribution to the Charles A. Dana Foundation Consortium on the genetic basis of manic-depressive illness, we held a **Workshop on Manic-depressive Illness** in June to introduce the findings of recent research to nonscientists. The meeting was modeled on the extremely successful meetings for journalists and congressional staff that had been sponsored by the Alfred P. Sloan Foundation here at Banbury Center. This workshop brought together journalists, congressional staff, and staff of the Dana Foundation and covered the

field of manic-depressive illness from clinical studies through genetics to the economic impact of the disorder.

Human Genome and Genetic Analysis Workshops

We have held a number of **Genetics Workshops for Nonscientists** for the Health Effects & Life Sciences Division of the Department of Energy. These workshops have proved popular, and the DOE made a grant to the DNA Learning Center and the Banbury Center for a further two workshops. Previous workshops targeted teachers, Congressional staff, theologians, bioethicists, journalists, lawyers, and patient advocates. The new series is targeting a different group who also need to understand modern human genetics, namely primary care physicians. To maximize the impact of the workshop, with the help of Bernard Rosof and Andrew Packard at Huntington Hospital, we invited directors of continuing medical education in New York State hospitals to participate. The workshop went very well and we are confident that they returned to their colleagues and encouraged them to take the same course. As a consequence of this workshop, I gave a total of five lectures at Long Island Jewish and Long Island College Hospitals.

Other Meetings

The Center is a valuable resource for our community and I was pleased that several groups came here during 1994. Banbury Center hosted seminars given by the nonprofit groups in the village of Lloyd Harbor in February and March. In August, West Side School held a faculty meeting and, in September, Huntington Hospital brought its Board of Trustees here. Holiday House, which provides summer vacations for disadvantaged girls, spent a day at the Center reviewing Holiday House's activities. The New York Biotechnology Association held a 1-day discussion meeting here and the Esther A. and Joseph Klingenstein Fund held a 2-day meeting for its Neuroscience Research Fellows. The Carnegie Council for Ethical Affairs and the Uehiro Foundation on Ethics and Education held a joint discussion meeting reviewing the impact of modern human genetics. The Albert B. Sabin Vaccine Foundation held a board meeting here. In addition, two groups of scientists from Cold Spring Harbor Laboratory came from the main campus to hold group meetings here.

Funding

Once again, the generosity of the members of the Cold Spring Harbor Laboratory Corporate Sponsor Program and other companies and foundations supported our program. It is difficult to overemphasize how important this funding is in enabling us to hold timely and exciting meetings. The Corporate Sponsors provided support for six meetings in 1993: **Secretory Pathways: The Molecular Basis for Their Specificity; Arabidopsis Genome; Targets for Specific Therapies in Leukemia; Protein Design/Folding; Telomeres; Evolution of Genes and Genomes.** Support for the latter meeting was also provided by the Alfred P. Sloan Foundation, which has played a critical role in promoting research in molecular evolution and in supporting Banbury Center.

Foundations provided funds for a significant proportion of Banbury Center meetings in 1994. Four foundations supported meetings on human genetic disorders: The Amyotrophic Lateral Sclerosis Association funded the meeting on **Candidate Gene Approaches to ALS**; The William Stamps Farish Fund provided support for the third of the series of meetings on complex human genetics (**Molecular Genetics of Diabetes**); the Charles A. Dana Foundation funded two meetings related to manic-depressive illness; and the Robert Wood Johnson Foundation supported the first of two meetings on genetics and health care. The Albert B. Sabin Foundation funded the meeting on **Planning for the Next Generation of Vaccines**, the first in the series of meetings on contemporary issues in vaccine development.

The outstanding 1994 Executives' meeting, the **Biology of Human Behavior**, was generously funded by J.P. Morgan. The Office of Health and Environmental Research of the Department of Energy funded the **Genetics Workshop for Nonscientists**.

Looking Forward to 1995

The 1995 Banbury Center program promises to be even more eclectic and interesting than usual. Genetics will continue to be the dominating theme of the program, but there will also be meetings on imaging, plant molecular biology, genomics, and cell biology. It is clear that the Center's activities are going to continue to expand and that this will require close coordination with other activities at the Laboratory. I want to thank David Stewart (Meetings Office), Susan Cooper (Public Affairs), Jim Hope (Catering), and Jack Richards (Buildings and Grounds) and their staffs for their help and forbearance.

Here at the Banbury Center, Bea Toliver and Ellie Sidorenko in the Banbury Center office and Katya Davey in Robertson House continue to make running a complex operation seem easy. Danny Miller and Andy Sauer worked their magic in keeping the Banbury Center grounds beautiful, but, after 10 years here, Danny moved to the main laboratory site. We were sorry to see him go but we were pleased to welcome Chris McEvoy, whose home is here on the estate, in his place. No doubt there will be changes, but the Center will remain dedicated to providing an environment in which scientists can reflect on their research with few interruptions from outside.

Jan Witkowski

Sammis Hall

MEETINGS

Secretory Pathways: The Molecular Basis for Their Specificity

February 27–March 2

FUNDED BY

Cold Spring Harbor Laboratory Corporate Sponsor Program

ARRANGED BY

J.E. Rothman, Memorial Sloan-Kettering Cancer Center, New York, New York
G. Warren, Imperial Cancer Research Fund, London, United Kingdom

SESSION 1: The Synapse
Chairperson: R.H. Scheller, Howard Hughes Medical Institute, Stanford University Medical Center, California

J.E. Rothman, Memorial Sloan-Kettering Cancer Center, New York, New York: Opening remarks.
R.H. Scheller, Howard Hughes Medical Institute, Stanford University Medical Center, California: Synaptic transmission.
T.C. Sudhof, Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas: Composition and regulation of the synaptic fusion complex.
P. DeCamilli, Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, Connecticut: Membrane traffic at the synapse.
T. Sollner, Memorial Sloan-Kettering Cancer Center, New York, New York: Intracellular vesicle docking and fusion.
T.F.J. Martin, University of Wisconsin, Madison: Characterization of ATP-dependent and Ca^{++}-activated steps of the regulated secretory pathway.

SESSION 2: Toxins
Chairperson: G. Warren, Imperial Cancer Research Fund, London, United Kingdom

G. Schiavo, University of Padua, Padova, Italy: Tetanus and botulism neurotoxins are zinc endopeptidases specific for the neuroexocytosis apparatus.
R. Jahn, Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, Connecticut: Clostridial neurotoxins as tools to study exocytosis in neurons.

SESSION 3: Mutants
Chairperson: H.F. Lodish, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts

B.J. Meyer, University of California, Berkeley: *C. elegans* mutants defective in neurotransmission.
T.L. Schwarz, Stanford University Medical Center, California: Synaptotagmin mutations in the fly.

SESSION 4: Yeast
Chairperson: H.R.B. Pelham, MRC Laboratory of Molecular Biology, Cambridge, United Kingdom

S. Ferro-Novick, Yale University School of Medicine, New Haven, Connecticut: Factors mediating the late stages of ER to Golgi transport in yeast.
R.W. Schekman, Howard Hughes Medical Institute, University of California, Berkeley: Mechanism and regulation of vesicle budding from the ER.
J.E. Gerst, Mount Sinai School of Medicine, New York, New York: Synaptobrevin-like proteins and their role in vesicle transport.
P. Novick, Yale University School of Medicine, New Haven, Connecticut: Components of the yeast secretory machinery that confer specificity to the final stage of the exocytic pathway.

J. Fernandez, J. White

SESSION 5: Rabs
Chairperson: W.E. Balch, The Scripps Clinic and Research Institute, La Jolla, California

S.R. Pfeffer, Stanford University School of Medicine, California: Functional analysis of rab 9 protein.
M. Zerial, European Molecular Biology Laboratory, Heidelberg, Germany: The GTPase molecular switch of rab proteins in the regulation of intracellular transport.

SESSION 6: Calcium
Chairperson: J.M. Fernandez, Mayo Clinic, Rochester, Minnesota

W. Almers, Max-Planck Institut für medical Forschung, Heidelberg, Germany: Fast steps in Ca-triggered exo- and endocytosis in neuroendocrine cells.
E. Neher, Max-Planck Institute for Biophysical Chemistry, Gottingen, Germany: Sources of secretory delays in neuroendocrine cells.
K.L. Wilson, Johns Hopkins University School of Medicine, Baltimore, Maryland: Calcium mobilization via IP3 receptors during nuclear vesicle fusion.

SESSION 7: Endosomes
Chairperson: J. Gruenberg, University of Geneva, Sciences II, Switzerland

P.D. Stahl, Washington University School of Medicine, St. Louis, Missouri: In vitro reconstitution of the endocytic pathway.
I. Mellman, Yale University School of Medicine, New Haven, Connecticut: Molecular sorting during intracellular transport.

SESSION 8: Hydrophobic Peptides
Chairperson: J.M. White, University of Virginia Medical Center, Charlottesville

D.M. Engelman, Yale University, New Haven, Connecticut: Peptide interactions with and within lipid environments Specificity of helix-helix interactions and the spontaneous insertion of a transmembrane helix.
F. Hughson, Harvard University, Cambridge, Massachusetts: Three-dimensional structure of the fusion-active conformation of influenza hemagglutinin.
G.E. Palade, University of California, San Diego, La Jolla: Closing remarks.

The *Arabidopsis* Genome

March 20–March 23

FUNDED BY

Cold Spring Harbor Laboratory Corporate Sponsor Program

ARRANGED BY

M. Bevan, John Innes Centre, Norwich, United Kingdom
J.R. Ecker, University of Pennsylvania, Philadelphia
R. Martienssen, Cold Spring Harbor Laboratory, New York

SESSION 1: Large-scale Genome Projects
Chairperson: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory, New York

J.R. Ecker, University of Pennsylvania, Philadelphia: Introductory remarks.
S.G. Oliver, UMIST, Manchester, United Kingdom: Yeast as a subject and a tool for genome analysis.
R. Wilson, Washington University School of Medicine, St. Louis, Missouri: Sequencing *C. elegans*.
M. Palazzolo, Human Genome Center, Berkeley, California: *Drosophila* human genome project.
W. Dietrich, Whitehead Institute Genome Center, Cambridge, Massachusetts: Construction of a dense genetic linkage map of the mouse.
L. Rowen, University of Washington, Seattle: Redundancy is our friend: Large-scale sequencing of the human and mouse T-cell receptor β loci.

SESSION 2: Physical Mapping and Markers
Chairperson: M. Beven, John Innes Center, Norwich, United Kingdom

E. Meyerowitz, California Institute of Technology, Pasadena: The *Arabidopsis* genome (structure, size, contents).
E. Richards, Washington University, St. Louis, Missouri: Molecular chromosome studies in *Arabidopsis*.
P.A. Scolnik, E.I. du Pont de Nemours & Co., Wilmington, Delaware: Mapping and sequencing the *Arabidopsis* genome: New high-throughput markers for genome and mutation analysis.
M. Zabeau, Keygene n.v., Wageningen, The Netherlands: Progress in physical mapping of the *Arabidopsis* genome using AFLP markers.
F.M. Ausubel, Massachusetts General Hospital, Boston: Use of RFLP/genomic subtraction to identify large numbers of CAPS (cleaved amplified polymorphic sequences), codominant ecotype-specific PCR-based markers, for *Arabidopsis*.
J.R. Ecker, University of Pennsylvania, Philadelphia: Progress toward a complete map of the *Arabidopsis* genome.
R. Schmidt, John Innes Centre, Norwich, United Kingdom: Strategies for completion of the physical maps for chromosomes 4 and 5.
H.M. Goodman, Massachusetts General Hospital, Boston: Progress on the physical map and sequence of chromosome II.

E. Richards, R. Schmidt, U. Grossniklaus, V. Sundaresan, P. Scolnik, K. Feldmann

SESSION 3: Gene Identification
Chairperson: R. Martienssen, Cold Spring Harbor Laboratory, New York

C.R. Somerville, Carnegie Institution of Washington, Stanford, California: MSU *Arabidopsis* EST project.
M. Caboche, INRA Versailles Cedex, France: Present and future of *Arabidopsis* genome projects in France.
C. Gigot, IBMP-CNRS, Strasbourg Cedex, France: Expressed sequence tags obtained by partial sequencing of cDNAs from *A. thaliana.*
R.W. Davis, Stanford University School of Medicine, California: A simple genetic map and a proposal of complete cDNA sequencing for the total genome for *A. thaliana.*
J. Ryals, Ciba Biotechnology, Research Triangle Park, North Carolina: Acquired resistance in *Arabidopsis:* A genetic approach—The limitations.
K.A. Feldmann, University of Arizona, Tucson: Utility of T-DNA-generated populations of *Arabidopsis* for gene cloning and reverse genetics.
V. Sundaresan, Cold Spring Harbor Laboratory, New York: Exon trapping with transposons.

SESSION 4: Sequencing and Informatics
Chairperson: H.M. Goodman, Massachusetts General Hospital, Boston

M. Bevan, John Innes Centre, Norwich, United Kingdom: *Arabidopsis* genome sequencing—The ESSA project.
W.R. McCombie, Cold Spring Harbor Laboratory, New York: Strategies for automated sequence analysis of the genomes of model organisms.
D. Searls, University of Pennsylvania School of Medicine, Philadelphia: Computational gene prediction: Comparative studies in vertebrates, invertebrates, and plants.
C. Fields, The Institute for Genome Research, Gaithersburg, Maryland: Managing and integrating information from high-throughput genome projects.
T.G. Marr, Cold Spring Harbor Laboratory, New York: Discussion about *Arabidopsis* database.

SESSION 5: Policy
Chairperson: J.R. Ecker, University of Pennsylvania, Philadelphia

Remarks by:
J.R. Ecker, University of Pennsylvania, Philadelphia
R.J. Cook, USDA-RNI-CGP, Washington, D.C.
M.W. Dilworth, National Science Foundation, Arlington, Virginia

Melatonin: Mechanisms and Actions

April 10–April 13

ARRANGED BY

A.J. Lewy, Oregon Health Sciences University, Portland

SESSION 1: Basic Physiology and Pharmacology
Chairperson: J. Redman, Monash University, Victoria, Australia

S.M. Reppert, Massachusetts General Hospital, Boston: Molecular biology of melatonin receptors.
M.L. Dubocovich, Northwestern University Medical School, Chicago, Illinois: Melatonin receptor: Pharmacology and circadian activity; melatonin analogs.
M.H. Stetson, University of Delaware, Newark: Melatonin phase hypothesis for seasonal reproductive rhythms.
B.D. Goldman, University of Connecticut: Storrs: Melatonin duration hypothesis for seasonal reproductive rhythms.

SESSION 2: Melatonin PRCs and Effects of Melatonin in Animals
Chairperson: H. Illnerova, Czech Academy of Sciences, Prague, Czech Republic

J. Redman, Monash University, Victoria, Australia: Melatonin entrainment and PRC in rodents.
V.M. Cassone, Texas A&M University, College Station: Effects of melatonin on the avian and mammalian circadian systems.
L.P. Morin, State University of New York, Stony Brook: Effects of pinealectomy on circadian rhythmicity in rodents.
M.U. Gillette, University of Illinois, Urbana: Mechanisms of melatonin on the suprachiasmatic nucleus.

A. Lewy, T. Wehr, H. Illnerova, M. Dubocovich

SESSION 3: Light Phase Response Curves (PRCs) and Effects of Light in Humans
Chairperson: M.U. Gillette, University of Illinois, Urbana

R.E. Kronauer, Harvard University, Cambridge, Massachusetts: Type-O (amplitude suppression) phase resetting in humans.

D.G. Beersma, University of Groningen, The Netherlands: Type-1 phase resetting in humans.

K.-I. Honma, Hokkaido University School of Medicine, Sapporo, Japan: A light PRC in humans: Aftereffect of entrainment?

D.S. Minors, University of Manchester, United Kingdom: The classical light PRC in humans.

S.S. Campbell, New York Hospital–Cornell Medical Center, White Plains: Alerting/energizing effects of light.

C.I. Eastman, Rush–Presbyterian–St. Luke's Medical Center, Chicago, Illinois: Treatment of shift workers with light and dark.

M. Terman, New York State Psychiatric Institute, New York: Treatment of winter depressives with light.

H. Illnerova, Czech Academy of Science, Prague, Czech Republic: Phase resetting with one pulse of light: Entrainment of the human melatonin rhythm.

SESSION 4: Melatonin PRCs and Effects of Melatonin in Humans
Chairperson: C.I. Eastman, Rush–Presbyterian–St. Luke's Medical Center, Chicago, Illinois

A.J. Lewy, Oregon Health Sciences University, Portland: The human melatonin PRC.

R.L. Sack, Oregon Health Sciences University, Portland: Phase resetting in blind people and shift workers.

J. Arendt, University of Surrey, Guildford, United Kingdom: Phase resetting in delayed sleep phase syndrome and air travelers.

B. Claustrat, Hopital Neuro-Cardiologique, Lyon, France: Phase resetting in air travelers. Human melatonin PRC pharmacokinetcs.

SESSION 5: Side Effects and Other Consequences of Melatonin: Melatonin Analogs
Chairperson: M. Terman, New York State Psychiatric Institute, New York

O. Tzischinsky, Brown University School of Medicine, Providence, Rhode Island: Melatonin possesses a delayed hypnotic effect that is time-dependent.

I.V. Zhdanova, Massachusetts Institute of Technology, Cambridge: Soporific effects of melatonin in humans.

C. Singer, Oregon Health Sciences University, Portland: Melatonin administration and sleep in the elderly.

D. Dawson, University of Adelaide, Woodville, Australia: Melatonin and "uncoupling" the clock: A neuroendocrine cause of age-related sleep disturbances.

B. Myers, Bowling Green State University, Ohio: Hypothermic effects of melatonin in humans.

T.A. Wehr, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland: Melatonin, temperature, sleep, and seasonal changes in humans.

R.J. Reiter, University of Texas Health Science Center, San Antonio: Melatonin, EMF, and scavenging for negative ions. Intracellular melatonin: Antioxidant actions and non-enzymatic degradation.

D.E. Blask, The Mary Imogene Bassett Hospital, Cooperstown, New York: Melatonin and cancer: Mechanisms of action and potential impact of EMF.

A.J. Lewy, Oregon Health Sciences University, Portland: General discussion.

Genetics of Learning and Memory

April 17–April 20

FUNDED BY

H. Robertson Memorial Fund

ARRANGED BY

A. Silva, Cold Spring Harbor Laboratory, New York
T. Tully, Cold Spring Harbor Laboratory, New York

SESSION 1
Chairperson: J.C. Hall, Brandeis University, Waltham, Massachusetts

Part I: Genes, Physiological Mechanisms, and Behavior: What Are the Interconnections?

A. Silva, Cold Spring Harbor Laboratory, New York: Genetics of learning and memory.
R. Hen, Columbia University, New York, New York: $5HT_{1B}$ receptor knockout: Behavioral consequences.
Y. Zhong, Cold Spring Harbor Laboratory, New York: The function of neuropeptides in mushroom body neurons of *Drosophila* learning mutants.

Part II: Genetic Strategies for the Dissection of Learning and Memory: Are There Common Patterns Amongst Evolutionary Divergent Organisms?

J.M. Wehner, University of Colorado, Boulder: Multiple behavioral and genetic strategies to study hippocampal-dependent learning and memory.
C. Rankin, University of British Columbia, Vancouver, Canada: Issues in the genetic dissection of learning in *C. elegans*.

SESSION 2
Chairperson: L.C. Griffith, Brandeis University, Waltham, Massachusetts

Part I: The Interplay between Genes, Development, and Learning: Can We Alter Learning without Causing Physiological and Structural Changes?

W. Quinn, Massachusetts Institute of Technology, Cambridge: Forward and reverse genetic studies of learning in *Drosophila*.
S.G.N. Grant, Columbia University, New York, New York: Fyn mutant mice: Biochemical, physiological, and behavioral deficits.
K.-F. Fischbach, Institut für Biologie III, Freiburg, Germany: Targeted misexpression of cell adhesion molecules in *Drosophila*.
K. Kaiser, University of Glasgow, United Kingdom: *Drosophila* mushroom bodies: Covert cellular organization and in vivo manipulation.

Part II: Strategies to Circumvent the "Developmental Problem": Is It Possible to Actually Study Learning without Interfering with It?

R.F. Lathe, University of Edinburgh, United Kingdom: Redirecting gene expression in the mouse hippocampus.
R. McKay, National Institute of Neurological Disorders and Stroke, Bethesda, Maryland: Transplantation of cultured stem cells: A new method to analyze the molecular mechanisms of neuronal function in mammals.

R. Bourtchouladze

SESSION 3
Chairperson: R. Greenspan, New York University, New York

S. Tonegawa, Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge: Electrophysiological and behavioral analyses of mouse mutants generated by gene targeting.

Discussion: Specificity of Gene Disruptions: How Specific Do They Have To Be?
Moderator: R. Greenspan, New York University, New York

SESSION 4
Chairperson: S.F. Heinemann, The Salk Institute, San Diego, California

Part I: Neurotransmitter: Release and Receptor Function: Mechanisms of Learning?

T.C. Sudhof, Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas: Genetic approaches to neurotransmitter release.
S. Nakanishi, Kyoto University Faculty of Medicine, Japan: Physiological functions of glutamate receptors in neuron plasticity and development.
P.H. Seeburg, University of Heidelberg, Germany: Genetic regulation of excitatory postsynaptic cation channels.

Part II: Functional Plasticity: What Are the Implications to Learning and Memory?

C.-F. Wu, University of Iowa, Iowa City: Physiological and developmental plasticity of neurons in *Drosophila* learning mutants.
J. Gordon, University of California, San Francisco: Using transgenic mice to dissect the mechanisms underlying ocular dominance plasticity.

SESSION 5
Chairperson: J. Roder, University of Toronto, Ontario, Canada

Part I: The Study of Plasticity in Cells, Circuits, and Behavior: Is There a Link?

T.J. O'Dell, University of California, Los Angeles, School of Medicine: Snaptic plasticity in transgenic mice expressing an α-CaMKII variant.
R. Malinow, Cold Spring Harbor Laboratory, New York: Acute expression of genes to dissect mechanisms of learning and memory.
J.O. McNamara, Duke University, Durham, North Carolina: Effect of null mutations of α-CaCMKII on epileptogenesis in transgenic mice.

C.I. Bargmann, University of California, San Francisco: Behavioral plasticity in olfactory responses of *C. elegans*.

Part II: Creating Biological Explanations of Cognitive Processes: Will Genetics Do It?

Y. Dudai, The Weizmann Institute of Science, Rehovot, Israel: Neurogenetic dissection of learning: How specific can it be?
A.M. Smith, American University in Cairo, Egypt: Genetics of learning and memory: Creating a new interdisciplinary paradigm.

SESSION 6
Chairperson: M. Stryker, University of California, San Francisco

E.R. Kandel, Howard Hughes Medical Institute, Columbia University, New York, New York: A molecular switch for long-term memory in *Aplysia* and mice.

Discussion: Developmental vs. Behavioral Plasticity: Are There Common Mechanisms and Common "Switches?"
Moderator: M. Stryker, University of California, San Francisco

SESSION 7: Issues in the Behavioral Analysis of Mutants: How Can We Integrate Data from Different Laboratories and from Different Model Systems?
Chairperson: M. Davis, Connecticut Mental Health Center, Yale University, New Haven

D. Wahlsten, University of Alberta, Edmonton, Canada: Standardizing and validating tests of mouse behavior: Genetic aspects.
M. Heisenberg, Theodor-Boveri-Institut (Biozentrum), Wurzburg, Germany: Learning in tethered flies.
B.H. Smith, The Ohio State University, Columbus: Higher-order conditioning in invertebrates; behavioral and genetic analyses.
D. Van Der Kooy, University of Toronto, Ontario, Canada: Mutations that block associative and/or nonassociative learning in *C. elegans.*
T. Tully and J. Yin, Cold Spring Harbor Laboratory, New York: Genetic dissection of memory.

Workshop on Human Molecular Genetics

April 21–April 24

FUNDED BY

Office of Health and Environmental Research, U.S. Department of Energy

ARRANGED BY

M. Bloom, DNA Learning Center, Cold Spring Harbor Laboratory, New York
D. Micklos, DNA Learning Center, Cold Spring Harbor Laboratory, New York
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory, New York

SESSION 1

D. Micklos, DNA Learning Center, Cold Spring Harbor Laboratory, New York: Mendelian view of the gene: From peas to eugenics.
J. Witkowski, Banbury Center, Cold Spring Harbor Laboratory, New York: Modern view of the gene.
J. Witkowski, Banbury Center, Cold Spring Harbor Laboratory, New York: PCR, RFLPs, and $(CA)_n$: What they are; what they do.

SESSION 2

S. Airhart, Oncor Science, Gaithersburg, Maryland: Cytogenetics in the age of DNA.

SESSION 3

D. Micklos, DNA Learning Center, Cold Spring Harbor Laboratory, New York: Laboratory: Using restriction enzymes to construct chromosome maps.

SESSION 4

D. Micklos, DNA Learning Center, Cold Spring Harbor Laboratory, New York: Laboratory: Construction of chromosome maps.

SESSION 5

M. Bloom, DNA Learning Center, Cold Spring Harbor Laboratory, New York: Cloning human disease genes.
P. Ward, Institute of Molecular Genetics, Baylor College of Medicine, Houston, Texas: DNA-based diagnosis for human genetic diseases.
C. Harris, Laboratory of Human Carcinogenesis, National Cancer Institute, Bethesda, Maryland: Cancer genetics: New advances, new surprises.
R. Tanzi, Neurogenetics Laboratory, Massachusetts General Hospital, Boston: Molecular genetics and biology of Alzheimer's disease.

SESSION 6

M. Bloom and D. Micklos, DNA Learning Center, Cold Spring Harbor Laboratory, New York: Laboratory: Fingerprinting your own DNA by polymerase chain reaction.

SESSION 7

M. Bloom and D. Micklos, DNA Learning Center, Cold Spring Harbor Laboratory, New York: Laboratory: Analyzing fingerprinting results.
C. Link, Human Gene Therapy Institute, Des Moines, Iowa: Human gene therapy trials.
T. Tully, Cold Spring Harbor Laboratory, New York: Genetics and behavior.
M. Saxton, Massachusetts Office on Disability, Boston: Genetics and cultural attitudes to disability.
P. Reilly, Shriver Center for Mental Retardation, Waltham, Massachusetts: Future of genetic testing and screening.

Workshop on Manic-depressive Illness

June 9–June 11

FUNDED BY

The Charles A. Dana Foundation

ARRANGED BY

K. Jamison, Johns Hopkins University Medical School, Baltimore, Maryland
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory, New York

SESSION 1

K. Jamison, Johns Hopkins University Medical School, Baltimore, Maryland: Clinical description of manic-depressive illness (MDI).
N. Rosenthal, National Institute of Mental Health, Bethesda, Maryland: Current status of treatments for MDI.
C. Gilliam, Columbia University, New York, New York: Developments in the molecular genetics of MDI.

SESSION 2

W. Drevets, Washington University, St. Louis, Missouri: Using new brain imaging techniques for studying mental disorders.

SESSION 3

M. Bloom, DNA Learning Center, Cold Spring Harbor Laboratory, New York: Laboratory: Fingerprint your own DNA using the polymerase chain reaction.

SESSION 4

T. Wehr, National Institute of Mental Health, Bethesda, Maryland: Biological rhythms and their significance in MDI.
P. Reilly, Shriver Center for Mental Retardation, Waltham, Massachusetts: Ethical and societal issues of discovering genes for human behavior.
R.D. Wyatt, National Institute of Mental Health Neuroscience Center, Washington, D.C.: Economic impact of MDI.

Workshop on Human Genetics and Health Care

August 28–August 31

FUNDED BY

The Robert Wood Johnson Foundation

ARRANGED BY

J.G. Davis, New York Hospital, Cornell University College of Medicine, New York
D.H. Lea, Foundation for Blood Research, Scarborough, Maine
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory, New York

SESSION 1: Overview of Current State of Providing Genetic Information
Chairperson: E. Thomson, National Institutes of Health, Bethesda, Maryland

P. Reilly, Shriver Center for Mental Retardation, Waltham, Massachusetts: Medical geneticists.
A.P. Walker, University of California, Irvine, Medical Center: Genetic counselors.
J.K. Williams, Iowa City, Iowa: Nurses.

SESSION 2: Current Models for Genetic Services
Chairpersons: A.P. Walker, University of California, Irvine, Medical Center
D.H. Lea, Foundation for Blood Research, Scarborough, Maine

B.R. Haas, New York Hospital–Cornell Medical Center, New York: Community outreach.
J.D. Schulman, Genetics & IVF Institute, Fairfax, Virginia: Private genetic services.
L. Djurdjinovic, Genetic Counseling Program, Binghamton, New York: Rural outreach genetic services.
N.L. Fisher, Medical Genetic Services, Seattle, Washington: Community hospital-based genetic services.
D.L. Wethers, St. Luke's-Roosevelt Hospital Center, New York, New York: Genetic services targeted to single gene disorders.
K.A. Schneider, Dana-Farber Cancer Institute, Boston, Massachusetts: Cancer genetics.
G. Cunningham, Department of Health Services-Genetic Disease Branch, Berkeley, California: State health department-initiated genetic services.
M. S. Lubinsky, Children's Hospital of Wisconsin, Milwaukee: Genetic services in an academic center.

Summary and discussion:
What can we learn from these different approaches?
Is it simplistic to think of a general model for delivering genetic services?

SESSION 3: Cultural and Consumer Issues in Providing Genetic Information
Chairperson: N.L. Fisher, Medical Genetic Services, Seattle, Washington

J. Mackta, Alliance of Genetic Support Groups, Chevy Chase, Maryland: Partnerships between consumers and healthcare professionals.
D. Punales-Morejon, Beth Israel Medical Center, New York, New York: Genetic counselling and ethno-cultural issues.
G. Wang, Chinatown Health Clinic, New York, New York: Incorporation of genetic information into primary healthcare for Asian populations.

Summary and discussion:
How to take account of different cultural backgrounds in viding genetic information?
How to ensure continuing and active interactions between consumers and genetic healthcare professionals?

SESSION 4: Genetic Services: Providers of Genetic Information
Chairpersons: N.L. Fisher, Medical Genetic Services, Seattle, Washington
J. Hanson, Office of the Assistant Secretary of Health, Washington, D.C.

R.B. Black, Columbia University School of Social Work, New York, New York: Social workers.
C. Scanlon, American Nurses Association, Washington, D.C.: Nurses and nurse practitioners.
J.G. Davis, New York Hospital, Cornell University College of Medicine, New York: Physicians.

Comments:
J.R. Allen, American Medical Association, Chicago, Illinois
W. Freeman, American Academy of Family Physicians, Albuquerque, New Mexico
A.L. Mathews, University of Colorado, Denver
M. Shannon, Health Resources and Services Administration, Rockville, Maryland
G. Anderson, New England Medical Center, Boston, Massachusetts

Summary and conclusions:
What are the current roles of different healthcare providers?
What resources and people are available to develop new routes for providing genetic information?

SESSION 5: Education of Primary Care Providers in Genetics
Chairpersons: J. Hanson, Office of the Assistant Secretary of Health, Washington, D.C.
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory, New York

N.A. Holtzman, Johns Hopkins Medical Institutes, Baltimore, Maryland: State of genetic knowledge of primary healthcare providers.
M.E. Carlin, 4 Tarrant County Hospital District, Fort Worth, Texas: Primary care physicians: A regional approach to continuing education.
A.L. Matthews, University of Colorado, Denver: Continuing education of nurses.
K. Greendale, New York State Department of Health, Albany: Genetic counselors' initiatives in providing education to other health professionals.
J.S. Lin-Fu, Maternal and Child Health Bureau, Rockville, Maryland: Maternal and Child Health initiatives in genetic education for primary care providers.
S. Feetham, National Institute of Nursing, National Institutes of Health, Bethesda, Maryland: National Institute of Nursing Research initiatives on genetic education.
D. Runkle, American Association for the Advancement of Science, Washington, D.C.: AAAS professional education outreach.

SESSION 6: Review of Recommendations: Where to Go from Here?
Chairperson: J.G. Davis, New York Hospital, Cornell University College of Medicine, New York

E.H. Thomson, National Institutes of Health, Bethesda, Maryland
D.H. Lea, Foundation for Blood Research, Scarborough, Maine
A.P. Walker, University of California, Irvine, Medical Center
N.L. Fisher, Medical Genetic Services, Seattle, Washington
J. Hanson, Office of the Assistant Secretary of Health, Washington, D.C.

Targets for Specific Therapies in Leukemia

September 11–September 14

FUNDED BY

Cold Spring Harbor Laboratory Corporate Sponsor Program

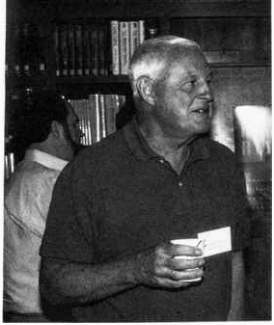
ARRANGED BY

B.A. Chabner, National Cancer Institute, National Institutes of Health, Bethesda, Maryland
B. Clarkson, Memorial Sloan-Kettering Cancer Center, New York, New York

Introduction: B. Clarkson, Memorial Sloan-Kettering Cancer Center, New York, New York

SESSION 1: Growth Regulation of Normal and Leukemic Cells
Chairperson: R.A. Weinberg, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts

D.H. Beach, Cold Spring Harbor Laboratory, New York: The cell cycle and cancer.
C. Greider, Cold Spring Harbor Laboratory, New York: Telomeres and telomerase in cancer.
J.N. Ihle, St. Jude Children's Research Hospital, Memphis, Tennessee: Role of JAKs and STATs in cytokine receptor signal transduction.
K. Kelly, National Cancer Institute, National Institutes of



B. Clarkson

C. Greider, R. Weinberg

Health, Bethesda, Maryland: Role of two immediate early genes (*PAC1* and *GEM*) in regulating signal transduction and cell cycle advancement in hematopoietic cells.

W. Kaelin, Dana-Farber Cancer Institute, Boston, Massachusetts: The cell cycle regulatory transcription factor E2F as a target for antineoplastic drug discovery.

T.D. Tlsty, University of North Carolina, Chapel Hill: Origin of drug-resistant tumor cells.

SESSION 2: Cell Adhesion/Homing/Angiogenesis
Chairperson: T.D. Tlsty, University of North Carolina, Chapel Hill

P.W. Kincade, Oklahoma Medical Research Foundation, Oklahoma City: Sex steroids as regulators of normal lymphopoiesis.

A. Freedman, Dana-Farber Cancer Institute, Boston, Massachusetts: Adhesion of follicular lymphoma cells: Regulation of homing and activation.

M.W. Long, University of Michigan, Ann Arbor: Role of cytoadhesion in hematopoietic cell development.

C.M. Verfaillie, University of Minnesota, Minneapolis: Interferon-x may reverse abnormal circulation and proliferation in CML.

SESSION 3: Follicular Lymphomas and Hairy Cell Leukemia
Chairperson: C.M. Croce, Thomas Jefferson University, Philadelphia, Pennsylvania

E. Beutler, The Scripps Research Institute, La Jolla, California: 2-chlorodeoxyadenosine: A lympho-lytic nucleoside designed to take advantage of a cell-specific metabolic pattern.

J.C. Reed, La Jolla Cancer Research Foundation, California: BCL.2 and chemoresistance in cancer.

SESSION 4: CML
Chairperson: B. Clarkson, Memorial Sloan-Kettering Cancer Center, New York, New York

D. Afar, Howard Hughes Medical Institute, University of California, Los Angles: Activation of multiple signals by the BCR-ABL oncogene.

A.M. Pendergast, Duke University Medical Center, Durham, North Carolina: Signaling by the BCR/ABL oncogene.

E.A. Sausville, National Cancer Institute, National Institutes of Health, Bethesda, Maryland: Drugs directed at pathogenetically relevant protein kinases: Novel strategies for the treatment of leukemia and lymphoma.

R. Van Etten, Center for Blood Research, Harvard Medical School, Boston, Massachusetts: New targets for therapy of Philadelphia-positive leukemia derived from studies of c-ABL and BCR/ABL.

J.Y.J. Wang, University of California, San Diego, La Jolla: BCR-ABL, mechanism of action and target of intervention.

SESSION 5: APL
Chairperson: R.A. Weinberg, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts

H. De The, CNRS, Paris, France: Acute promyelocytic leukemia and RA.
R.P. Warrell, Jr., Memorial Sloan-Kettering Cancer Center, New York, New York: Retinoids as targeted cancer therapies: Clinical, pharmacologic, and molecular studies.

SESSION 6: New Therapies/Conclusions
Chairperson: B.A. Chabner, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

D. Cheresh, The Scripps Research Institute, La Jolla, California: Cell adhesion, angiogenesis, and apoptosis.
J.R. Bertino, Memorial Sloan-Kettering Cancer Center, New York, New York: p53 and drug resistance in leukemia.
A.B. Deisseroth, University of Texas M.D. Anderson Cancer Center, Houston: Interferon inducible transcriptional regulatory factors using MDR-1 vectors and autologous bone marrow transplantation.
L. Fairbairn, Paterson Institute for Cancer Research, Manchester, United Kingdom: Gene therapy to reduce hematotoxicity and secondary hematopoietic neoplasms following antitumor treatment.
A.I. Oliff, Merck Research Laboratories, West Point, Pennsylvania: Pharmaceutically realistic targets in molecular oncology.
I.H. Pastan, DCBD, National Cancer Institute, National Institutes of Health, Bethesda, Maryland: Recombinant immunotoxins for the therapy of leukemia and lymphoma.
M. Feldman, Weizmann Institute of Science, Rehovot, Israel: Cancer metastasis: Immunotherapy via peptide and gene therapy.

Protein Design/Folding

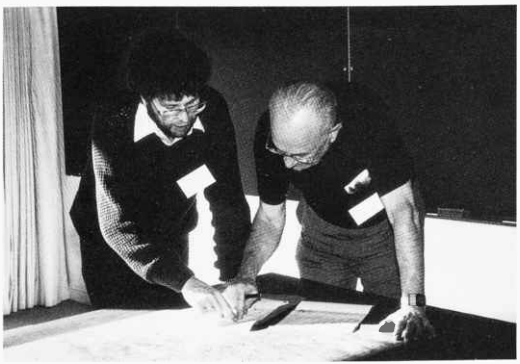
October 16–October 19

FUNDED BY

Cold Spring Harbor Laboratory Corporate Sponsor Program

ARRANGED BY

B. Honig, Columbia University, New York, New York
R.L. Jernigan, National Cancer Institute, National Institutes of Health, Bethesda, Maryland
H.A. Scheraga, Cornell University, Ithaca, New York



J. Skolnick, H. Scheraga

SESSION 1

F.M. Richards, Yale University, New Haven, Connecticut: Nonpolar, solvent-accessible, surface area: Can we measure it experimentally?

C.R. Matthews, Pennsylvania State University, University Park: Size and structure in early folding intermediates of dihydrofolate reductase.

D.M. Rothwarf, Cornell University, Ithaca, New York: Folding/unfolding pathways of ribonuclease A.

J.S. Weissman, Yale University, New Haven, Connecticut: GroEl-mediated folding proceeds by multiple rounds of binding and release of nonnative polypeptides.

P.E. Wright, The Scripps Research Institute, La Jolla, California: NMR structural characterization of protein folding pathways and folding intermediates.

K.A. Sharp, University of Pennsylvania, Philadelphia: Cyclic dipeptides as models for protein folding and association energetics.

SESSION 2

G.D. Rose, Washington University, St. Louis, Missouri: Prediction of protein helices from a stereochemical code.

A.-S. Yang, Columbia University, New York, New York: Energetics of the coil-helix transition for poly-L-alanine in water.

L. Holm, EMBL, Heidelberg, Federal Republic of Germany: The slow death of the protein folding problem. (When will we know all natural protein structures?)

D. Eisenberg, University of California, Los Angeles: 3D profiles for protein folding and design.

R.L. Jernigan, DCBDC, National Cancer Institute, National Institutes of Health, Bethesda, Maryland: Coarse-grained interaction energies.

S. Rackovsky, Mount Sinai School of Medicine, New York, New York: Studies of the protein-folding code.

SESSION 3

W.L. Jorgensen, Yale University, New Haven, Connecticut: Thermal and urea-induced unfolding of proteins with molecular dynamics simulations.

R.M. Levy, Rutgers University, Piscataway, New Jersey: Thermodynamics of solvation and pKas in proteins.

D.G. Covell, PRI/NCI-FCRDC, Frederick, Maryland: Role of surface hydrophobicity in protein-protein recognition.

B. Honig, Columbia University, New York, New York: The free energy balance in protein folding.

M.R. Pincus, State University of New York Health Science Center, Brooklyn: Use of the electrostatically driven Monte Carlo. Method for exploring the conformational space around folded proteins and identification of effector domains of the *ras* p21 protein using this method.

J. Hermans, University of North Carolina at Chapel Hill: Do molecular dynamics forcefields correspond to stable proteins?

SESSION 4

E. Shaknovich, Harvard University, Cambridge, Massachusetts: Design of stable and fast-folding sequences and their mechanism.

J. Skolnick, The Scripps Clinic Research Institute, La Jolla, California: De novo simulations of globular protein folding.

A. Sali, Harvard University, Cambridge, Massachusetts: Thermodynamics and kinetics of protein folding.

C.L. Brooks, Carnegie Mellon University, Pittsburgh, Pennsylvania: Protein folding pathways and thermodynamics studied by molecular dynamics.

H.J.R. Weintraub, R.W. Johnson Pharmaceutical Research Institute, Raritan, New Jersey: The need for reasonably accurate structure predictions in drug discovery.

H. A. Scheraga, Cornell University, Ithaca, New York: The multiple-minima problem in protein folding.

SESSION 5

Z. Huang, University of California, San Francisco: Prion infectivity: A case of protein folding and refolding.

R. Friesner, Columbia University, New York, New York: Computational studies of protein folding.

A.T. Hagler, Biosym Technologies, Inc., San Diego, California: On the effect of long-range interactions on protein structure, specificity, and ligand-binding free energies.

S.C. Harvey, University of Alabama at Birmingham: New modeling methods for ribonucleoprotein complexes.

J.A. McCammon, University of Houston, Texas: Kinetic issues in the design of proteins.

W.G. Guida, Ciba-Geigy Company, Summit, New Jersey: How protein conformation has influenced the design of inhibitors of purine nucleoside phosphorylase.

Telomeres

October 23–October 26

FUNDED BY

Cold Spring Harbor Laboratory Corporate Sponsor Program

ARRANGED BY

E. Blackburn, University of California, San Francisco
T. de Lange, The Rockefeller University, New York, New York
C. Greider, Cold Spring Harbor Laboratory, New York

SESSION 1: Telomerase
Chairperson: H. Cooke, Western General Hospital, Edinburgh, United Kingdom

C. Greider, Cold Spring Harbor Laboratory, New York: Telomerase, biochemistry reconstitution.
J. Lingner, University of Colorado, Boulder: Hypotrichous telomerase RNA structure and function.
D. Shippen, Texas A&M University, College Station: Differential use of the RNA template by the *Euplotes* and *Oxytricha* telomerases.
E. Blackburn, University of California, San Francisco: Telomerase, telomerase RNA, and mutant telomeres in yeast.
D. Gottschling, University of Chicago, Illinois: TLC1: The template RNA component of *S. cerevisiae* telomerase.

SESSION 2: Telomere Dynamics and Replication: Small Eukaryotes
Chairperson: E. Blackburn, University of California, San Francisco

V. Lundblad, Baylor College of Medicine, Houston, Texas: Characterization of EST-mediated pathway for telomere replication.
T.D. Petes, University of North Carolina, Chapel Hill: Mutations that affect telomeres in yeast.
V.A. Zakian, Fred Hutchinson Cancer Research Center, Seattle, Washington: Telomere replication in *Saccharomyces*.
B.J. Brewer, University of Washington, Seattle: Telomeres and other elements that delay replication origin activation.
E. Henderson, Iowa State University, Ames: Telomere length regulation in *Tetrahymena*.

B. Brewer, S. Gasser, E. Blackburn

SESSION 3: Telomere Dynamics in Human Aging and Cancer
Chairperson: T. de Lange, The Rockefeller University, New York, New York

C.B. Harley, Geron Corporation, Menlo Park, California: Telomeres and telomerase in cell aging and cancer.
J.W. Shay, University of Texas Southwestern Medical Center, Dallas: Telomerase activity in human tissues and tumors; manipulation of telomere length in immortal cells.
S. Bacchetti, McMaster University Medical Center, Ontario, Canada: Telomerase in cell immortalization and tumor progression.

SESSION 4: Telomeric Chromatin, Silencing, and Telomere Binding Proteins
Chairpersons: T.D. Petes, University of North Carolina, Chapel Hill
J. Haber, Brandeis University, Waltham, Massachusetts

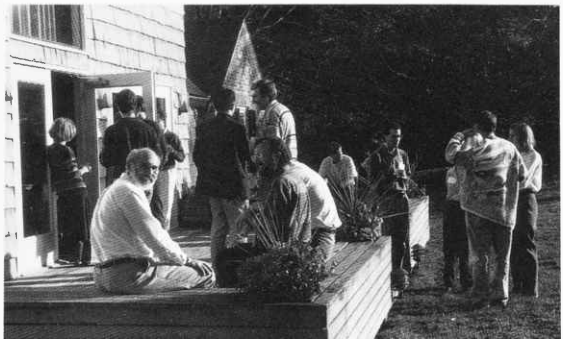
D. Gottschling, University of Chicago, Illinois: Transcriptional silencing in *S. cerevisiae.*
D.M. Shore, Columbia University College of Physicians & Surgeons, New York, New York: Regulation of transcriptional silencing at HM loci and telomeres in yeast.
A.J. Lustig, Memorial Sloan-Kettering Cancer Center, New York, New York: Mechanisms of action of the Rap1p in telomere silencing and size control.
R. Allshire, Western General Hospital, Edinburgh, United Kingdom: Fission yeast telomeres and position effect variegation.
H. Cooke, Western General Hospital, Edinburgh, United Kingdom: Position effects at mammalian telomeres.
S.M. Gasser, Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland: Subnuclear organization of telomeres and repressed chromatin in yeast.
J. Berman, University of Minnesota, St. Paul, Minneapolis: Gene products involved in telomere organization in the nucleus.
T. de Lange, The Rockefeller University, New York, New York: Mammalian telomeric chromatin.
C. Price, University of Nebraska, Lincoln: The telomere protein homolog: A novel telomere replication factor?
Y. Hiraoka, Communications Research Laboratory, Kobe, Japan: Dynamics of telomeres in meiotic prophase.

SESSION 5: Dipteran Telomeres
Chairperson: V.A. Zakian, Fred Hutchinson Cancer Research Center, Seattle, Washington

R.W. Levis, Fred Hutchinson Cancer Research Center, Seattle, Washington: *Drosophila* telomeric DNA: Structure and maintenance.
M. Pardue, Massachusetts Institute of Technology, Cambridge: *Drosophila* telomeres: Transposable elements earning an honest living.
H. Biessman, University of California, Irvine: *Drosophila* telomeres: DNA organization and elongation by Het-A retrotransposition.
J.-E. Edstrom, University of Lund, Sweden: Complex repeats at chromosome ends of *C. pallidivittatus.*

SESSION 6: Chromosome Healing and Subtelomeric Recombination
Chairperson: C. Greider, Cold Spring Harbor Laboratory, New York

J. Haber, Brandeis University, Waltham, Massachusetts: Mechanism of new telomere formation to repair broken yeast chromosomes.
F. Muller, University of Fribourg, Switzerland: Nematodes as a model system for the analysis of telomere function.
W.R.A. Brown, Oxford University, United Kingdom: Dissecting mammalian chromosomes with telomeric DNA.
A. Sherf, Institut Pasteur, Paris, France: Chromosome breakage and healing in the human malaria parasite *P. falciparum.*
P. Borst, The Netherlands Cancer Institute, Amsterdam: Control of telomeric expression sites for VSG genes of trypanosomes.



Coffee Break

J.P. Morgan/Cold Spring Harbor Laboratory Executive Conference on the Biology of Human Behavior

October 28–October 30

ARRANGED BY

J.D. Watson, Cold Spring Harbor Laboratory, New York
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory, New York

SESSION 1

E. Wilson, Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts: Animal to human behavior: The evolution of complexity.

SESSION 2

S. Snyder, Johns Hopkins University School of Medicine, Baltimore, Maryland: Neural messengers and brain activity.
M. Raichle, Washington University School of Medicine, St. Louis, Missouri: Images of the mind.
L. Squire, University of California, San Diego, School of Medicine, La Jolla: Learning and memory systems of the brain.

SESSION 3

T. Tully, Cold Spring Harbor Laboratory, New York: Learning and memory in fruit flies.

A. Silva, Cold Spring Harbor Laboratory, New York: Learning and memory in mice.

SESSION 4

G. Uhl, National Institute on Drug Abuse, Baltimore, Maryland: Recent advances in molecular neurobiology and genetics.
P. McGuffin, University of Wales College of Medicine, Cardiff: Genetic approaches to psychiatric disorders.

D. Hamer, National Cancer Institute, Bethesda, Maryland: Genetics of human behaviors.
H.E. Fisher, Rutgers University, New Brunswick, New Jersey: Marriage, adultery, and divorce.

H. Fisher, H. Solomon, J.D. Watson

Grass Genomes

October 31–November 2

FUNDED BY

USDA Plant Genome Program
The Rockefeller Foundation
United Kingdom Biotechnology and Biological Sciences Research Council

ARRANGED BY

J. Bennetzen, Purdue University, West Lafayette, Indiana

SESSION 1: Presentations on Current Research Status

J. Bennetzen, Purdue University, West Lafayette, Indiana, and M. Gale, John Innes Centre, Norwich, United Kingdom: Introduction.
J. Gale, John Innes Centre, Norwich, United Kingdom: Comparative genetic maps.
S. McCouch, Cornell University, Ithaca, New York: Comparative genetic maps.
J. Bennetzen, Purdue University, West Lafayette, Indiana: Physical maps.
T. Sasaki, STAFF Institute, Tsukuba Ibaraki, Japan: Monocot cDNAs; saturation/specificity.
S. Cartinhour, USDA - NAL, Beltsville, Maryland: Database integration.

SESSION 2: Discussion Group

Expected/potential benefits of an IGGI program.
What is available (technology, resources, organization)?
What is needed (technology, resources, organization)?

SESSION 3: Discussion Group

Discussion/revision of IGGI document outline prepared from discussions and presentations on previous day.
Conclusions and designation of next steps and parties responsible.

Candidate Gene Approaches to ALS

November 2–November 5

FUNDED BY

The Amyotrophic Lateral Sclerosis Association

ARRANGED BY

R.H. Brown, Massachusetts General Hospital East, Charlestown
R.L. Nussbaum, National Center for Human Genome Research, National Institutes of Health, Bethesda, Maryland

Introduction
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory, New York
R.V. Abendroth, The Amyotrophic Lateral Sclerosis Association, Milwaukee, Wisconsin

SESSION 1: Genetics of ALS
Chairperson: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory, New York

R.H. Brown, Massachusetts General Hospital East, Charlestown: Overview of inherited motor nerve diseases.
M.A. Pericak-Vance, Duke University Medical Center, Durham, North Carolina, and J.L. Haines, Massachusetts

General Hospital East, Boston: Approaches to linkage analysis in familial ALS: Present resources and strategies.

J. de Belleroche, Charing Cross & Westminster Medical School, London, United Kingdom: A survey of superoxide dismutase mutations in 100 U.K. families and exclusion studies in extensive pedigrees lacking SOD-1 mutations.

M. Devoto, Columbia University, New York, New York: Theoretical considerations in analysis of mono- and multi-genic disease loci.

SESSION 2: Pathophysiology of ALS
Chairperson: T. Siddique, Northwestern University Medical School, Chicago, Illinois

M. Chalfie, Columbia University, New York, New York: Cell death genes in nematodes.

D.W. Choi, Washington University School of Medicine, St. Louis, Missouri: Excitotoxicity and neuronal death.

D.E. Merry, University of Pennsylvania School of Medicine, Philadelphia: Bcl-2, CAG repeats, and motor neurons.

B. Demple, Harvard School of Public Health, Boston, Massachusetts: Anti-oxidant gene families.

T. Siddique, Northwestern University Medical School, Chicago, Illinois: SOD1 transgenic mice.

D.W. Cleveland, Johns Hopkins University School of Medicine, Baltimore, Maryland: SOD1 and neurofilament L transgenic mice.

D.A. Figlewicz, University of Rochester Medical Center, New York: Mutations in neurofilament H in ALS.

P.N. Leigh, Institute of Psychiatry, London, United Kingdom: Cytoskeletal pathology in ALS.

G.D. Yancopoulos, Regeneron Pharmaceuticals, Inc. Tarrytown, New York: Neurotrophic growth factors and their receptors.

SESSION 3: Open Discussion: Candidate ALS Genes
Chairperson: A. Tobin, University of California, Los Angeles

SESSION 4: Approaches to Gene Mapping, Genomic Analysis, and Mutational Screening
Chairperson: R.L. Nussbaum, National Center for Human Genome Research, National Institutes of Health, Bethesda, Maryland

D. Patterson, Eleanor Roosevelt Institute, Denver, Colorado: Development of YAC contig maps.

R.A. Gibbs, Baylor College of Medicine, Houston, Texas: Genomic sequencing for gene searching.

M. Borodovsky, Georgia Institute of Technology, Atlanta: Computational sequence analysis.

W.M. Barnes, Washington University School of Medicine, St. Louis, Missouri: Long PCR.

E.A. Rose, Perkin-Elmer Cetus Instruments, Emery-ville, California: Applications of long PCR.

P. Nisson, Life Technologies, Gaithersburg, Mary-land: Exon trapping.

V.C. Sheffield, University of Iowa Hospitals & Clinics, Iowa City: Efficient identification of disease loci and mutation detection.

Evolution of Genes and Genomes

November 9–November 12

FUNDED BY

Alfred P. Sloan Foundation
Cold Spring Harbor Laboratory Corporate Sponsor Program

ARRANGED BY

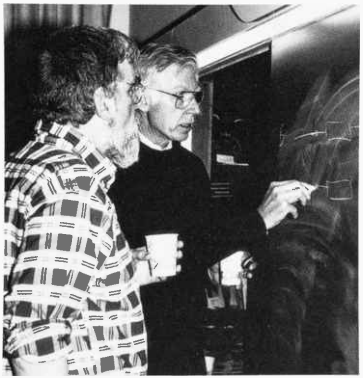
S. Brenner, University of Cambridge School of Medicine, United Kingdom
W. Gilbert, Harvard University, Cambridge, Massachusetts
J. Lake, University of California, Los Angeles
E.S. Lander, Massachusetts Institute of Technology Center for Genome Research, Cambridge

SESSION 1: Contemporary Sequences and the Past
Chairperson: J. Lake, University of California, Los Angeles

R.F. Doolittle, University of California, San Diego, La Jolla: Evolution of the earliest proteins.
P. Green, University of Washington, Seattle: Ancient conserved gene families.
M.W. Gray, Duke University, Durham, North Carolina: The organelle genome megasequencing project: Exploring mitochondrial DNA evolution.

SESSION 2: Analysis of Populations
Chairperson: D. Botstein, Stanford University School of Medicine, California

M.E. Kreitman, University of Chicago, Illinois: Role of weak selective forces on rates of molecular evolution.
J.H. Gillespie, University of California, Davis: What can be inferred about the causes of molecular evolution from species comparisons of DNA sequences?
C.H. Langley, University of California, Davis: Recombination and DNA sequence polymorphism: Distributions of transposable elements and of single nucleotide differences.
D.A. Powers, Stanford University, Pacific Grove, California: A multidisciplinary approach toward resolving the selectionist/neutralist controversy.
M.-C. King, University of California, Berkeley: Human genome diversity.
S. Paabo, University of Munich, Germany: Human population history and the mitochondrial genome.



R. Doolittle, F. Ruddle

M.-C. King

SESSION 3: Origin and Evolution of Eukaryotic Genes
Chairperson: W. Gilbert, Harvard University, Cambridge, Massachusetts

L. Hood, University of Washington, Seattle: DNA sequence of the T-cell receptor loci of humans and mice; 80 million years of molecular evolution.
J. Lake, University of California, Los Angeles: Searching for the prokaryotic origins of eukaryotes.
L. Simpson, Howard Hughes Medical Institute, University of California, Los Angeles: Evolution of RNA editing in kinetoplastid protozoa.
D. Helfman, Cold Spring Harbor Laboratory, New York: Alternative RNA splicing.

SESSION 4: Analysis of Populations
Chairperson: W. Gilbert, Harvard University, Cambridge, Massachusetts

M. Akam, University of Cambridge, United Kingdom: Evolution of *HOX* genes in arthropods.
F.H. Ruddle, Yale University, New Haven, Connecticut: Hox cluster evolution.

SESSION 5: Evolution of Genomes
Chairpersons: W. Gilbert, Harvard University, Cambridge, Massachusetts
J. Lake, University of California, Los Angeles

D. Sankoff, University of Montreal, Quebec, Canada: The mathematical inference of evolution via genome rearrangement.
P. Green, University of Washington, Seattle: Progress on sequencing the *C. elegans* genome and its analysis.
W.F. Doolittle, Dalhousie University, Halifax, Nova Scotia, Canada: Archaebacterial genomes: Why we are sequencing *Sulfolobus solfataricus*?
J.H. Miller, University of California, Los Angeles: Preliminary sequence analysis of the archaebacteria *Pyrobaculum aerofilum*.
S.J. O'Brien, National Cancer Institute, National Institutes of Health, Frederick, Maryland: Evolving genes and genomes: Lessons from the Felidae.
T. Helentjaris, University of Arizona, Tucson: Internal duplication of the maize genome and what it reveals about the evolution of genome structure.
J.E. Womack, Texas A&M University, College Station: Comparative gene mapping in cattle, mice, and humans: Contributions to understanding genome evolution.

Molecular Genetics of Diabetes

December 4–December 7

FUNDED BY

The William Stamps Farish Fund

ARRANGED BY

G.I. Bell, Howard Hughes Medical Institute, University of Chicago, Illinois
J. Todd, John Radcliffe Hospital, Oxford, United Kingdom

SESSION 1: Genetics of Diabetes and Obesity in Animal Models
Chairperson: A. Lernmark, Karolinska Hospital, Stockholm, Sweden

L.S. Wicker, Merck Research Laboratories, Rahway, New Jersey: Molecular genetics of IDDM in the NOD mouse.
E.H. Leiter, The Jackson Laboratory, Bar Harbor, Maine: Linking genotype to phenotype in mouse models of diabetes.
J.M. Friedman, The Rockefeller University, New York, New York: Genetic analysis of rodent obesity.
P. Nishina, The Jackson Laboratory, Bar Harbor, Maine: Genetics of modifying factors in NIDDM and obesity in mouse models.

SESSION 2: Genetics of Insulin-dependent (Type 1) Diabetes Mellitus (IDDM)
Chairperson: G.J. Thomson, University of California, Berkeley

J. Todd, University of Oxford, United Kingdom: Genetics of type-1 diabetes: Exclusion mapping.
L.L. Field, Health Sciences Center, Calgary, Canada: Genetics of IDDM.
E.A.M. Gale, St. Bartholomew's Hospital, London, United Kingdom: Integrated strategies for prediction of IDDM.

SESSION 3: Clinical Studies: The Diabetic Phenotype
Chairperson: C.R. Kahn, Joslin Diabetes Center, Boston, Massachusetts

L. Groop, Malmo General Hospital, Sweden: Metabolic heterogeneity of NIDDM.
P.Z. Zimmet, International Diabetes Institute, Melbourne, Australia: Latent autoimmune diabetes in adults: Genetics and autoimmunity.

SESSION 4: Genetics of Noninsulin-dependent (Type 2) Diabetes Mellitus (NIDDM)
Chairperson: G.I. Bell, Howard Hughes Medical Institute, University of Chicago, Illinois

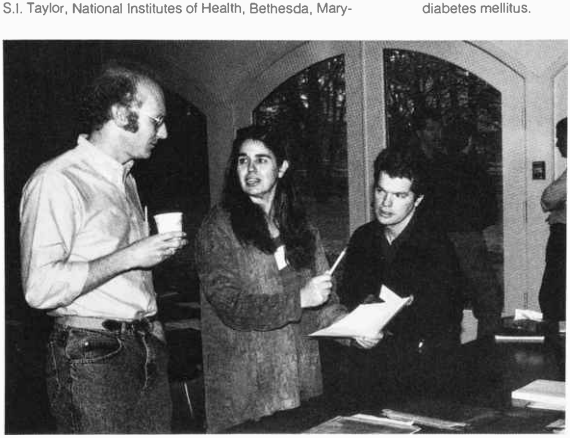
A.D. Roses, Duke University Medical Center, Durham, North Carolina: Molecular genetics of the Alzheimer diseases: A model for studies of complex genetic disorders.
P. Froguel, Human Polymorphism Study Center. Paris, France: MODY: A paradigm for genetics of NIDDM.
K.S. Polonsky, The University of Chicago, Illinois: Distinctive alterations in B-cell function associated with different genetic forms of diabetes.
F.S. Collins, National Center for Human Genome Research, National Institutes of Health, Bethesda, Maryland: Genetics of NIDDM in Finland.

SESSION 5: Genetic Studies of NIDDM In High-Risk Populations
Chairpersons: A.T. Hattersley, Queen Elizabeth Hospital, Birmingham, United Kingdom
N. Iwasaki, Tokyo Women's Medical College, Japan

C. Bogardus, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Phoenix, Arizona: NIDDM genes in Pima Indians.
M.P. Stern, University of Texas Health Science Center, San Antonio: Search for type II diabetes susceptibility genes in Mexican Americans.

SESSION 6: Candidate Genes: Genetics of Insulin Secretion and Insulin Action
Chairperson: P. Froguel, Human Polymorphism Study Center, Paris, France

M.A. Permutt, Washington University, St. Louis, Missouri: Genetics of rare inherited disorders of pancreatic B-cell function.
S.I. Taylor, National Institutes of Health, Bethesda, Maryland: Molecular genetics of insulin resistance.
G.I. Bell, Howard Hughes Medical Institute, University of Chicago, Illinois: Candidate genes and genetic studies of diabetes mellitus.



P. Concannon, G. Thomson, J. Todd

SESSION 7: Approaches for Mapping Genes for Complex Traits
Chairperson: R.S. Spielman, University of Pennsylvania, Philadelphia

N. Risch, Yale University, New Haven, Connecticut: Assessing the genetic contribution to type 1 diabetes.
J. Ott, Columbia University, New York, New York: Detecting linkage and association in the haplotype relative risk design.
M.L. Boehnke, University of Michigan, Ann Arbor: Mapping and exclusion of genes for complex traits: Application to NIDDM.
B.K. Suarez, Washington University School of Medicine, St. Louis, Missouri: Nonparametric linkage analysis and linkage disequilibrium analysis.
E.S. Lander, Whitehead Institute, Cambridge, Massachusetts: Genetic dissection of complex traits.

Planning for the Next Generation of Vaccines

December 11–December 14

FUNDED BY

The Albert B. Sabin Vaccine Foundation

ARRANGED BY

P.K. Russell, The Johns Hopkins University, Baltimore, Maryland

SESSION 1: Future Directions in Vaccine Science
Chairperson: R. Rabinovich, NIAID, NIH, Bethesda, Maryland

F.J. Malinoski, Lederle-Praxis Biologicals, Wayne, New Jersey: Glycoconjugate technology and future combination vaccines.
M.A. Liu, Merck Research Laboratories, West Point, Pennsylvania: DNA vaccines: A new approach to vaccination.
E. Paoletti, Virogenetics Corporation, Troy, New York: Attenuated poxvirus recombinants.
S. Plotkin, Pasteur Merieux Connaught, Marnes la Coquette, France: How vaccine development of the future may affect vaccination schedules and cost.
R.W. Chesnut, Cytel Corporation, San Diego, California: Immune stimulation.
G.A. Siber, Public Health Biological Laboratories, Boston, Massachusetts: Overview and discussion.

P. Freeman, P. Russell

SESSION 2: Perspectives of the Biotechnology Industry
Chairperson: F. Cano, Aviron Corporation, Burlingame, California

T.P. Stagnaro, Univax Biologics, Inc., Rockville, Maryland: Bacterial vaccines and their role as immunizing agents.
L.C. Gordon, Ora Vax Inc., Cambridge, Massachusetts: Disease and product profile for financability of vaccine development.
T. Elliott, Prime Capital Management Company, Inc., Stamford, Connecticut: Venture capital: A partial overview.
J.L. Read, Aviron, Burlingame, California: Overview and discussion.

SESSION 3: Perspectives of the Large Vaccine Companies
Chairperson: S. Lemon, University of North Carolina, Chapel Hill

L.E. Long, Lederle-Praxis Biologicals, Wayne, New Jersey: Lederle-Praxis Biologicals perspective.
R. Rappuoli, Biocine S.p.A., Siena, Italy: Perceived vaccine value and implications on discovery and development.
M. De Wilde, SmithKline Beecham Biologicals, Rixensart, Belgium: Changes in the vaccine industry and areas for partnership with the public sector.
C.M. Grant, Connaught Laboratories, Inc., Swiftwater, Pennsylvania: Vaccine economics and the impact of globalization.
R. Ellis, Merck & Company, Inc., Rahway, New Jersey: Overview and discussion.

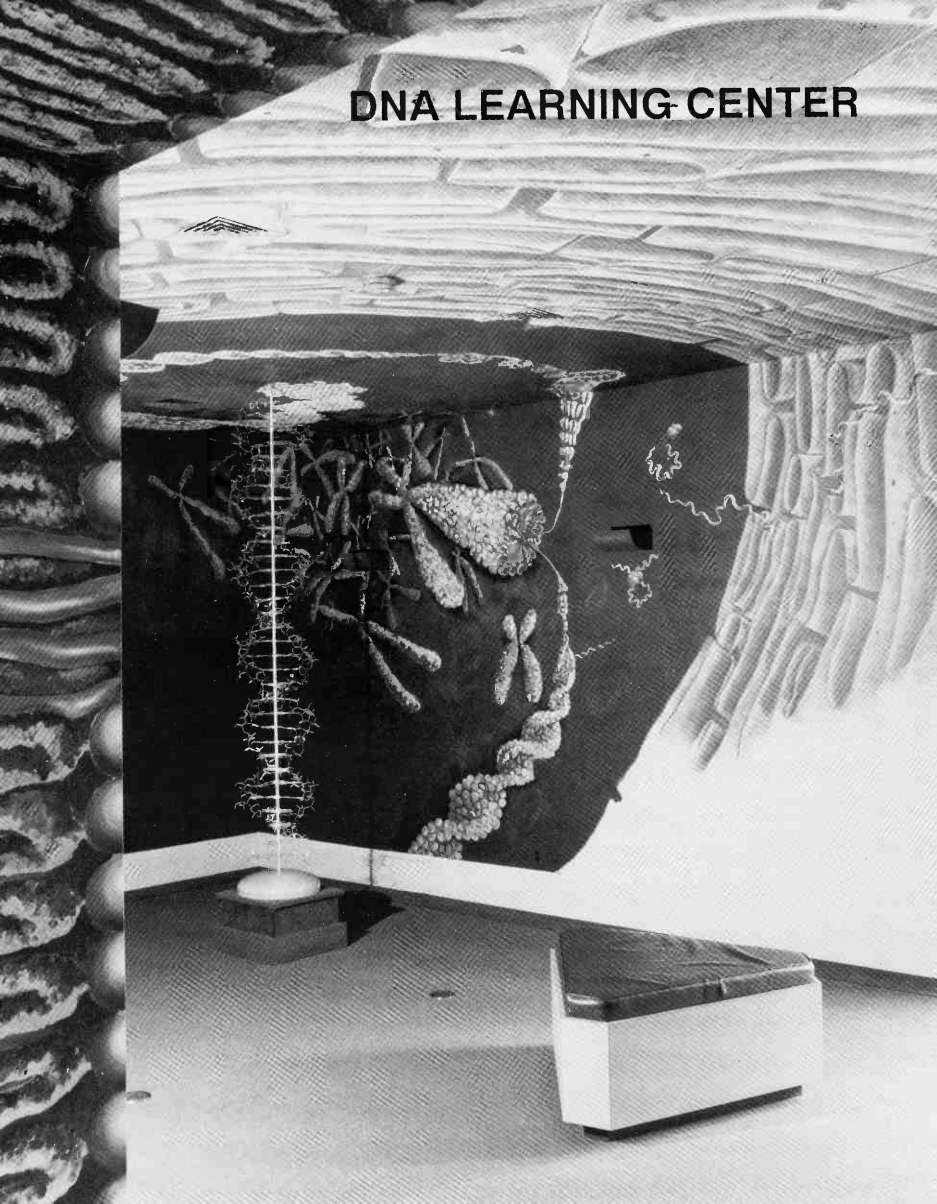
SESSION 4: Policy Discussions from the Public Sector
Chairperson: M.T. Osterholm, Minnesota Department of Health, Minneapolis

R. Rabinovich, NIAID/NIH, Bethesda, Maryland: Planning for the next generation of vaccines: Vaccine research and development supported by NIAID/NIH.
R. Bernier, Centers for Disease Control, Atlanta, Georgia: A status report on the childhood immunization initiative.
C. Broome, Centers for Disease Control and Prevention, Atlanta, Georgia: Surveillance opportunities for improved vaccine development.
M.C. Hardegree, Center for Biologics Evaluation and Research, FDA, Rockville, Maryland: Role of FDA in the regulation of vaccines.
A. Robbins, U.S. Public Health Service, Boston, Massachusetts: Overview and discussion.

SESSION 5: Policy Development
Chairperson: P. Freeman, University of Massachusetts, Boston

E.K. Marcuse, Children's Hospital and Medical Center, Seattle, Washington: Integrating public and private sectors to accomplish universal childhood immunization.
R. Widdus, National Vaccine Program Office, Washington, D.C.: Behavior of the vaccine development "system."
R. Goldberg, Springfield, New Jersey: Reinventing vaccine policy.
P.K. Russell, The Johns Hopkins University, Baltimore, Maryland: Overview and discussion.

F. Cano


DNA LEARNING CENTER

DNA LEARNING CENTER

David A. Micklos, Director
Mark V. Bloom, Assistant Director
Susan M. Lauter, Designer
Robert Muratore, Computational Scientist
Judy L. Cumella, Program Coordinator

Jane P. Conigliaro, Education Manager
Malissa A.G. Hewitt, Laboratory Instructor
Diane S. Jedlicka, Laboratory Instructor
Diane Esposito, Laboratory Instructor
Flavio Della Seta, Laboratory Instructor

Time flies. It hardly seems possible, but it was ten years ago that Cold Spring Harbor Laboratory offered its first summer training course in recombinant DNA technology for high school faculty. The DNA Learning Center did not exist then, so the workshop was held in a classroom at the Wheatley School in Nassau County.

The course was not called *DNA Science*, as it is today. The title then, *Recombinant DNA for Beginners*, was borrowed from the 1971 record album, *Songs for Beginners*, by Graham Nash. This was Nash's first solo album after the split-up of Crosby, Stills, Nash, and Young—a band propelled into the public consciousness by Woodstock and in many ways emblematic of that awkward time in American life. Nash's album was composed largely of simple songs with which nearly any beginning guitar player or vocalist could easily tag along and so, in some sense, feel like a pop musician. Our course was likewise intended—a collection of relatively simple experiments that could make high school students feel like molecular biologists. In 1990, when our manuscript was formally printed, we opted for the more enduring title, *DNA Science*. This simple but catchy synopsis of molecular genetics—looking at biology through the lens of DNA—had been mentioned casually in conversation by Jim Watson. The name has stuck, because I now hear biology educators using the term DNA science in a generic sense.

The project to develop molecular genetic experiments for student use had its origin in an unlikely collaboration between Dave Micklos, then public affairs and development director at the Laboratory, and Greg Freyer, then a postdoctoral fellow in Richard Roberts' lab group. Beginning in 1984, Greg volunteered to prepare gels of *Lambda* restriction digests for Dave to show to school students who came for tours of the Laboratory. The stained gels were illuminated on a long-wavelength UV "black light" Dave found in a lab storeroom. This was a relict from the mid-1970s, when ethidium bromide staining was developed at Cold Spring Harbor by Phil Sharp, Bill Sugden, and Joe Sambrook. Specialized mid-wavelength transilluminators were not initially available; however, "black lights" were available for illuminating the posters that were a popular form of interior decoration for young people.

Students proved fascinated with those simple "DNA fingerprints" of the *Lambda* virus, and we began thinking about hands-on experiments that might be done in high school biology classes. At about the same time, Fran Roberts became superintendent of Cold Spring Harbor Schools and sent a letter to Jim Watson asking how the school district and the Laboratory could work together in education. Fran, who had just returned from a tour of duty at the National Endowment for the Humanities in Washington, D.C., suggested that we submit a joint

Greg Freyer (*left*), Dave Micklos, and student during first *DNA Science* summer workshop in 1985.

grant proposal to the newly resynthesized education directorate of the National Science Foundation (NSF). Our proposal to develop a workshop to introduce high school teachers to recombinant DNA was turned down by NSF in 1984.

After this setback, local educators Mike Glennon and Ed Tronolone suggested what seemed a preposterous idea—to get several well-heeled school districts of Long Island to each contribute $10,000 toward teacher training and equipment purchase. By January 1985, $60,000 had been raised from six charter districts of the Cold Spring Harbor Curriculum Study, and we committed to have the first training workshop ready for summer.

The workshop would be based upon a laboratory sequence conceived by Greg and contained in a slim "manuscript" consisting of 10—12 pages of lab notes comprehensible only to a bench scientist. Happily, Rich Roberts gave Dave a half-counter of bench space at which the labs could be fleshed out before being loosed on high school teachers. In the spring, the labs were first tested with teachers during afternoon sessions at Cold Spring Harbor High School. Unfortunately, the pivotal lab involving the recombination of ampicillin- and kanamycin-resistant genes did not work. Anjun Bagwat, another postdoc in Rich Roberts' lab, solved that problem simply by including a recovery at 37°C prior to plating on kanamycin. By summer, every experiment worked well, and we conducted the first full-scale workshop at the Wheatley School in Williston Park.

Our horizons expanded in 1986, when we obtained our first real grant from Citibank to purchase and equip the first Vector Mobile DNA Laboratory. That summer, we perfected our capability for long-range instruction, conducting workshops in Massachusetts, New Hampshire, Wisconsin, Illinois, Chicago, and California. Our first NSF grant in 1987 allowed us to expand our national program and set this pattern of summer training that has extended over the ensuing years. Also in 1987, we further increased our reach when we joined with Carolina Biological Supply Company to formally publish the labs and to develop teaching kits to support wider dissemination of the *DNA Science* labs. This fruitful collaboration was brokered by Neil Patterson, the dean of modern biochemistry and molecular biology publishing, who, among many accomplishments, had published Jim Watson's epic *Molecular Biology of the Gene*.

The development of *DNA Science* is one of the first examples of bench molecular biologists making a substantive commitment to help bring biology education into the gene age. Although this educational role is now accepted and legitimized at high levels of the biological research establishment, this was not the case in 1984, especially at a "pure" science place like Cold Spring Harbor Laboratory. At that time, research biologists were generally thought to have only one responsibility—to the bench. Rich Robert's blessing for Greg to participate in the project, donating enzymes and lab supplies, and providing lab space for Dave were almost without precedent at the time.

But Rich's help also made good sense. During the 1970s, prior to the advent of biotechnology supply houses, the Roberts lab had been a major source of restriction enzymes. During that period, his group isolated approximately half of known restriction enzymes, which were made available free of charge. Researchers visiting Cold Spring Harbor for symposia and courses would literally line up outside his lab for samples of enzymes with new cutting specificities. So, just as the Roberts lab had helped popularize the use of restriction enzymes in research, it also encouraged their new use as educational tools. Our historical connection to Rich became even more precious, when in 1993 he was awarded the Nobel Prize for the codiscovery of RNA splicing.

Receipt of Key Grants

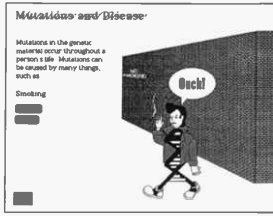
In July, we were awarded a 5-year grant of $400,000 from the Howard Hughes Medical Institute through the new Precollege Science Education Initiative for Biomedical Research Institutions. The DNALC grant was the second largest of awards to 42 institutions. The Hughes program will support our ongoing work with local school districts to vertically integrate genetics instruction at the elementary, middle, and high school levels. Significant resources will also be used to provide intensive enrichment for minority students at several New York City schools and to link these resource-poor schools to Long Island schools with well-developed genetics education programs. The grant provides large-scale support for our *BioMedia* Computer Laboratory to model uses of high-level computing in biology education, including the *Student Allele Database*, which allows students to electronically submit personal DNA fingerprints and compare them with those of other students from around the world. Related collaborations with Helen Donis-Keller at Washington University, St. Louis, and John Kruper at the University of Chicago are also supported.

In October, we were notified that the NSF had approved 3-year funding of $218,503 for "A Novel Mechanism for Introducing Human Genome Research in Freshman Biology Classes." Under the new project, to begin in 1995, college faculty will be trained to implement our polymerase chain reaction (PCR) experiment on human *Alu* insertion polymorphisms and to use the *Student Allele Data* base. Twenty-four participants will attend each of four workshops per year held at sites around the country. Faculty from 2-year and minority institutions will be targeted in promotional mailings, and one workshop per year will be sited at a historically minority institution. This grant follows on 4 years of continuous funding from the Undergraduate Faculty Enhancement Program and complements our NSF training program for high school faculty.

Building Our Computational Capability

In the spring, we released the *Genetic Computer Arcade* through the Carolina Biological Supply Company. Developed by designer Sue Lauter, this animated and interactive computer "primer" helps students build mastery of genetics concepts and gives examples of real-life applications of modern genetic technology. The program can be played on virtually any Macintosh computer with a color monitor; CD-ROM, laser disk player, other devices, or specialized software *are not* needed. Each of five "chapters" incorporates photographs, animation, and scientific data. Modules include Gregor Mendel explaining his classic experiments with peas; "Gene" the DNA person demonstrating types and causes of mutations; and case studies illustrating forensic DNA fingerprinting, DNA diagnosis, and gene therapy. The programs build knowledge sequentially, with the introductory topics appropriate for bright elementary students and the more advanced topics appropriate for high school students.

With 3-year capital support from the Stone Foundation, we have been building a sophisticated computer network for staff and student use. Our local area network of 12 Macintosh Quadras in the *BioMedia* Laboratory and 4 Sun Sparcstations in the staff office are all linked through a Sun10-30 server. However, it was becoming increasingly difficult for the Laboratory computer center to provide support for our substantial network, and we were unable to provide students regular access to the *BioMedia* Laboratory.



A scene from "Human Genetic Disease," one of five programs in the *Genetic Computer Arcade*.

Robert Muratore (*right*) assists students in the *BioMedia* Computer Laboratory.

This situation was remedied in July, with the arrival of full-time computational biologist, Robert Muratore. With a bachelor's degree in bioengineering from Johns Hopkins, a master's degree in mechanical and aerospace engineering from Princeton, and a doctorate in biophysics from Syracuse, Robert is exactly the sort of hands-on person we were seeking. Prior to joining our staff, Robert was on the faculty at SUNY Geneseo, where he developed a new biophysics program and taught computer science. His thesis research at Syracuse and postdoctoral work at the National Institutes of Health involved developing computer programs to image brain activity using antiprotons. Earlier work at the Space Studies Institute at Princeton and Hughes Aircraft Company included designing a fault-tolerant electronic interface for the *Galileo* planetary probe now en route to Jupiter.

Two events at year's end dramatically increased our computational capability. First, we took delivery of a Silicon Graphics Indigo2 workstation, a fast graphics terminal of the type used to create digital effects in movies such as *Jurassic Park*. The Silicon Graphics machine will allow us to begin work on *Journey to the Center of the Cell*, a recreation of a three-dimensional microspace of a cell based on electron micrographical data. Second, the DNALC was connected to the main Laboratory via a fiber optic cable. This linkage allows our Sun server to communicate at ethernet speeds with the Laboratory's *Phage* server. This provides us with extremely fast access to Internet and other international networks. To encourage student and faculty use of our computational facilities, we now schedule free Internet access on Thursday afternoon each week.

Also at year's end, plans were finalized for renovation of a computer/design office in the east basement, which will mark the completion of an 8-year effort to redevelop our 1925 school building as a modern science center. The office will house computational biologist Robert Muratore and designer Sue Lauter, as well as several interns. The space will facilitate a synthesis of these two functions, which will be increasingly important as we begin development of three-dimensional computer and video programs. The facility will become the command center for our local computer network, as well as our node on international networks. Under the new configuration, the *Multitorium*, *Bio2000* Teaching Laboratory, and research/prep laboratory will be added to the network.

Ad for PCR kits in Carolina Biological's Biotechnology catalog.

Progress in Making PCR Widely Available to Biology Students

Although the revolutionary importance of polymerase chain reaction (PCR) was recognized by the 1993 Nobel Prize in Chemistry, the cost, biochemical complexity, and patent protection of the process have kept it beyond the reach of biology instruction. Believing that PCR offers the *only* practical means for students to examine their own DNA and the best means to introduce human molecular genetics, we have made it our business to remove the obstacles to its use in precollege and freshman college classes. Tangible evidence of success came in September with the release of the first educational PCR kits by Carolina Biological Supply Company, under license from Perkin-Elmer. One kit amplifies viral DNA using hand thermal cycling, and the other kit amplifies the D1S80 human polymorphism using automated cycling.

During the summer, we began to shift emphasis to the class of *Alu* insertion polymorphisms, which work brilliantly with the mouthwash/chelex extraction we advocate, show very little nonspecific amplification, and can be analyzed conclusively on agarose gels. *Alu* is a family of repeated DNA sequences, approxi-

mately 300 nucleotides in length, that have the ability to copy themselves and insert randomly throughout the genome. Some *Alu* elements have inserted so recently in evolutionary time that they have not been inherited with the same frequency by different groups of people. Such *Alu* insertions are termed dimorphic ("two forms") because chromosomes can be scored for the presence or absence of the insertion. Because *Alu* insertions at a particular chromosome locus have only two alleles (+ or –), they make simple case studies for studying population genetics.

Robert Muratore worked quickly to develop a prototype *Student Allele Database* that allows students to tabulate *Alu* insertion data and test Hardy-Weinberg Equilibrium. The database also contains *Alu* insertion data from several Mendelian populations in Africa, Greenland, Sardinia, and the Caribbean. Users can compare any two populations using contingency Chi-square and calculate simple genetic distances. Initially, the database template and test data are available on diskette or can be downloaded from Genentech's *Access Excellence* gateway on America Online. Soon, Internet users will be able to access the database through our World Wide Web home page and manipulate data in real time.

Robert also led design development of a inexpensive thermal cycler, which we hope will make PCR accessible to high school and college instructors. The device consists of computer software and an analog-digital interface that cycles temperature in a Plexiglas reaction vessel by alternately activating an immersion heater and a solenoid valve controlling cold water flow. Water-flow regulation was simplified and a custom analog-digital controller was designed to receive commands from both Macintosh and PC computers. In collaboration with John Kruper, head of academic computing of the Biological Science Division of the University of Chicago, development of a much-improved Macintosh controller is nearing completion.

Training Workshops Reach Faculty and Opinion Leaders Nationwide

With funding from the National Science Foundation, we continued our tradition of offering high-level lab training to high school and college faculty. Dave Micklos took the standard-bearing *DNA Science* far afield, reaching 100 high school teachers at workshops in Oklahoma City, Colorado Springs, Moscow (Idaho), and San Juan. Mark Bloom instructed 47 college faculty at *Advanced DNA Science* workshops in San Antonio and Boston. The *Leadership Institute in Human and Molecular Genetics*, also known as DNA Boot Camp, drew together top high school faculty representing 22 states, Sweden, and Australia. In addition to practical laboratory and computer work, the institute included seminars on an array of topics by Laboratory scientists and visiting faculty:

Jack Ballantyne, Suffolk County Examiner's Office: Forensic DNA typing.
Susan Brady, Lawrence Hall of Science: Partnerships.
Xiaodong Cheng, Cold Spring Harbor Laboratory: Structure of DNA/*Hha*I methylase cocrystal.
Seth Grant, Columbia University: Molecular basis of memory.
Fred Gillam, Sachem High School: Laboratory program in molecular genetics.
John Horton, Cold Spring Harbor Laboratory: X-ray crystallography.
Paul Hough, Brookhaven National Laboratory: DNA-binding proteins.
John Kruper, University of Chicago: Computational biology.

Ruji Kobayashi, Cold Spring Harbor Laboratory: High-performance liquid chromatography and peptide synthesis.
Gerry Latter, Cold Spring Harbor Laboratory: Two-dimensional protein electrophoresis.
Elaine Lawson, Institute of Medicine: Assessing genetic risks.
Sue Levi-Pearl, Tourette Syndrome Association: Genetic disease support groups.
Dan Marshak, Cold Spring Harbor Laboratory: Protein kinases and mass spectrometry.
Dick McCombie, Cold Spring Harbor Laboratory: DNA sequencing.
Rick Michitsch, Oncogene Science: Expression screening for therapeutic agents.
Bruce Stillman, Cold Spring Harbor Laboratory: Research highlights.
Spencer Teplin, Cold Spring Harbor Laboratory: DNA synthesis.
Tim Tully, Cold Spring Harbor Laboratory: Genes and behavior.
James Watson, Cold Spring Harbor Laboratory: Open question and answer.
Jan Witkowski, Banbury Center: DNA screening and diagnosis.

With funding from the Department of Energy, we continued our collaboration with Banbury Center to introduce nonresearchers to the science behind the Human Genome Project. In the past, the project targeted "opinion leaders" in federal agencies, the media, education, law, and ethics who influence policy and education. The 1994 workshop drew education directors from 15 hospitals in New York State, with the expectation that they will begin the process of educating doctors about the growing impact of molecular genetics in medicine. In addition to an experiment to illustrate the basis of DNA diagnosis and background briefings, participants heard high-level seminars about topics on the interface of molecular genetics, medicine, and society:

Susan Airhart, Oncor Science: Cytogenetics in the age of DNA.
Charles Link, Iowa Methodist Hospital: Human gene therapy trials.
Philip Reilly, Shriver Center for Mental Retardation: Future of genetic testing and screening.
Marsha Saxton, Massachusetts Office on Disability: Genetics and cultural attitudes to disability.
Rudolph Tanzi, Massachusetts General Hospital: Molecular genetics and biology of Alzheimer's disease.
Tim Tully, Cold Spring Harbor Laboratory: Genetics and behavior.
Patricia Ward, Baylor College of Medicine: DNA-based diagnosis for human genetic diseases.

Expanding Hands-on Opportunities for Local Students

When we first developed the *DNA Science* curriculum, we were one of only a handful of institutions offering laboratory training in molecular genetics for precollege faculty. During the last decade, we have maintained the only continuously funded program operating on a nationwide scale. Our expertise at administering off-site workshops has provided a "jump-start" for local initiatives in many states. However, as more and more local universities have accepted the challenge to provide lab training for teachers, the need for off-site workshops has decreased. Fortunately, at the same time, demand for local programs and use of our excellent facilities is increasing. So we find that, after securing our reputation

through national programs, we are now devoting more effort to fostering innovative biology instruction in the school systems in our own backyard.

Realizing that systematic genetics education needs to begin when children are still forming fundamental attitudes and behaviors, we are focusing our effort at the middle-school level (grades 5–8). The flagship of our commitment to younger learners is *Genetics as a Model for Whole Learning*, which uses genetics as a paradigm for integrated learning and which incorporates concepts across disciplines and relates science to the student's life and culture. Working almost single-handedly, Education Manager Jane Conigliaro has aided faculty at 15 schools to introduce genetics modules in their elementary and middle school classes, reaching 1590 students in 1994. We anticipate that these students will provide an eager audience for the advanced genetics activities currently available at the high schools in their districts. Thus, in the small school districts of Long Island, we are beginning to see working models of science education for the gene age, incorporating hands-on learning about genetics at several stages in child and adolescent development.

The *Bio2000* Laboratory was kept very busy during the academic year. Labs on bacterial transformation, DNA restriction analysis, and human DNA fingerprinting were performed by 3180 high school students, and experiments on Mendelian genetics, cell study, and DNA extraction were performed by 780 5th–7th graders. This was a 24% increase in total lab visits over 1993, made possible through the excellent instruction offered by part-time instructors Flavio Della Seta, Diane Esposito, Diane Jedlicka, and Malissa Hewitt. The *Great Moments in DNA Science* Honors Student Seminar Series, now in its 10th year, continued to be a popular element of our annual calendar of events, drawing the attendance of 575 local students and teachers. Speakers and topics were:

Greg Freyer, Columbia University: Fixing DNA.
Tim Tully, Cold Spring Harbor Laboratory: Genes and memory.
Xiaodong Cheng, Cold Spring Harbor Laboratory: A surprising structure of DNA.

We continued to expand summer learning opportunities for local students, thanks in part to additional venues at Portledge School (Locust Valley), Central Islip School, Roslyn Middle School, and the American Museum of Natural History. High school students including 233 minority students received laboratory training during three sessions of *DNA Science*. Eight sessions of *Fun With DNA* served a total of 159 elementary students, including 74 minority students. Three sessions of the workshop *World of Enzymes* attracted 62 students.

Xiaodong Cheng presents his research to advanced biology high school students.

Karin Glaizer and Elizabeth Woerner present "Linda," a study of brain tumor gene therapy for the *World of Enzymes* summer workshop.

Multimedia and Exhibit Programs

Although we devote our major effort to curriculum development and lab instruction, we are also committed to providing stimulating multimedia presentations and exhibits for our visitors. We were happy to find that Cablevision's multimedia show, *Long Island Discovery*, drew 15,000 viewers during its first year of operation, more than twice the number of DNALC visitors prior to the show's opening in October 1993.

By year's end, the staff was focused on the development of three new exhibits to fill renovated galleries on the main level: *Story of a Gene*, *The World of Barbara McClintock*, and the *Cold Spring Harbor Nobels*. *Story of a Gene* is a comprehensive presentation on the biochemistry, physiology, medical uses, and

(*Left*) The Walk of Life construction in progress. (*Right*) Melissa Hewitt, lab instructor turned painter, works on a dungeon wall. (*Below left*) Denise Sauer puts finishing touches on exhibit entry sign.

social aspects of human growth hormone (HGH). The exhibit will encourage visitors to integrate microscopic through macroscopic perspectives, including (1) expression of the HGH gene, as illustrated in the *Cellarium* mural; (2) physiology of human growth; (3) the danger of isolating HGH from human cadavers and the link with kuru and Creutzfeldt-Jakob disease; and (4) ethical issues arising from ready availability of the hormone produced by recombinant DNA technology. The exhibit employs two- and three-dimensional displays, computer multimedia, video, and audio—many elements that will encourage visitor interaction.

The World of Barbara McClintock, being developed in cooperation with the Public Affairs Department, will recreate McClintock's laboratory using equipment and personal effects saved after her death. The object is to provide visitors with a glimpse of the day-to-day intellectual life of an extraordinary individual. *Cold Spring Harbor Nobels* shows the prizes and highlights the common thread of Nobel-winning experiments by Al Hershey, James Watson, Barbara McClintock, and Richard Roberts. All three exhibits will be geared to a level appropriate for bright elementary students and will also be appropriate for the general public. Curriculum guides, including vocabulary, questions, and readings, will help students get the most out of their exhibit visit and follow up on their interests.

Corporate Advisory Board

In the fall, we bid farewell to Corporate Advisory Board Chairman Doug Fox, when he left his position at Times Mirror to become Chief Operating Officer of Landmark Communications of Norfolk, Virginia, which owns the Weather Channel. Under Doug's leadership, the Corporate Advisory Board has become an effective advocate for the DNALC, raising substantial unrestricted annual funds and increasing our visibility in the Long Island business community. Our disappointment at Doug's departure was muted by our good luck that the chairmanship was taken over by Rick Clark. A partner at Price Waterhouse, Rick showed his commitment to our cause when he worked closely with Doug to organize the first annual Cold Spring Harbor Laboratory Golf Tournament. Held at Piping Rock Club on June 7, the tournament netted $47,750, which was added to

Dave Micklos with Corporate Advisory Board members (from left to right) Arthur Herman, Rick Clark, Pat Peterson, Michael Aboff, and Michael Vittorio.

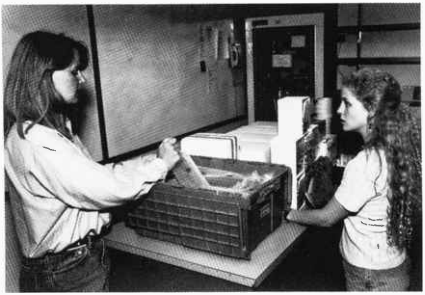
the DNALC Annual Fund. Rick's service to the Laboratory began in 1993 when he hosted the CSHL Association outreach event at the Manhattan office of Price Waterhouse. His connection to us is strengthened through his wife Jill, who is associate development director for the Laboratory.

The Corporate Advisory Board draws strength from Vice-Chairman Gary Frashier, President and CEO of Oncogene Science, who is our link to the growing biotechnology industry on Long Island. Other members of the executive committee represent the gamut of Long Island businesses: Rocco Barrese, founding partner at the patent law firm Dilworth and Barrese; Howard Blankman, founder of the public relations firm Howard Blankman Inc.; Tom Calabrese, managing director at NYNEX; Bob Diller, Vice President of the scientific equipment firm Brinkmann Instruments; Art Herman, chairman of A.D. Herman Construction; Pat Peterson, President of the realty company Daniel Gale Agency; and Peter Schiff, general partner of Northwood Ventures.

Staff and Interns

Administering our rigorous schedule of student field trips, student summer workshops, teacher-training institutes, and various follow-up activities has become an increasingly complicated task. Each program typically requires an informational mailing of 400 or more pieces, answering queries, taking reservations, processing applications, sending confirmations, and tracking payments. Many programs take place off-site and require coordination with a local organizer; several programs require yearly mail surveys. Five part-time staff and five interns provide cost-effective support and instruction but further complicate scheduling. Since her arrival at the beginning of the year, Judy Cumella has made herself indispensable in choreographing this complex dance of people and activities. She functioned single-handedly during parts of the summer when Mark and Dave were teaching off-site and Sue was out on maternity leave. In recognition of her key organizational role and the increasingly complicated nature of our activities, Judy was promoted to Program Coordinator in the fall.

The recruitment of part-time lab instructor Malissa Hewitt enabled us to offer daily afternoon labs for elementary and middle-school students. Malissa has an undergraduate degree in early childhood/elementary education and has taught elementary science at the School of the Holy Child in Old Westbury and kindergarten at the Gardens Schools. She is currently working on her master's degree in secondary biology education.



Malissa Hewitt and Stacey Trotter organize supplies in the *Bio2000* Teaching Laboratory.

We continued to get excellent laboratory, computational, and design support from interns drawn from neighboring high schools and colleges. Designer Sue Lauter was assisted by Donna Conversano and Denise Sauer. An art student at the New York Institute of Technology, Donna had participated in the painting of the Cellarium Mural in summer 1993. During the academic year, she added finishing touches to the 1993 building renovation, including hand-lettering the names of 35 Nobel Laureates in genetics whose names appear on the Multitorium frieze. Donna and Denise overpainted areas of the Cellarium mural to more accurately portray the cellular expression of human growth hormone (HGH), including the addition of transcriptional activators, signal recognition apparatus, and barrel depiction of HGH protein. Denise is an art student at SUNY Farmingdale.

Computational biologist Robert Muratore was assisted by Chris Como (Cold Spring Harbor High School) and Mark Teoh (Rice University). In addition to helping to maintain our growing computer network, each worked on multimedia projects for the *Story of a Gene* exhibit. Chris has developed an animated introduction by "Gene" the gene person, and Mark has assembled a software package to create a "morph" illustrating human growth.

The laboratory instructional staff was ably assisted by high school interns Andy Diller (Sachem), Ken Bassett (Massapequa), Jessica Hinton (Huntington), and Jermel Watkins (Central Islip). In the fall, we bid farewell to Andy, who began his freshman year at Rutgers, and welcomed Jermel, whose father Jerry is a biology instructor at Central Islip High School. Jerry was a participant in the NSF-sponsored *Leadership Institute* and coordinates our effort to develop Central Islip as the hub of an Instructional Resource Cluster serving the underprivileged students in Brentwood and Wyandanch. Assisting at *Fun With DNA* summer camps were lab aides Tara Marathe (Colby College), Daryn Berger (Walt Whitman High School), Andrea Conigliaro (St. Anthony's High School), and Michael Conigliaro (Cold Spring Harbor High School).

Publications

Bloom, M. 1994. Polymerase chain reaction. *Carolina Tips* **57:**

Bloom, M., G. Freyer, and D. Micklos. 1994. *Laboratory DNA science: An introduction to recombinant DNA technology and methods of genome analysis.* Benjamin/Cummings, Redwood, California (in press).

Micklos, D. 1994. Go with the odds: Believe DNA. *Newsday.* September 27, 1994.

Micklos, D. 1994. Genetic testing: An educational imperative to our schools. In *Assessing genetic risks: Implications for health and social policy*, vol. 2. National Academy Press, Washington, D.C.

Micklos, D. and J. Kruper. 1994. Genetics education in American schools. *Biotechnology Education* **4:** 2.

1994 Workshops, Meetings, and Collaborations

January 19	Long Island Partnership Meeting, DNALC
	Corporate Advisory Board Meeting, DNALC
January 22	Site visit by David Hicks, St. Paul's School, Concord, New Hamphsire
January 26–28	National Science Foundation Grant Review, Washington, D.C.
March 5–6	National Science Foundation Follow-up Workshop, *Advanced DNA Science*, University of Puerto Rico, Rio Piedras
March 8–10	National Institutes of Health Grant Review, Washington, D.C.
March 12–13	National Science Foundation Follow-up Workshop, *Advanced DNA Science*, University of Washington, Seattle
March 17	Laboratory for SEED Program, SUNY, Old Westbury, DNALC
March 22–24	Carolina Biological Supply Company, Burlington, North Carolina
March 30	Corporate Advisory Board Meeting, DNALC
March 31–April 1	National Science Teachers Association Meeting, Los Angeles, California
April 5	*Access Excellence* Advisory Committee, Genentech, Inc., South San Francisco, California
April 14	National Institute of Social Sciences Lecture, Harvard Club, New York, New York
	Great Moments In DNA Science Honors Student Seminar, DNALC
April 20	*Great Moments In DNA Science* Honors Student Seminar, DNALC
April 21–23	Department of Energy Workshop, *Human Genetics and Genome Analysis*, DNALC and Banbury Center
April 27–28	National Science Foundation Grant Review, Ann Arbor, Michigan
April 30–May 1	Department of Education Follow-up Workshop, *Advanced DNA Science*, Golden West College, Los Angeles, California
May 3	*Great Moments In DNA Science* Honors Student Seminar, DNALC
May 12–14	National Science Foundation Grant Review, Washington, D.C.
May 17	Hutton House Lecture, Long Island University, Old Westbury, New York
May 20	New York Biotechnology Association Meeting, DNALC
May 24	Hutton House Lecture, DNALC
June 6–10	National Science Foundation Workshop, *DNA Science*, Colorado College, Colorado Springs
June 13–17	National Science Foundation Workshop, *DNA Science*, Oklahoma School of Science and Mathematics, Oklahoma City
June 13–24	National Science Foundation Workshop, *Advanced DNA Science*, Boston University, Boston, Massachusetts
June 20–24	National Science Foundation Workshop, *DNA Science*, University of Idaho, Moscow
June 27–July 1	*DNA Science* Workshop, DNALC
July 1	Site visit by Laurence Smaje, Wellcome Trust, London, England
July 4–8	*World of Enzymes* Workshop, Portledge School, Locust Valley, New York
July 4–29	National Science Foundation *Leadership Institute*, DNALC
July 11–15	*Fun With DNA* Workshop, Portledge School, Locust Valley, New York
July 25–28	*Fun With DNA* Workshop, Portledge School, Locust Valley, New York
	Fun With DNA Minority Workshop, American Museum of Natural History, New York, New York
August 1–5	National Science Foundation Workshop, *DNA Science*, University of Puerto Rico, Rio Piedras
	Fun With DNA Workshop, DNALC
August 1–12	National Science Foundation Workshop, *Advanced DNA Science*, Trinity University, San Antonio, Texas
August 8–12	*Fun With DNA* Workshop, DNALC
	Fun With DNA Minority Workshop, Roslyn Elementary School, New York

	Fun With DNA Minority Workshop, American Museum of Natural History, New York
August 15–19	*Fun With DNA* Minority Workshop, DNALC
August 18–19	Advanced Placement Workshop, Stanford University, Palo Alto, California
August 22–26	*World of Enzymes* Workshop, DNALC
August 29–Sept. 2	*DNA Science* Workshop, DNALC
September 12–14	*Decade of PCR* Meeting, CSHL
September 22	Seminar, CSHL Association, DNALC
September 20	Site visit by Helen Donis-Keller Laboratory, Washington University Medical School, St. Louis, Missouri
September 26–30	World Health Organization Workshop, *DNA Science*, University of Panama, Panama City
October 13	Site visit by Treopia Washingon, Columbus Center, Baltimore, Maryland
October 17	Corporate Advisory Board Meeting, DNALC
November 2	Seminar, Adelphi University, Garden City, New York
November 12–13	National Science Foundation Follow-up Workshop, *DNA Science*, Oklahoma School of Science and Mathematics, Oklahoma City
November 12–17	Department of Energy Contractor's Meeting, Santa Fe, New Mexico
November 16–19	National Association of Biology Teachers Meeting, St. Louis, Missouri
December 8	Laboratory Seminar, Theodore Roosevelt American Inn of Court, DNALC
December 9–10	National Science Foundation Follow-up Workshop, *DNA Science*, University of Puerto Rico, Rio Piedras
December 12	*Women In Science and Engineering Laboratory*, DNALC

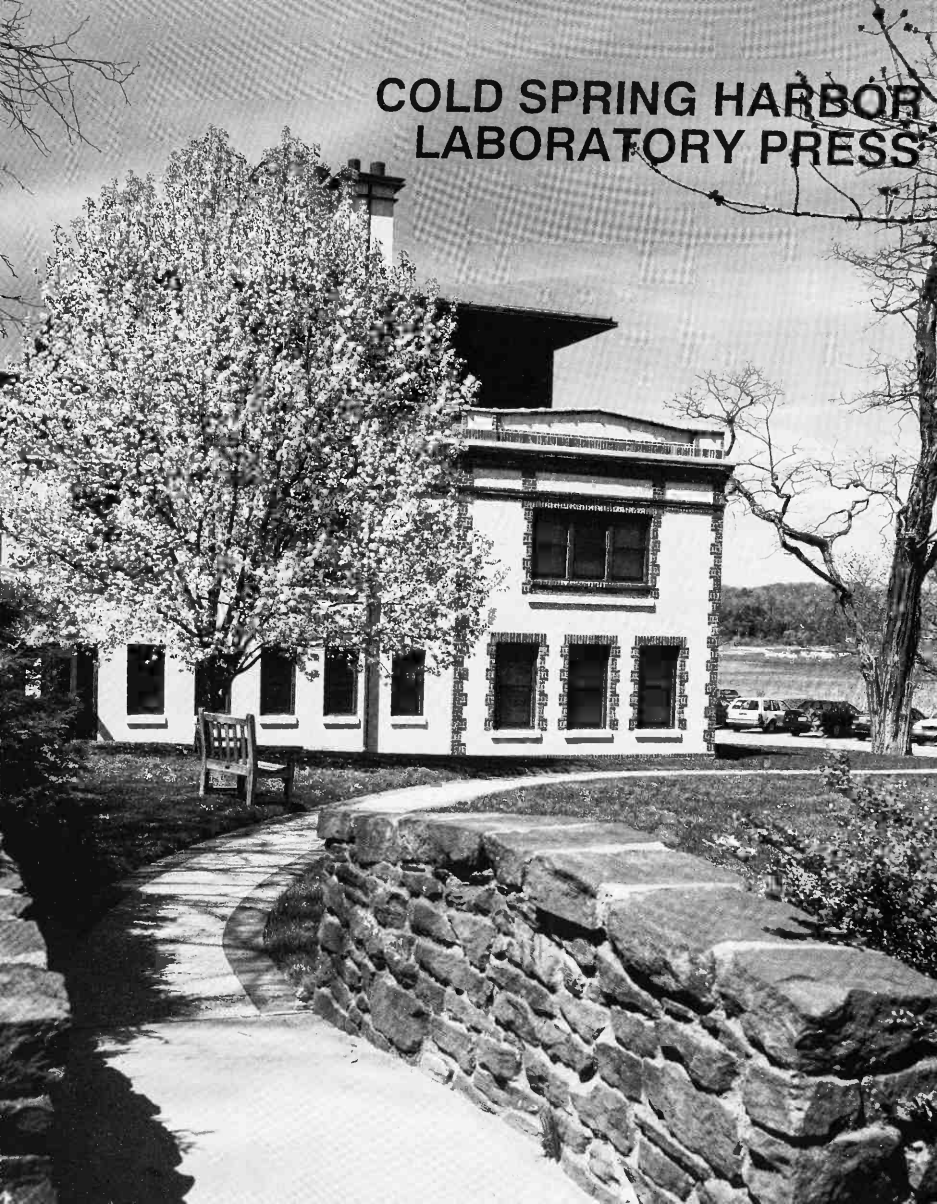
Sites of Major 3–10-day Faculty Workshops 1985–94

KEY: High School
College
Middle School

ALABAMA	University of Alabama, Tuscaloosa 1987, 1988, 1989,1990
ARIZONA	Tuba City High School 1988
ARKANSAS	Henderson State University, Arkadelphia 1992
CALIFORNIA	University of California, Davis 1986
	San Francisco State University 1991
	University of California, Northridge 1993
COLORADO	Colorado College, Colorado Springs 1994
CONNECTICUT	Choate Rosemary Hall, Wallingford 1987
FLORIDA	North Miami Beach Senior High School 1991
	University of Western Florida, Pensacola 1991
	Armwood Senior High School, Tampa 1991
GEORGIA	Fernbank, Inc., Atlanta 1989
	Morehouse College, Atlanta 1991
HAWAII	Kamehameha Secondary School, Honolulu 1990
ILLINOIS	Argonne National Laboratory 1986, 1987
	University of Chicago 1992
INDIANA	Butler University, Indianapolis 1987
IDAHO	Univeristy of Idaho, Moscow 1994
IOWA	Drake University, Des Moines 1987
KENTUCKY	Murray State University 1988
	University of Kentucky, Lexington 1992
	Western Kentucky University 1992
LOUISIANA	Jefferson Parish Public Schools, Harvey 1990
	John McDonogh High School, New Orleans 1993

MANITOBA	Red River Community College, Winnipeg 1989
MARYLAND	Annapolis Senior High School 1989
	McDonogh School, Baltimore 1988
	Montgomery County Public Schools 1990-92
	St. John's College, Annapolis 1991
MASSACHUSETTS	Beverly High School 1986
	Dover-Sherborn High School, Dover 1989
	Randolph High School 1988
	Winsor School, Boston 1987
	Boston University 1994
MICHIGAN	Athens High School, Troy 1989
MISSISSIPPI	Mississippi School for Math & Science, Columbus 1990-91
MISSOURI	Washington University, St. Louis 1989
NEW HAMPSHIRE	St. Paul's School, Concord 1986, 1987
NEVADA	University of Nevada, Reno 1992
NEW YORK	Albany High School 1987
	Bronx High School of Science 1987
	Columbia University, New York 1993
	Cold Spring Harbor High School 1985, 1987
	DeWitt Middle School, Ithaca 1991, 1993
	DNA Learning Center 1988-94
	DNA Learning Center 1990, 1992
	DNA Learning Center 1990-92
	Fostertown School, Newburgh 1991
	Huntington High School 1986
	Irvington High School 1986
	Junior High School 263, Brooklyn 1991
	Lindenhurst Junior High School 1991
	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, 1988, 1989, 1990
	Titusville Middle School, Poughkeepsie 1991, 1993
	Wheatley School, Old Westbury 1985
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
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
SOUTH CAROLINA	Medical University of South Carolina, Charleston 1988
	University of South Carolina, Columbia 1988
TEXAS	J.J. Pearce High School, Richardson 1990
	Langham Creek High School, Houston 1991
	Taft High School, San Antonio 1991
	Trinity University, San Antonio 1994
UTAH	University of Utah, Salt Lake City 1993

VERMONT	University of Vermont, Burlington 1989
VIRGINIA	Jefferson School of Science, Alexandria 1987
	Mathematics and Science Center, Richmond 1990
WASHINGTON	**University of Washington, Seattle 1993**
WASHINGTON, DC	**Howard University 1992**
WEST VIRGINIA	Bethany College 1989
WISCONSIN	Marquette University, Milwaukee 1986, 1987
	University of Wisconsin, Madison 1988, 1989
WYOMING	University of Wyoming, Laramie 1991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LABORATORY PRESS

1994 PUBLICATIONS

General Books

DNA and Chromosomes
Symposia on Quantitative Biology 58

Vaccines 94: Modern Approches to New Vaccines Including Prevention of AIDS
E. Norrby, F. Brown, R.M. Chanock, and H.S. Ginsberg (eds.)

Manipulating the Mouse Embryo: A Laboratory Manual, Second Edition
B. Hogan, R. Beddington, F. Costantini, and E. Lacy

Biology of Drosophila
M. Demerec (ed.)

Gene Therapy: Fact and Fiction in Biology's New Approaches to Disease
T. Friedmann

CSHL Monograph Series

The Biology of Heat Shock Proteins and Molecular Chaperones
R.I. Morimoto, A. Tissières, and C. Georgopoulos (eds.)

Arabidopsis
E.M. Meyerowitz and C.R. Somerville (eds.)

Cellular Receptors for Animal Viruses
E. Wimmer (ed.)

Current Communications in Cell & Molecular Biology Series

Apoptosis II: The Molecular Basis of Apoptosis in Disease
L.D. Tomei and F.O. Cope (eds.)

Video Tapes

SDS-Polyacrylamide Gel Electrophoresis: A Video Guide
M. Basta

Stories from the Scientists
University of California, San Francisco

Hybridomas and Monoclonal Antibodies: A Video Guide
University of Pittsburgh

Cancer Surveys Series

Vol. 19/20: *Trends in Cancer Incidence and Mortality*
R. Doll, J.F. Fraumeni, Jr., and C.S. Muir (eds.)

Vol. 21: *Palliative Medicine: Problem Areas in Pain and Symptom Management*
G.W. Hanks (ed.)

Journals

Genes & Development (Volume 8, 24 issues)
T. Grodzicker and N. Hastie (eds.)

PCR Methods and Applications (Volume 3, 3 issues; Volume 4, 3 issues)
D. Bentley, R. Gibbs, and R. Myers (eds.)

Learning & Memory (Volume 1, 4 issues)
R. Davis, E. Kandel, R. Morris, C. Shatz, L. Squire, and C. Stevens (eds.)

Other

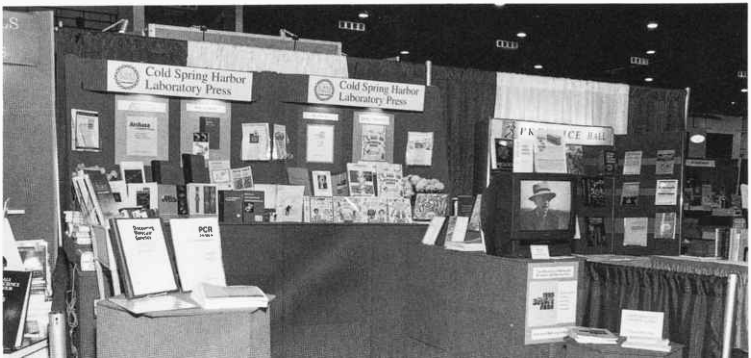
CSHL Annual Report 1993

Banbury Center Annual Report 1993

Administration and Financial Annual Report 1993

Abstract/program books for 14 CSHL meetings

The CSHL Press sales exhibit at all of the academic society meetings attended each year.



COLD SPRING HARBOR LABORATORY PRESS

Our publishing program continued to expand and diversify in 1994. Twelve new books and three new videotapes were published, bringing our titles in print to over 200. One journal doubled in frequency and another was launched. An entirely new venture began—the creation of a directory of products and services for laboratory work—and we embarked successfully on the global information highway. Program income rose by 4% to $4.49 million, and the operating margin was reduced by one-time adjustments in book inventory value.

Book Publishing

New titles were added in all categories of the book catalog. The most notable was the second edition of *Manipulating the Mouse Embryo* by Brigid Hogan, Rosa Beddington, Frank Costantini, and Elizabeth Lacy. First published in 1986, this manual was highly successful in its intention of catalyzing the interaction between molecular biology and mammalian development. The new edition is completely revised and updated, with additional striking illustrations, and has quickly become another laboratory essential to stand alongside the classics *Molecular Cloning* and *Antibodies*, which continue to sell strongly.

Techniques manuals, the monograph series, and the Symposium continue to be the mainstays of our book program. A change was evident in this year's Symposium volume, *DNA and Chromosomes*—not in its cover color, a time-honored pigment John Cairns called "oxidized blood," but in its paper. With so much color artwork to be included, the cream stock selected by Reginald Harris in 1933 and used ever since, had to be replaced by a bright white sheet that framed the art to advantage. Reversion is unlikely.

The CSHL monograph series lengthened with the addition of three new titles. *The Biology of Heat Shock Proteins and Molecular Chaperones* was edited by Richard Moromoto, Alfred Tissières, and Costas Georgopoulos. A review of cellular viral receptors was edited by Lab Trustee Eckard Wimmer, and Elliot Myerowitz and Christopher Sommerville assembled *Arabidopsis*, an essential work of reference on this important experimental organism that is the first of several planned titles in the resurgent field of plant genetics.

Not every new title can be mentioned here (see opposite for a complete list) but the year was particularly notable for the growing success of several newer and novel classes of publication. Science books for children continued to be extraordinarily popular, including our own new publication, the imaginative book/tape combination *Double Talking Helix Blues* by Joel and Ira Herskowitz and Judy Cuddihy, which attracted mass media attention nationwide. Our videotape series continued to prosper, with strong sales for the cassette library *Winding Your Way Through DNA*. Three new tapes were published, two instructional programs, on SDS-PAGE electrophoresis and hybridomas, and *Stories from the Scientists*, an educational program on the Watson/Crick and Boyer/Cohen partnerships produced in collaboration with the University of California at San Francisco.

We also experimented with the republication of out-of-print books that had either historic interest or continued utility for today's investigators. Demerec's 1950 classic *Biology of* Drosophila falls into both categories and was so eagerly seized by the fly community that it quickly required a second printing. We also republished the 1972 Banbury Center volume *Gene Therapy*, the first and for a long time the only book on this now widely discussed topic, using new print-on-demand technology.

In producing this year's crop of new titles, the book publishing group led by Nancy Ford rose to the challenge of producing more books, in more formats, with more color, and in addition took on new responsibilities, managing the duplication of audiotapes and videotapes and the production of packaging and other ancillary materials.

Ira Herskowitz and Joel Herskowitz, creators of the Double-talking Helix Blues.

Journal Publishing

Each of the past several years has seen significant change in our journals operation and 1994 was no exception. In January, *Genes & Development* shifted smoothly from monthly to twice monthly publication. This was a decision taken in 1993 to speed up paper publication time and reduce the average issue size, in correct anticipation of increased demand to publish in the journal. After citation analysis for 1993 by the Institute for Scientific Information, *Genes & Development* was once again the leading journal in the categories of genetics and developmental biology. However, its content pages were notable for excellent papers on topics such as signal transduction and cell cycle control, aspects of cell biology in which genetic dissection has made a substantial impact. The editors, Terri Grodzicker and Nick Hastie, with invaluable advice from the editorial board and other reviewers, took care to ensure that the journal remained first class. The efforts of the entire journal team were rewarded by a 4% increase in circulation during the year, with increased penetration of academic libraries, and a satisfying 100% increase in operating margin. The progress of *Genes & Development* in 1994 confirmed its place among the handful of outstanding journals in biology, a source of pride and satisfaction for both CSHL Press and its co-publishers, the Genetical Society of Great Britain.

PCR Methods and Applications, having doubled in frequency to bimonthly publication in August 1993, continued to receive a steady flow of papers. Those accepted appeared alongside a series of specially commissioned articles which will be reassembled to form an introductory lab manual on PCR technology in 1995. The journal was scored for the first time by citation analysis, placing fifth among all the journals in biotechnology, with the highest impact factor of any journal devoted to a single technique. This recognition acknowledges the work of the editors, Eric Green, Richard Gibbs, Rick Myers, and David Bentley, and their advisors. The journal also benefited from the worldwide distribution of sponsored subscriptions by the Perkin-Elmer Corporation.

Since its launch in 1990, the journal has been a success editorially and financially and its circulation in 1994 remained stable while its operating margin doubled. However, the powerful and flexible technology of PCR has become more established and we began to consider what opportunities for evolution might be available to the journal. The technique has become a mainstay of the ever-expanding field of genome studies and we received strong encouragement to reposition the journal as a new forum for studies using PCR and other techniques to map and sequence the genomes of many species. The decision was made to relaunch *PCR Methods and Applications* as *Genome Research* in mid 1995.

In 1993, a commitment was made to launch a third journal, *Learning & Memory*, in 1994. The first issue of this bimonthly publication appeared in May. Its academic editors, Ron Davis, Eric Kandel, Richard Morris, Larry Squire, Carla Shatz, and Chuck Stevens, decided that they would best serve this new journal by accepting from the start only high-quality papers. Research on the biological

basis of learning and memory is new and fascinating, so our fledgling journal must compete for papers with the best known serials in science. As a result, issues have been produced less regularly than we would wish. Nevertheless, their standard is excellent, the editors and their advisors are critical and authoritative, and we have in place the beginnings of a core journal for this exciting, emergent field.

These changes in the journals program increased the demands on the editorial and production staff, headed by Judy Cuddihy, who responded efficiently to ensure that the issues appeared error-free, on time, and on budget throughout the year.

The Laboratory Manual Source Book

Our laboratory manuals have always included a list of the products required to carry out the techniques described in the book, and where possible a suggested supplier of each product. Readers value this information and as the number of manuals has grown, the lists have expanded and have begun to overlap.

We reasoned that users of the manuals would benefit even more from a complete, fully indexed compendium of laboratory products cross-referenced to all their potential suppliers, particularly if it offered readers ready access to technical advice and further information. It also seemed likely that suppliers would welcome the chance to place their advertisements in front of a large, targeted audience of interested users. Market research confirmed that both users and suppliers found the idea attractive, and we were encouraged to embark on a major new publication, *The Lab Manual Source Book*, that would be distributed free to all manual users and supported financially by the sale of advertising space within it. To manage this publication, we were fortunate to attract back to Cold Spring Harbor, Joan Boyce, a former lab manager who had moved on to a successful career in sales at Boehringer Mannheim. With her strong technical and commercial background, she was able to assemble within just a few months a database of over 16,000 products from more than 570 companies, divided into 1700 product categories. In December, this information was carefully formatted into a 192-page book and 35,000 copies were distributed internationally to users of our lab manuals. The book was warmly received and is being widely used, encouraging us to proceed with an updated and expanded 1996 edition.

Marketing, Fulfillment, and Finance

Our marketing activities, managed by Ingrid Benirschke, remain centered on direct mail, augmented by advertisements in journals and exhibits at the larger society meetings. Three issues of the *Notebook* announced the new titles and two subject-specific catalogs designed by Jim Suddaby were dispatched to targeted recipients worldwide. A complete catalog of publications was also produced for the first time in 2 years, at 116 pages the largest ever from the Press. In addition to the customary meetings in genetics, cell biology, neuroscience, and microbiology, the marketing department attended the annual meeting of the National Biology Teachers Association for the first time and learned some valuable lessons about the potential of our children's book program and videotape series in that marketplace. The conviction that the *Double Talking Helix Blues* had widespread popular appeal prompted attempts at more widespread promotion, which were rewarded with invitations to bookstore signings and features on the book and its creators in major newspapers, cable and network television, and national public radio.

At a more local level, our bookstore in Grace Auditorium, run by Connie Hallaran, brought our publications to the attention of the 5000 attendees at the Laboratory's meetings and courses. Although the bookstore is cramped, patrons also appreciated gift items, travel necessities, and an interesting mix of science-related books from other publishers. These were particularly valued by visitors from other countries where books published in the United States are hard to get and expensive.

Our own marketing activities in other countries were much discussed during the year, with the aim of improving our books' availability in a cost-effective fashion. Several radical strategies were discussed and discarded. In the interim, direct mail to recipients in Europe was increased and exclusive distribution arrangements with agents in Japan, India, and the Pacific Rim were maintained. The proportion of books sold abroad went up.

As a result, close attention was paid to the methods and costs of distributing our publications overseas. The complex and ever-changing world of international and national delivery was managed by Guy Keyes, whose staff at our Plainview facility dispatched more than 40,000 books and processed over 85,000 journal issues during the year. Our computer system now contains records for 50,000 customers worldwide. For many of these individuals and institutions, the Plainview staff is their only direct contact with Cold Spring Harbor, and the scientific community's evident goodwill toward the Press owes much to the care and competence with which the staff conducts the required order processing, customer service, and dispatch.

The Plainview computer is also the source of all our publication sales information. From these data, Nancy Hodson, promoted this year to Business Manager, derives an increasingly sophisticated array of reports, analyses, and projections that help us to understand the underlying dynamics of our business and make better financial decisions.

The World Wide Web

1994 was the year in which the much heralded era of electronic publishing became reality. The Press was alerted early, thanks to our colleagues in the Laboratory's Computational Biology group. The group drew our attention to the new software program Mosaic which made it possible to view hyperlinked multimedia over the international computer network known as the World Wide Web. To a scientific information company, Mosaic's power and mass appeal were obvious, and we quickly initiated the construction of a site on the Web that would deliver on-demand information about our books, journals, meetings, courses, and public events. The site's architect was Corprew Reed from the Computational Biology group, working with staff from the Press and Meetings and Public Affairs Departments. *Cold Spring Harbor Laboratory Online* (CLIO) opened in May, the first Web site to be associated with a science publisher. As the year progressed, the Web and its business potential became front page news everywhere, as thousands of companies joined the network and consumers clamored for access to this new commercial frontier. By year's end, after including our Web address in all print catalogs and advertisements, we found we had a new and effective means of communicating directly with our readers, particularly those abroad, who visited our site in the thousands each month. We learned a great deal from this project and experiments began that will make it possible in future years to deliver editorial content as well as marketing information through CLIO.

New Projects

From being a highly respected book publisher less than a decade ago, the Press has become a provider of information in many formats. More than 25 new projects of different types were initiated in 1994. We continue to aspire to excellence in both the assembly of information, by engaging first-class authors, and its presentation, by employing talented and committed staff. Our reputation in traditional publishing is strong. Now we must rise to the challenge of new media, in which experienced authors are rare, development costs are large, the market is uncertain, and the business risks are high. In five years, the professional use of scientific information will be profoundly different. The nature of this change is being intensely discussed. To be ill-prepared for it would be to squander our special place in the communication of science, and our hard-won reputation for innovation and quality.

John R. Inglis

FINANCE

FINANCIAL STATEMENTS

BALANCE SHEET
Year ended December 31, 1994
with comparative amounts for 1993

ASSETS

	Operating Funds						
	Unrestricted			Endowment &	Land, Building, &	Total All Funds	
	Undesignated	Designated	Restricted	Similar Funds	Equipment Funds	1994	1993
Cash and cash equivalents	$2,676,444	1,150,000	1,333,406	5,295,944	3,497,983	13,953,777	16,921,939
Marketable securities	2,291,694	–	–	29,681,513	157,153	32,130,360	29,690,918
Accounts receivable:							
Publications (less allowance for doubtful accounts of $20,500 in 1994 and 1993)	470,706	–	–	–	–	470,706	436,288
Other	163,780	–	–	–	–	163,780	138,252
Grants receivable	–	–	2,454,025	–	–	2,454,025	1,883,040
Accrued interest receivable	–	–	–	465,017	–	465,017	259,517
Publications inventory	1,512,132	–	–	–	–	1,512,132	1,439,940
Other assets, principally prepaid expenses	484,570	–	–	–	852,754	1,337,324	1,230,600
Investment in employee residences	–	–	–	–	2,047,999	2,047,999	2,009,967
Land, buildings, and equipment:							
Land and improvements	–	–	–	–	7,372,872	7,372,872	5,825,328
Buildings	–	–	–	–	58,793,446	58,793,446	56,621,938
Furniture, fixtures, and equipment	–	–	–	–	3,301,440	3,301,440	2,961,229
Laboratory equipment	–	–	–	–	9,834,902	9,834,902	8,839,803
Library books and periodicals	–	–	–	–	365,630	365,630	365,630
	–	–	–	–	79,668,290	79,668,290	74,613,928
Less accumulated depreciation and amortization	–	–	–	–	21,829,861	21,829,861	19,182,465
Land, buildings, and equipment, net	–	–	–	–	57,838,429	57,838,429	55,431,463
Construction in progress	–	–	–	–	936,028	936,028	1,326,536
Total assets	$7,599,326	1,150,000	3,787,431	35,442,474	65,330,346	113,309,577	110,768,460

LIABILITIES AND FUND BALANCES

	Operating Funds			Endowment & Similar Funds	Land, Building, & Equipment Funds	Total All Funds	
	Unrestricted						
	Undesignated	Designated	Restricted			1994	1993
Liabilities:							
Accounts payable and accrued expenses	$ 963,259	–	123,567	–	156,687	1,243,513	1,268,874
Notes payable	–	–	–	–	963,877	963,877	1,008,364
Bonds payable	–	–	–	–	30,000,000	30,000,000	30,000,000
Deferred revenue	1,534,789	–	3,663,864	–	221,809	5,420,462	5,673,094
Total liabilities	2,498,048	–	3,787,431	–	31,342,373	37,627,852	37,950,332
Fund balances:							
Unrestricted–undesignated	5,101,278	–	–	–	–	5,101,278	3,474,376
Unrestricted–designated	–	1,150,000	–	–	–	1,150,000	1,350,000
Endowment and similar funds	–	–	–	35,442,474	–	35,442,474	33,770,391
Land, buildings, and equipment:							
Expended	–	–	–	–	31,882,880	31,882,880	31,970,878
Unexpended–Donor restricted	–	–	–	–	57,094	57,094	242,786
Unexpended–Board authorized	–	–	–	–	2,047,999	2,047,999	2,009,697
Total fund balances	5,101,278	1,150,000	–	35,442,474	33,987,973	75,681,725	72,818,128
Total liabilities and fund balances	$7,599,326	1,150,000	3,787,431	35,442,474	65,330,346	113,309,577	110,768,460

STATEMENT OF SUPPORT, REVENUE AND EXPENSES, AND CHANGES IN FUND BALANCES
Year ended December 31, 1994
with comparative amounts for 1993

	Operating Funds						
	Unrestricted			Endowment &	Land, Building, &	Total All Funds	
	Undesignated	Designated	Restricted	Similar Funds	Equipment Funds	1994	1993
Support and revenue:							
Public support	$1,964,892	–	7,260,160	1,914,818	84,562	11,224,432	10,377,960
Government grant awards	–	–	11,905,278	–	–	11,905,278	11,550,559
Indirect cost allowances	8,554,344	–	–	–	–	8,554,344	8,510,065
	10,519,236	–	19,165,438	1,914,818	84,562	31,684,054	30,438,584
Other revenue:							
Program fees	1,701,306	–	–	–	–	1,701,306	1,535,762
Rental income	403,216	–	–	–	–	403,216	289,891
Publications	4,390,023	–	–	–	–	4,390,023	4,319,267
Dining services	1,854,745	–	–	–	–	1,854,745	1,661,674
Rooms and apartments	1,498,087	–	–	–	–	1,498,087	1,451,719
Distribution from Robertson Funds	665,000	–	1,120,000	–	41,450	1,826,450	1,575,000
Investment income	225,827	–	–	2,154,024	61,488	2,441,339	3,098,475
Royalty & licensing fees	337,398	–	–	–	–	337,398	283,870
Miscellaneous	160,808	–	–	–	–	160,808	146,282
Total other revenue	11,236,410	–	1,120,000	2,154,024	102,938	14,613,372	14,361,940
Total support and revenue	21,755,646	–	20,285,438	4,068,842	187,500	46,297,426	44,800,524
Expenses:							
Program services:							
Research	–	–	14,878,837	–	–	14,878,837	14,630,971
Summer programs	1,263,629	–	4,414,434	–	–	5,678,063	4,660,085
Publications	4,309,043	–	–	–	–	4,309,043	4,134,244
Banbury Center conferences	244,766	–	604,278	–	–	849,044	579,458
DNA Education Center programs	18,008	–	342,845	–	–	360,853	449,428
Total program services	5,835,446	–	20,240,394	–	–	26,075,840	24,454,186
Supporting services:							
Direct research support	1,308,939	–	–	–	–	1,308,939	1,196,259
Library	548,470	–	–	–	–	548,470	505,105
Operation and maintenance of plant	5,371,772	–	–	–	–	5,371,772	5,008,217
General and administrative	3,604,612	–	–	247,997	–	3,852,609	3,522,136
Dining services	1,885,701	–	–	–	–	1,885,701	1,659,112
Interest	–	–	–	–	1,199,557	1,199,557	926,162
Total supporting services	12,719,494	–	–	247,997	1,199,557	14,167,048	12,816,991

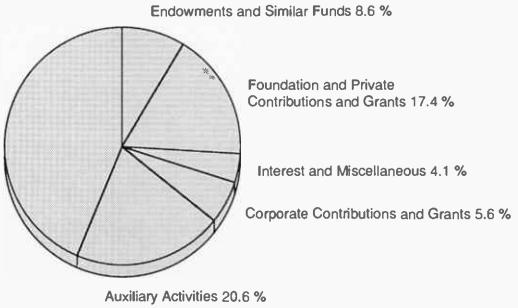
Depreciation and amortization	–	–	–	–	2,668,167	2,668,167	2,521,642
Unrealized loss on marketable securities	–	–	–	498,305	24,469	522,774	48,937
Total expenses	18,554,940	–	20,240,394	746,302	3,892,193	43,433,829	39,841,756
Excess (deficiency) of support and revenue over (under) expenses before designation	$3,200,706	–	45,044	3,322,540	(3,704,693)	2,863,597	4,958,768
Designation:							
Release of funds designated for research program	200,000	(200,000)	–	–	–	–	–
Excess (deficiency) of support and revenue over (under) expenses and designation	3,400,706	(200,000)	45,044	3,322,540	(3,704,693)	2,863,597	4,958,768
Other changes in fund balances:							
Capital expenditures	(2,362,648)	–	(1,106,657)	–	3,469,305	–	–
Transfer to restricted funds	(467,251)	–	1,061,613	(594,362)	–	–	–
Transfer to endowment funds	(567,899)	–	–	567,899	–	–	–
Transfer to unrestricted funds	1,623,994	–	–	(1,623,994)	–	–	–
Net increase (decrease) in fund balances	1,626,902	(200,000)	–	1,672,083	(235,388)	2,863,597	4,958,768
Fund balances at beginning of year	3,474,376	1,350,000	–	33,770,391	34,223,361	72,818,128	67,859,360
Fund balances at end of year	$5,101,278	1,150,000	–	35,442,474	33,987,973	75,681,725	72,818,128

Copies of our complete, audited financial statements, certified by the independent auditing firm of KPMG, Peat, Marwick & Co., are available upon request from the Comptroller, Cold Spring Harbor Laboratory.

COLD SPRING HARBOR LABORATORY

SOURCES OF REVENUE

YEAR ENDED DECEMBER 31, 1994



COMPARATIVE OPERATING HISTORY
1990-1994
(Dollars in Thousands)

	1990	*1991*	*1992*	*1993*	*1994*
Income:					
Main Lab:					
Grants & contracts	$13,535	15,172	16,800	18,136	19,293
Indirect cost reimbursement	6,558	7,170	8,388	8,383	8,460
Other	3,976	5,056	5,520	6,049	6,808
CSH Press	4,223	3,079	3,709	4,319	4,390
Banbury Center	1,120	1,090	1,104	1,281	1,569
DNA Learning Center	585	744	822	796	824
Total income	29,997	32,311	36,343	38,964	41,344
Expenses:					
Main Lab:					
Grants & contracts	13,535	15,172	16,800	18,136	19,293
Operation & maintenance of plant	3,759	3,904	4,241	4,777	5,141
General & administrative	2,414	2,468	2,634	2,785	2,909
Other	2,973	3,375	4,141	4,385	4,847
CSH Press	3,708	3,488	3,548	4,134	4,309
Banbury Center	1,125	1,063	1,070	1,226	1,498
DNA Learning Center	615	752	843	768	798
Total expenses	28,129	30,222	33,277	36,211	38,795
Excess before depreciation and (designation) release of funds	1,868	2,089	3,066	2,753	2,549
Depreciation	(1,485)	(1,898)	(2,358)	(2,522)	(2,668)
(Designation) release of funds (1)	(250)	(100)	(600)	0	200
Net operating excess	$ 133	91	108	231	81

The above amounts are presented on a combined basis for all funds for which Cold Spring Harbor Laboratory prepares operating budgets.

(1) Funds designated to underwrite future direct and indirect expenses of the neuroscience and other research programs.

FINANCIAL SUPPORT OF THE LABORATORY

Cold Spring Harbor Laboratory, Banbury Center, and the DNA Learning Center receive a substantial portion of their funding through grants from the Federal Government and through grants, capital gifts, and annual contributions from private foundations, corporations, and individuals. The following section summarizes funding that occurred during 1994.

GRANTS January 1, 1994–December 31, 1994

COLD SPRING HARBOR LABORATORY

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*1994 Funding*[+]
FEDERAL GRANTS			
NATIONAL INSTITUTES OF HEALTH			
Program Projects	Cancer Research Center, Dr. Herr	1/92–12/96	$3,365,364
	Cancer Center Support, Dr. Stillman	8/90–7/95	2,754,715
Research Support	Dr. Arndt	1/91–12/94	71,931
	Dr. Bar-Sagi	7/91–6/95	255,788
	Dr. Beach	5/94–4/95	282,250*
	Dr. Beach	4/93–3/97	182,519
	Dr. Cheng	4/93–3/97	190,492
	Dr. Enikolopov	9/94–8/98	196,786*
	Dr. Franza	9/85–11/94	291,638
	Dr. Franza	10/91–6/94	110,940
	Dr. Futcher	1/91–12/94	154,233
	Dr. Futcher	4/93–3/97	251,933
	Dr. Garrels	1/90–3/95	902,386
	Dr. Gilman	9/92–6/97	353,469
	Dr. Greider	12/94–11/98	264,749*
	Dr. Greider	8/91–7/96	260,870
	Dr. Helfman	4/94–3/98	276,994*
	Dr. Helfman	8/93–5/98	215,825
	Dr. Hernandez	7/92–6/96	252,935
	Dr. Hernandez	9/91–8/96	231,012
	Dr. Herr	3/92–2/96	219,150
	Dr. Krainer	7/94–6/98	312,002*
	Dr. Kuret	7/89–6/94	425,413
	Dr. Lisitsyn	7/94–6/99	268,129*
	Dr. Malinow	5/94–4/97	118,883
	Dr. Marr	8/94–5/97	366,303*
	Dr. Martienssen	8/92–7/94	178,562
	Dr. Mathews	2/92–1/97	281,111
	Dr. Mathews	9/93–8/98	306,372
	Dr. Mathews	9/94–9/97	26,000*
	Dr. McCombie	4/94–3/95	678,702*
	Dr. Roberts	4/91–3/95	230,492
	Dr. Skowronski	12/93–11/97	309,446
	Dr. Spector	4/90–3/95	271,633
	Dr. Stillman	7/83–5/96	302,719
	Dr. Stillman	7/91–6/95	197,428
	Dr. Tonks	8/91–5/96	326,444
	Dr. Tully	4/94–3/97	307,329*
	Dr. Wigler	7/92–4/99	1,728,220
Fellowships	Dr. Buchkovich	7/94–5/95	26,144*
	Dr. Jones	10/91–9/94	53,731
	Dr. Zhang	9/92–8/97	88,679
Training Support	Training in Cancer Cell Biology and Tumor Virology, Dr. Helfman	7/94–2/99	179,001*

* New Grants Awarded in 1994

\+ Includes direct and indirect cost

Grantor	Program/Principal Investigator	Duration of Grant	1994 Funding+
Course Support	Advanced Bacterial Genetics, Dr. Grodzicker	5/93–4/98	54,096
	Cancer Research Center Workshops, Dr. Grodzicker	4/92–3/97	236,697
	Neurobiology Short-term Training, Dr. Hockfield	5/82–4/96	142,341
	CSHL Analysis Large DNA Molecules, Dr. Grodzicker	1991–1996	108,906
	Essential Computational Genomics for Molecular Biologists, Dr. Marr	1991–1996	26,284
	Advanced In Situ Hybridization and Immunocytochemistry, Dr. Spector	1992–1997	48,805
	Molecular Biology & Development of *Xenopus Laevis*, Dr. Grodzicker	4/93–3/96	11,841
Meeting Support	Genome Mapping & Sequencing Conference	4/91–3/96	31,148
	Zebrafish Development and Genetics	4/94–3/95	14,500 *
	Heat Shock Proteins and Molecular Chaperones	15/94–4/95	4,000 *
	CSHL 59th Symposium: Molecular Genetics of Cancer	6/94–5/95	15,000 *
	Mouse Molecular Genetics	7/94–6/95	11,018 *
	Epstein-Barr Conference	8/94–7/95	11,000 *
	Gene Therapy Conference	9/94–8/95	22,000 *
	Ribosome Synthesis Conference	9/94–8/95	2,000 *
NATIONAL SCIENCE FOUNDATION			
Research Support	Dr. Cline	9/94–8/95	72,915
	Dr. Grotewold	11/94–10/98	114,248 *
	Dr. Ma	5/94–4/97	127,000 *
	Dr. Ma	8/94–7/98	109,901 *
	Dr. Martienssen	2/93–7/94	50,000
	Dr. Martienssen	8/94–7/97	150,000 *
	Dr. Peterson/Stillman	11/91–10/94	106,487
	Dr. Sundaresan	7/92–7/95	130,000
Fellowship Support	Dr. Pena	6/92–6/96	35,000
	Dr. Springer	12/93–11/95	32,755
Equipment Support	Dr. X. Cheng	6/94–5/96	155,300 *
Training Support	Undergraduate Research Program, Dr. Herr	6/91–5/97	50,000
Course Support	Arabidopsis Molecular Genetics	6/94–5/95	60,000 *
	Macromolecular Crystallography	8/94–7/97	45,000 *
	Computational Neuroscience: Vision	8/94–7/95	30,000 *
	Molecular Genetics of Fission Yeast	9/94–8/99	80,000 *
Construction Support	Greenhouse	12/92–5/95	140,000
Meeting Support	The Biology of Heat Shock	4/94–3/95	5,000 *
	Zebrafish Development and Genetics	5/94–4/95	11,400 *
	Mouse Molecular Genetics	7/94–6/95	10,000 *
	Molecular Biology and Development of *Xenopus Laevis*	5/94–4/95	4,166 *
	Ribosome Synthesis	7/94–6/95	5,000 *
	Learning & Memory	9/94–8/95	5,500 *
DEPARTMENT OF ENERGY			
Research Support	Dr. Marr	7/94–2/97	425,313 *
Meeting Support	CSHL 59th Symposium: Molecular Genetics of Cancer	1994	9,700 *
Course Support	Macromolecular Crystallography	1994	14,550 *

* New Grants Awarded in 1994
+ Includes direct and indirect cost

Grantor	Program/Principal Investigator	Duration of Grant	1994 Funding+
U.S. DEPARTMENT OF AGRICULTURE			
Research Support	Dr. Ma	9/94–9/96	65,678*
	Dr. Martienssen	9/94–9/97	61,443*
	Dr. Sundaresan	8/94–9/97	72,000*
U.S. DEPARTMENT OF ARMY			
Research Support	Dr. Futcher/Dr. Marshak	6/94–6/98	195,414*
	Dr. Wigler/Dr. Lisitsyn	7/94–8/98	200,000*
NONFEDERAL GRANTS			
Research Support			
Alzheimer's Association	Dr. Nawa	12/93–11/94	30,000
American Cancer Society	Dr. Gilman	7/91–5/95	100,000
	Dr. Ma	7/94–6/97	32,000*
	Dr. Marshak	7/93–6/95	103,000
	Dr. Sutton	1/92–12/94	95,000
	Dr. Wigler, Professorship	1986–2012	50,000
	Dr. Wigler, Supply Allowance	1994	10,000*
American Heart Association	Dr. Helfman	7/91–6/96	35,000
Amplicon Corporation	Dr. Wigler	6/94–5/97	660,000*
Baxter Foundation	Dr. Skowronski	6/92–5/95	33,694
Arnold & Mabel Beckman Foundation	Dr. Silva	7/94–6/96	100,000*
Sara Chait Foundation	Dr. Marshak	12/91–11/96	25,000
J.W. Cleary	Muscular Dystrophy Research	11/93–10/94	26,899
Council for Tobacco Research	Dr. Greider	7/92–6/95	52,000
	Dr. Helfman	7/91–6/95	80,000
	Dr. Spector	7/92–6/95	80,000
Charles E. Culpeper Foundation	Dr. Spector	5/93–4/95	60,000
The Daphne Seybolt Culpeper Foundation	Cancer Research	1994	5,000*
Gertrude Elion Cancer Research Award	Dr. Grieder	7/94–6/95	30,000*
Ford Foundation	Dr. Pena	3/93–2/94	2,500
Geron Corporation	Dr. Greider	2/94–1/97	192,550*
Greenwall Foundation	Dr. Marshak	7/92–6/94	81,179
Irving A. Hansen Memorial Foundation	Dr. Tonks	7/92–6/94	17,569
Hitachi Foundation	Dr. Nawa	3/92–2/95	10,000
Human Frontier	Dr. Futcher	6/92–5/96	80,000
Science Program	Dr. Tully	5/92–8/95	53,462
ICI Seeds	Dr. Martienssen	1/92–12/94	7,324
ISIS Pharmaceuticals, Inc.	Dr. Spector	9/93–3/95	27,067
Johnson & Johnson	Dr. Skowronski	5/92–4/95	90,000
Esther A. & Joseph Klingenstein Fund, Inc.	Dr. Cline	7/94–6/95	33,333*
	Dr. Malinow	7/94–6/95	33,333*
	Dr. Nawa	7/92–6/95	33,333
	Dr. Silva	7/93–6/96	33,333
Long Island Breast Coalition	Dr. Wigler	11/94–1/95	20,000*
Robert Leet & Clara Guthrie Patterson Trust	Dr. Cline	7/94–6/95	50,000*
March of Dimes	Dr. Barker	7/93–6/94	40,436

* New Grants Awarded in 1994
+ Includes direct and indirect cost

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*1994 Funding+*
Lucille P Markey Charitable Trust	Neurobiology Support	7/90–6/96	314,581
MSCI/US Agency for International Development	Dr. Marshak	11/91–10/94	106,488
Mathers Charitable Foundation	Neurobiology Research Support	8/91–7/94	286,235
The McKnight Endowment Fund for Neuroscience	Dr. Cline	7/91–6/94	17,452
Mellam Family Foundation	Dr. Tonks	12/93–11/95	50,000
Memorial-Sloan Kettering (NIH)	Dr. Marshak	9/94–7/97	162,920*
	Dr. Tonks	9/94–7/97	173,103*
John Merck Fund	Dr. Tully	9/91–5/94	60,000
NYU Consortium (NIH)	Dr. Marshak	5/92–4/97	70,800
Nanoprobes, Inc.	Dr. Spector	6/94–5/95	24,000*
Oncogene Science, Inc.	Monoclonal Agreement	6/92–5/95	190,365
OSIRIS	Dr. Marshak	10/94–6/95	101,790*
Oxnard Foundation	Dr. Gilman	2/91–1/94	50,000
Samuel Freeman Charitable Trust	Freeman Laboratory of Cancer Cell Biology	7/89–6/94	100,000
Sloan Foundation, Inc.	Dr. Malinow	4/94–9/94	8,022*
Lauri Strauss Leukemia Foundation, Felix Schnyder Memorial Fund	Dr. Gilman	5/94–4/95	10,000*
St. Giles Foundation	Dr. Beach/Dr. Wigler	3/93–2/96	50,000
Whitehall Foundation	Dr. Silva	9/93–8/96	45,000
	Dr. Zhong	1/94–12/96	40,000*
Equipment Support			
Arrow Electronics	Equipment	7/94–12/94	10,000*
Louis Berkowitz Foundation	Equipment	7/94–6/95	25,000*
The Daphne Seybolt Culpeper Foundation	Equipment	1994	10,000*
Ira DeCamp Foundation	Equipment	7/94–6/95	300,000*
Edward S. Moore Foundation	Equipment	7/94–12/94	10,000*
The Perkin Fund	Equipment	1/93–12/95	50,000
Harold A. Peterson Trust	Equipment	11/93–12/94	300,000
Pritchard, Inc.	Equipment	12/94–11/95	75,000*
Fannie E. Rippel Foundation	Equipment	12/93–12/94	250,000
Fellowships			
Rita Allen Foundation	Dr. Hengartner	9/94–8/99	30,000*
	Dr. Hernandez	10/89–9/94	30,000
American Cancer Society	Dr. Flanagan	7/92–6/95	28,000
	Dr. Hinkley	9/92–8/95	28,000
	Dr. Marcus	7/93–11/94	10,718
American Heart Association	Dr. Yung-Chih Wang	7/93–6/95	30,000
Austrian Science Foundation	Dr. Gimona	4/94– 4/96	27,800*
Swedish Cancer Society Cancer Fonden–Sweden	Dr. Berg	9/93–9/95	25,000
Cancer Research Institute	Dr. Flint	1/92–12/94	31,500
Cancer Research Fund of the Damon Runyon - Walter Winchell Foundation	Dr. Conklin	11/93–10/96	30,000
	Dr. Hannon	1/92–12/94	31,000
	Dr. Horton	9/92–8/95	31,000
	Dr. Jung	5/92–4/95	31,000
	Dr. Liang	9/94–8/97	26,000*
	Dr. Sun	5/92–4/95	31,000
	Dr. Weinreich	1/94–12/96	26,000*

* New Grants Awarded in 1994
+ Includes direct and indirect cost

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*1994 Funding*+
Jane Coffin Childs	Dr. Mirzayan	7/93–6/96	27,000
	Dr. Verreault	10/94–9/97	26,500 *
Cowan Fellowship Support	Neurobiology Fellowship Support	12/93–12/94	25,000 *
DFG Deutsche Forschung Gemeinschaft	Dr. Giese	1/93–6/95	19,026
Eppley Foundation	Dr. Silva	11/93–10/94	10,000
European Molecular Biological Organization	Dr. Giese	1/93–5/94	9,930
Fonds Dela Resherchen En Sante Du Quebc	Dr. Autexier	11/91–11/96	16,819
Glaxo Research Institute	Fellowship Support	1/94–12/94	100,000 *
Goldring International Group, Inc.	Fellowship Support	7/94–6/97	30,000 *
Human Frontier	Dr. Della Seta	4/93–3/95	39,526
Science Program	Dr. Grossniklaus	11/94–10/96	41,200 *
	Dr. Hamaguchi	8/94–7/95	48,700 *
	Dr. Hanamura	7/93–6/95	39,500
	Dr. Steiner	4/94–3/96	31,400 *
	Dr. Vignais	8/93–7/95	40,500 *
	Dr. Watakabe	12/93–11/95	42,700
Japan Funds	Dr. Hidaka	9/94–12/95	20,109 *
Kaplan & Kilsheimer	Dr. Tobin	12/94–12/95	3,000 *
Leukemia Society	Dr. Rong Li	7/94–6/95	31,320 *
Life Science Research Foundation	Dr. Collins	6/92–5/95	35,000
	Dr. Kaufman	7/92–6/95	35,000
Ministerio De Education Y Ciencia, Madrid	Dr. Blasco	6/94–6/95	16,000 *
Medical Research Council of Canada	Dr. Demetrick	11/91–11/95	43,260
Pew Scholars Program	Dr. Greider	7/90–6/94	50,000
	Dr. Krainer	7/92–6/96	50,000
	Dr. Tonks	7/91–6/95	50,000
	Dr. Zhong	7/94–6/98	50,000 *
Andrew Seligson Memorial Fellowships	Fellowship Support	9/90–5/94	78,200 *
Wellcome Trust	Dr. Frenguelfi	11/93–10/95	33,000
Wendt Fellowships	Neurobiology Fellowship Support	1/94–12/96	50,000 *
Training Support			
Bio-Rad Laboratories	Summer Undergraduate Program	1994	1,000 *+
Burroughs Wellcome Foundation	Summer Undergraduate Program	1994	6,600 *
Hanson White Industries	Summer Undergraduate Program	1994	6,600 *
Howard Hughes Medical Institute	Graduate Student Support	1994	25,000 *
Dr. Ira Herskowitz	Summer Undergraduate Program	1994	100 *
Phillips Petroleum Co.	Summer Undergraduate Program	1994	1,000 *
St. Paraskevi Greenlawn	AIDS Research	1994	1,000 *
Elaine E. & Frank T. Powers	Summer Undergraduate Program	1994	2,500 *
Course Support			
Grass Foundation	Neurobiology Scholarships	5/94–4/95	22,000 *
Howard Hughes Medical Institute	Advanced Neurobiology	1991–1995	266,170
Esther A. & Joseph Klingenstein Fund, Inc.	Advanced Neurobiology	5/94–4/97	60,000 *
Meeting Support			
Affinity Bioreagents, Inc.	Heat Shock	1994	1,500 *
American Cyanamid	Molecular Approaches to Immune Intervention in Infectious Diseases	1994	15,000 *

* New Grants Awarded in 1994
+ Includes direct and indirect cost

Grantor	Program/Principal Investigator	Duration of Grant	1994 Funding+
Amgen, Inc.	Zebrafish	1994	3,000 *
Applied Science, Inc.	Zebrafish	1994	500 *
Council for Tobacco Research, U.S.A., Inc.	Cytoskeleton & Cell Function	1994	1,500 *
Genetics Institute	Mouse Molecular Genetics	1994	500 *
Institute for Biological Recognition & Catalysis, Inc.	Advanced Molecular Genetics	1994	1,000 *
Kureba Corp.	Zebrafish	1994	5,000 *
March of Dimes	Cytoskeleton & Cell Function	1994	3,000 *
Marine Bio	Zebrafish	1994	500 *
Merck Foundation	Zebrafish	1994	500 *
Otsuka Pharmaceutical Factory, Inc.	Zebrafish	1994	5,000 *
Pet Warehouse	Zebrafish	1994	500 *
Pharmaceutical Basic Research Laboratories	Heat Shock Meeting	1994	800 *
Pharmaceutical Biotech	Molecular Approaches to Immune Intervention in Infectious Diseases	1994	15,000 *
Ribo Gene, Inc.	Translational Control	1994	25,000 *
Social Development Bio	Zebrafish	1994	2,500 *
StressGen Biotechnologies Corp.	Zebrafish	1994	2,500 *

BANBURY CENTER

Grantor	Program/Principal Investigator	Duration of Grant	1994 Funding+
FEDERAL GRANTS			
DEPARTMENT OF ENERGY	Human Genetics for Nonscientists: Practical Workshops for Policy Makers and Opinion Leaders	1994	66,579 *
NONFEDERAL GRANTS			
The Charles A. Dana Foundation	Research on the Genetic Basis of Manic-Depressive Illness	1994	527,878
The Esther A. & Joseph Klingenstein Fund, Inc.	Neuroscience Conference	1994	11,000 *
The Rockefeller Foundation	Grass Genome Conference	1994	3,000 *
Alfred P. Sloan Foundation	Evolution of Genes/Genome Conference	1994	18,000 *
Ms. Von Stade	Manic Depressive Program	1994	5,000 *
The William Stamps Farish Fund	Molecular Genetics of Diabetes	1993–1996	39,827
The Robert Wood Johnson Foundation	Human Genome Meeting	1994	145,616 *
The Amyotrophic Lateral Sclerosis Association	Candidate Gene Approaches to ALS	1994	33,876 *
Carnegie Council on Ethics & International Affairs	Science & Ethics: Genetic Research	1994	7,151 *
Geron Corporation	Telomeres Meeting	1994	9,391 *
Albert B. Sabin, Foundation Inc.	Next Generation of Vaccines	1994	29,851 *

* New Grants Awarded in 1994
+ Includes direct and indirect cost

DNA LEARNING CENTER

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*1994 Funding+*
FEDERAL GRANTS			
NATIONAL SCIENCE FOUNDATION			
	A Two-part program to Develop and Support Nationwide Corps of Human & Molecular Genetics Resource Teachers at the Secondary Level, Mr. Micklos	4/93–3/96	283,918
	Laboratory-based Instruction in Molecular & Human Genetics for Teaching Faculty, Dr. Bloom	5/93–4/95	291,275
U.S. DEPARTMENT OF EDUCATION			
	College Faculty Enhancement Dr. Bloom	1991–1994	87,336
NONFEDERAL GRANTS			
Harweb Foundation	Core Support	1994	1,000
Howard Hughes Medical Institute	High School Faculty Enhancement	1994	42,393
Howard Hughes Medical Institute	Precollege Science Education Initiative for Biomedical Research Institutions	7/94–6/99	80,000 *
New York State Legislature	Middle School Program	1994	75,000
Stone Foundation	Equipment	1994	64,412
E.S. Webster Foundation	Core Support	1991–1994	20,000
Weezie Foundation	Exhibit	1991–1994	9,886

The following schools each awarded a grant of $5,000 in 1994 for the Genetics as a Model for Whole Learning Program:

Great Neck Public Schools
Half Hollow Hills Central School District
Jericho Union Free School District
Locust Valley Central School District

The following schools awarded a grant for Curriculum Study in 1994 of $850:

Commack Union Free School District
East Williston Union Free School District
Garden City Union Free School District
Great Neck Public Schools
Half Hollow Hills Central School District
Harborfields Central School District
Herricks Union Free School District
Island Trees Union Free School District
Jericho Union Free School District
Lawrence Union Free School District
Locust Valley Central School District
Manhasset Union Free School District
Massapequa Union Free School District
Northport-East Northport Union Free School District
North Shore Central School District
Oyster Bay-East Norwich Central School District
Plainedge Union Free School District
Portledge School
Port Washington Union Free School District
Roslynm Public Schools
Sachem Central School District
South Huntington Union Free School District
Syosset Central School District

of $1,500:

East Meadow Union Free School District

of $2,000:

Ramaz School

* New Grants Awarded in 1994

\+ Includes direct and indirect cost

Cold Spring Harbor Laboratory is a nonprofit research and educational institution chartered by the University of the State of New York. Less than half (44.1%) of our annual support is derived from Federal grants and contracts, and thus we rely heavily on support from the private sector: foundations, corporations, and individuals. Contributions from the private sector are tax exempt under the provisions of Section 501(c)(3) of the Internal Revenue Code. In addition, the Laboratory has been designated a "public charity" and therefore may 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: You can generally deduct the full amount of the gift on your income tax return, and, of course, you need pay no capital gains tax on the stock's appreciation.

We recommend any of the following methods:

(1) Have your broker sell the stock and remit the proceeds to Cold Spring Harbor Laboratory.

(2) Deliver the stock certificates to your broker with instructions to open an account for Cold Spring Harbor Laboratory and hold the securities in that account pending instructions from Cold Spring Harbor Laboratory.

(3) Send the *unendorsed* stock certificates directly to the Laboratory: Comptroller, Cold Spring Harbor Laboratory, One Bungtown Road, Post Office Box 100, Cold Spring Harbor, New York 11724. In a separate envelope, send an *executed* stock power.

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.

Life insurance and 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 for continuing good.

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.

For additional information, please contact the Director of Development, Cold Spring Harbor Laboratory, One Bungtown Road, Post Office Box 100, Cold Spring Harbor, NY 11724, or call 516-367-8840.

CAPITAL AND PROGRAM CONTRIBUTIONS

January 1, 1994–December 31, 1994

Contributions of $5,000 and above

In 1994, the Laboratory received significant support in the form of capital and program contributions from individuals, foundations, and corporations.

Arrow Electronics, Inc.
Louis Berkowitz Family Foundation
Gladys Brooks Foundation
Daphne Seybolt Culpeper Foundation
Ira DeCamp Foundation
Goldring Family Foundation
David H. Koch Foundation
Marks Family Foundation
Dextra Baldwin McGonagle Foundation
Edward S. Moore Foundation
1 in 9: Long Island Breast Cancer Coalition
Dr. David Pall
Robert Leet and Clara Gunthrie Patterson Trust
William and Maude Pritchard Charitable Trust
Alan and Edith Seligson
H. Turner Slocum

Total $731,750

ANNUAL CONTRIBUTIONS

Corporate Sponsor Program

The Corporate Sponsor Program, now in its 11th year, provides support for meetings held both in Grace Auditorium on the main Laboratory campus and at Banbury Center. The support provided by this Program is critical to the Laboratory's meetings program by enabling us to plan ahead for the following year's meetings. We are very grateful to the Corporate Sponsors for their willingness to contribute to the Program in a period where there are increasing financial constraints on the pharmaceutical and biotechnology industries. These difficulties were reflected in a small decline in membership to 35 in 1994. Furthermore, mergers in these industries are continuing to reduce the numbers of companies to whom we can apply. Thus, the funding provided by the 1994 Corporate Sponsors (listed below) was especially appreciated.

The members of the Program receive special privileges in acknowledgment of their contributions. We waive all on-site fees for eight representatives of each company at our meetings. Three of these scientists may attend meetings at Banbury Center, where attendance is otherwise only by invitation of the organizers. Corporate Sponsors receive gratis copies of Cold Spring Harbor Laboratory Press publications including the journals *Genes & Development*, *Learning & Memory*, and *PCR*. Grace Auditorium is made available to Corporate Sponsor Program members for sponsorship of scientific meetings on topics of their own choice. These meetings are of the same excellent standard as the regular meetings and will garner kudos from being held here at the Laboratory. They are strictly limited in number. In 1994, Perkin-Elmer held the very popular and successful meeting *A Decade of PCR* to celebrate both the technique and the award to Kary Mullis of a Nobel Prize for Chemistry in 1993.

In addition, we acknowledge our Sponsors in all relevant publications, including the book of abstracts given to every participant. The names of the sponsoring companies are listed on the poster describing the meetings and mailed to approximately 7000 scientists throughout the world.

Alafi Capital Company
American Cyanamid Company
Amgen Inc.
Becton Dickinson and Company
Biogen
Boehringer Mannheim Corporation
Bristol-Myers Squibb Company
Chugai Pharmaceutical Co., Ltd.
Ciba-Geigy Corporation
Diagnostic Products Corporation
The Du Pont Merck Pharmaceutical Company
Forest Laboratories, Inc.
Genentech, Inc.
Glaxo
Hoffmann-La Roche Inc.
Johnson & Johnson
Kyowa Hakko Kogyo Co., Ltd.
Life Technologies, Inc.
Mitsubishi Kasei Institute of Life Sciences
Monsanto Company
New England BioLabs, Inc.
Oncogene Science, Inc.
Pall Corporation
The Perkin-Elmer Corporation
Pfizer Inc.
Research Genetics
Sandoz Research Institute
Schering-Plough Corporation
SmithKline Beecham Pharmaceuticals
Sterling Winthrop Inc.
Sumitomo Pharmaceuticals Co., Ltd.
Takeda Chemical Industries, Ltd.
Toyobo Co., Ltd.
Wyeth-Ayerst Research
Zeneca Group PLC

Total	**$720,500**

Cold Spring Harbor Laboratory Association (CSHLA)

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Mrs. James M. Large, Vice President
Gordon E. Lamb, Secretary/Treasurer

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President's Report

The year 1994 was a banner year for science here at Cold Spring Harbor Laboratory. The excitement of this success has infected the members of the Cold Spring Harbor Laboratory Association and the community. It is rewarding to find photos of Tim Tully and Alcino Silva in the *New York Times* and to read in *Newsday* of Mike Wigler's and Nicholai Lisitsyn's RDA work on genetic changes that occur in breast cancer and in colon and prostate cancer.

Despite the advances in science, however, our neighbors are concerned about the growth of the Laboratory. The Association has made a concerted effort this year to reach out to the community to dispel misunderstandings, to supply historical and current facts, and to familiarize outsiders with the impact that this basic research can have on our health and on the health of our children and grandchildren. Personal contact, letters, and frequent tours have, I think, helped to increase awareness of the Laboratory's work. Two hundred new members have joined the Association, bringing the total to 741 members (of course, some members fail to renew or forget to return the following year). We are growing, and we aim to continue growing as we expand the membership geographically.

The events sponsored by the Association have taken on a cyclical rhythm. The year began with our Annual Meeting, and we were fortunate to have a well-known alumnus and friend of the Lab, Dr. Robert Pollack, a Professor at Columbia, as our speaker. He explained DNA and RNA and proteins as the building blocks of our genome. Based on his recent book, *Signs of Life*, his interpretation helped us to understand our need to know our own relationship to what the genome will reveal.

The rhythm of scientific lay talks continued at Grace Auditorium in the spring, with a presentation on epilepsy by Dr. James McNamara, Duke University Medical Center. In the fall, a lecture on human behavior was given by Dr. Helen Fisher, a research associate with the American Museum of Natural History. In December, Drs. Jeffrey Friedman and Kenneth Polonsky spoke on diabetes. Dr. Fisher's talk, "The Evolution of Marriage, Adultery, and Divorce: The Future of the Family," attracted a large audience. Each of the above lecturers was attending a meeting at the Banbury Conference Center and kindly took time out of their busy schedules to present these talks at Grace.

On a beautiful day in May, a special tour of the Laboratory was arranged for the Cosmopolitan Club of New York City. Also in May, members of the Association joined the North Shore and The Three Harbors Garden Clubs to hear Dr. Gene Elder Likens' talk on "Human Accelerated Environmental Change—An Ecologist's View."

The Dorcas Cummings lecture held at the height of the Cold Spring Harbor Symposium was given this year by Dr. Harold Varmus, recently appointed director of the National Institutes of

Hot Jazz on Cold Spring Harbor—Jerry Jerome and his skilled musicians.

Health. His talk, "Why is it Important to Understand the Genetic Basis of Cancer?" interested all who attended. After the lecture, guests and visiting scientists were transported to nearby homes of Association members for dinner. This is one of the very nicest customs, in which worldwide celebrated scientists, here for the Symposium, join our scientists to be guests of Association members. Special thanks go to the following who hosted dinners in 1994:

Mr. & Mrs. Morgan Browne
Mr. & Mrs. John P. Cleary
Mr. & Mrs. Norris Darrell
Mr. & Mrs. John Grace
Mr. & Mrs. Gordon S. Hargraves
Mr. & Mrs. Henry U. Harris, Jr.
Mrs. Wendy Vander Poel Hatch
Mr. & Mrs. George N. Lindsay
Mr. & Mrs. David L. Luke III
Mr. & Mrs. Robert Merrill
Dr. & Mrs. Larry Nathanson
Mr. & Mrs. Robert O'Connor
Mr. & Mrs. James F. Stebbins
Mr. & Mrs. Byam K. Stevens
Prof. & Mrs. John Stevenson
Mr. & Mrs. Philip R. von Stade

Our benefit event this year was a summer outdoor jazz concert. Jane Spingarn was able to obtain Jerry Jerome and his skilled musicians, and she and Hope Reese chaired the event. Everyone who attended "Hot Jazz on Cold Spring Harbor" gave rave reviews. Many thanks to Jane and Hope for their hard work and for bringing a new audience to Cold Spring Harbor Laboratory. Special thanks goes to the Spingarns for contributing $2,000 to underwrite the musicians. The Jazz Concert Committee consisted of:

Mrs. John R. Reese
Mr. & Mrs. James L. Spingarn
Mrs. Lionel E. Chaikin
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Solicitation mailings for membership in the Association are done in the spring and fall. All major donors and many Friends and Family letters are personalized by our Directors. This year we solicited every resident of Laurel Hollow and selected School District #2 residents, and we prospected numerous Long Island lists. This is a worthwhile exercise that I suspect will bring even better returns in future years.

The final report shows 174 major donors of more than $1,000 and includes one gift increased by $85,000! Our 200 new members helped to bring us an all time high of $600,967. These funds will go to support young postdoctoral fellows and their laboratories. Today with federal funds hard to come by, these unrestricted moneys are essential to the excellence of Cold Spring Harbor research.

On Sunday evening, October 23, Lorraine Grace gave a lovely cocktail/buffet reception at her home, a "thank you" party honoring the Association's Benefactors, Patrons, and Associates. Four of the postdoctoral fellows who have been recipients of Annual Fund grants as well as several of the Laboratory's senior scientists were guests at the event. Many thanks to Mrs. Grace for a very good time and for giving many of us a chance to get to know one another better.

As I turn over the presidency to John Cleary, I feel elation that the Association is performing its support of the Laboratory well. It will miss the energy of the four wonderful Directors who rotate off this year: Mary Jeanne Harris, Gordon Lamb, Anne R. Meier, and Marjorie von Stade. I thank them and all the other Directors who have worked with me, especially the tireless Carol Large and the enthusiastic Jim Spingarn.

I offer my best wishes to John Cleary; to the new Directors Nick Bartilucci, David Deming, Laurie Genovese, Joyce Green, Bob Marcus, and Gerry Olin; and to Susan Cooper, Jean Schwind, and the new staff. I would also like to extend goodbyes and best wishes to Joan Pesek and Debi Rizzieri in all their future endeavors. It has been a great experience for me.

March 3, 1995

Mary D. Lindsay

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Benefits	7,531
Other	915
Total	**$600,967**

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The goal of the Laboratory's two student intern programs, the Undergraduate Research Program for select college students and the Partners for the Future Program for top high school students, is to produce future research scientists and to encourage awareness among young people in general.

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An important objective of the Corporate Advisory Board of the DNA Learning Center is to provide a sustainable level of annual funding for Learning Center programs. As a means of reaching this objective, the Board conducts an Annual Fund and in June 1994 held the first annual Cold Spring Harbor Laboratory Golf Tournament with the proceeds benefitting the DNA Learning Center.

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